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The Martian Environment

The ease with which hydrogen escapes from Mars has been a crucial factor in shaping the planet's environment. As a result of hydrogen loss the atmosphere of Mars has been oxidizing throughout virtually its entire history. Under such circumstances amino acids are not readily synthesized abiogenically. If small amounts of such compounds were formed they would be relatively quickly destroyed. Accordingly, the probability that life has originated on Mars is trivial.

Philip H. Abelson
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Studies on an Extracellular Nuclease of Staphylococcus aureus

An extracellular nuclease produced during the growth of *Staphylococcus aureus* has been shown to catalyze the hydrolysis of phosphodiester bonds in both DNA and RNA. This dual specificity is exhibited by preparations of the enzyme which appear to be homogeneous by physical and chemical examination. The protein consists of a single polypeptide chain that is free of disulfide bonds and sulfhydryl groups and contains approximately 128 amino acid residues. The molecule contains four methionine residues which have been used as sites of attack by cyanogen bromide to produce five peptide fragments. On the basis of amino-acid analysis, end-group determinations, and the separation and study of trypsin fragments of each, these have been arranged in the proper order along the chain. Degradation of each tryptic fragment by the method of Edman has established the internal sequences of essentially all of these component peptides.

Both strains V8 and "Foggi" appear to produce the same nuclease on the basis of peptide maps, amino-acid analysis, and a variety of physical studies. The internal structure of the nuclease is quite compact (intrinsic viscosity, 0.04; S_w^{20}, 1.7; helix content estimated from optical rotatory measurements, about 50 percent) Indeed, the single tryptophan residue is resistant to Koshland's reagent in the absence of high concentrations (about $6M$) of guanidinium ions. The structure undergoes a marked "melting" over a remarkably narrow range (57° to 62°C) as estimated by changes in this range in intrinsic viscosity and b_0. Calcium ions are required for activity ($0.02M$ or higher) and a wide variety of other cations tested cannot substitute for this obligatory requirement.

Christian B. Anfinsen
Hiroshi Taniuchi, James N. Heins
J. Robert Suriano
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Structural Studies on Human Erythrocyte Carbonic Anhydrases

Human carbonic anhydrases (CA) B and C are compact globular proteins of molecular weight near 30,000, with one zinc atom per molecule. Titration and spectrophotometric studies [Riddiford *et al., J. Biol. Chem.* **240**, 3305 (1965)] indicate that most of the tyrosine, tryptophan, and histidine residues are in the interior of the native protein, and are shielded from solvent. Optical rotatory dispersion (ORD) data reveal Cotton effects in the region 260 to 300 mμ, which disappear on denaturation. [Myers and Edsall, *Proc. Nat. Acad. Sci. U.S.* **53**, 169 (1965)]. The shorter wavelength minimum is near 222 mμ, with a maximum near 204 mμ. A single term Drude equation adequately describes the ORD over the range 350 to 600 mμ. All the data suggest that there is little α-helix in the native proteins, perhaps none.

On acid denaturation, the ORD pattern for CA B changes in a manner suggesting some helix formation in the denatured protein; CA C shows similar but less striking changes. At *p*H 10 to 12, CA B undergoes changes in local conformation; about *p*H 12 gross changes take place. CA C appears to behave differently at high *p*H, more like a random coil. Heat denaturation of CA B occurs abruptly near 55°C; temperature difference spectra show a blue shift on denaturation above 270 mμ, and a red shift below 250 mμ. Some aggregation occurs on denaturation in urea solution, but some native enzyme can be recovered on removing the urea.

John McD. Armstrong
Carole L. Lindblow, John T. Edsall
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Biosynthesis of Lactose with a Soluble Bovine Milk Transferase

A soluble enzyme, UDP-D-galactose-D-glucose-β-4-galactosyl transferase, has been found in bovine milk capable of synthesizing lactose from UDP-D-galactose according to the following reaction.

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

Approximately 80 percent of the ^{14}C-labeled D-galactose can be incorporated into the lactose from the radioactive substrate. Some of the properties of this enzyme, which has been purified 70-fold, have been determined. The enzyme appears to be specific for UDP-D-galactose. None of the ^{14}C-labeled D-galactosyl nucleotides containing bases other than uridine (guanosine, adenosine, cytidine, thymidine) can serve as substrate for the formation of lactose. The following compounds are listed in order of their decreasing inhibition activity on the enzyme: PP$_i$, ITP, UTP, UDP, P$_i$, UMP, TTP, α-D-galactose 1-P, lactose, uridine, and dUMP. No inhibition can be shown with ATP, CTP, or D-galactose. The pattern of enzyme inhibition appears to be in accord with the high enzyme affinity shown for UDP-D-galactose ($K_m=5.0 \times 10^{-4}M$) and lower affinity shown for D-glucose ($K_m=2.5 \times 10^{-2}M$).

Alpha-D-Glucose 1-phosphate, α-D-galactose 1-phosphate, L-glucose, D-xylose, maltose, or α-D-methyl-D-glucoside will not act as an acceptor for the D-galactose moiety of UDP-D-galactose to form corresponding oligosaccharides. However, *N*-acetyl-D-glucosamine is 25 percent as effective an acceptor as D-glucose. The product appears to be *O*-β-D-galactosyl-*N*-acetyl-D-glucosamine. No reversal of the enzymic reaction can be demonstrated when lactose and UDP are used as substrates for the formation of UDP-D-galactose and D-glucose.

The bovine milk lactose synthetase is activated by divalent cations. It shows maximum activation by Mn^{++}, and inhibition by EDTA and Hg^{++}. The enzyme has a temperature optimum of 42°C and a *p*H optimum of 7.5.

HELENE BABAD
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Improved Porphyrin Analysis for Carbonaceous Meteorites

Porphyrin compounds generally regarded as fossil biogenic substances are frequently found in terrestrial rocks of all ages, even as old as 2.8 billion years and at levels as low as 0.002 ppm of the rock. Chlorin compounds are closely related to porphyrins, and are abundant in living plants, soils, dusts, and recent sediments. Samples of the Orgueil meteorite showed the presence of pigments indistinguishable from fossil porphyrins of ancient terrestrial rocks. Chlorins appeared to be absent. Detection of porphyrin pigments in carbonaceous meteorites is hampered by the limited availability of sample material and the presence of intensely colored organic sulfur compounds. New refinements in porphyrin analyses using thin layer chromatography are lowering the limits of detection to well below the 0.1-microgram level reported for porphyrin-like compounds in the Orgueil samples—which corresponded to 0.01 ppm of the meteorite—so that analyses may be carried out on type II and type III meteorites such as Cold Bokkeveld, Murray, and Mokoia.

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Improving the Effectiveness of Large Telescopes

A family of two-mirror correctors has been devised that in combination with any paraboloidal telescope of even the largest size will yield a greatly increased photographic field without the need for refracting elements of any form. Such correctors will be of particular use in the ultraviolet and for space-borne telescopes into the far ultraviolet, with performance limited only by diffraction over angular fields appreciably larger than available heretofore.

As a byproduct of the investigation, the author has found that two spherical mirrors, one convex and the other concave, already can produce a high state of correction. The fact that so simple a corrector exists and can be effective when used with a telescope as large as the 200-inch is an unexpected and mathematically curious result.

The fully optimized two-mirror corrector, however, requires an ellipsoidal convex secondary with a slightly turned-down edge and a spherical concave tertiary mirror. Such a mirror-pair, for a full range of diameters from very small to quite large according to the size of photographic plate desired, will produce a strictly flat photographic field free of spherical aberration, coma, and astigmatism to a more than satisfactory state of correction.

JAMES G. BAKER
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Mating Preferences in Dogs

Five beagle bitches and five males of the same strain were reared together from puppyhood and observed in a series of standardized mating tests over a period of two years. Every bitch was tested with each male several times in the course of two normal estrous periods separated by at least six months, and at other times when the female was in artificial estrus induced by estrogen treatment. Even at the height of physiological estrus some females were highly selective, readily accepting some males and vigorously rejecting the sexual approach of others. Bitches differed in the intensity and exclusiveness of their mating preferences. Some males were entirely acceptable to certain females but unacceptable to others. The sexual preferences of the five females remained stable and consistent from one estrous period to the next. The basis for individual choices by the bitches has not yet been determined but does not appear to bear any relation to the sexual efficiency of the males.

FRANK A. BEACH
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Amyloid: An Aberration in Protein Formation

From human liver, spleen, and kidney containing amyloid deposits proteins can be extracted with 6- to 8M urea solutions which are not found in extracts of non-amyloid containing tissues. A prominent entity in the material extracted is a globular protein of molecular weight about 13,500. This protein, which may represent ¼ or more of the total material recoverable from amyloid tissue, has phenylalanine as the *N*-terminal amino acid, no cysteine, and probably no threonine. Tyrosine, tryptophan, and phenylalanine represent 7.0, 3.0, and 9.4 percent of the material, respectively. In these and other respects the protein does not correspond, to our knowledge, to any of the presently characterized proteins of serum or tissues.

The sources of amyloid constituents are unknown. Association of amyloid with chronic inflammations, hyperimmunization, and plasma cell myeloma has engendered the idea that the material seen in these extracellular deposits may be antigen-antibody gamma globulin or its fragments and associated items, for example, complement. Immunologic examination of our extracts, of whole tissue, and mechanically purified amyloid material does not support this notion. The presence of the unusual protein suggests a new set of possible sources for amyloid: (i) the material is a normal protein (or proteins) produced in abnormal amounts; (ii) that we are dealing with an abnormal protein; or (iii) that this is a protein fragment of a normal constituent (for example, mucopolysaccharide) produced under conditions leading to amyloid formation.

EARL P. BENDITT
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Allosteric Transitions of an Induced Enzyme Manifested by Fluorescence Enhancement

A purified induced enzyme from yeast, uridine diphosphate (UDP)-galactose 4-epimerase, can exist in various allosteric catalytically active forms. These forms can be studied by means of fluorescence techniques since bound reduced diphosphopyridine nucleotide (DPN) of the induced epimerase elicits fluorescence with a high quantum yield.

The enhancement factor as based on the fluorescence of free 1.4 DPNH amounts to 30-fold. The specific substrates UDP-galactose or UDP-glucose reduce bound DPN but without any enhancement of fluorescence. However, a new uridine nucleotide-glucose compound which can be formed from 5′-uridylic acid and D-glucose (or D-galactose) is able to reduce the epimerase DPN under conditions of highly enhanced fluorescence. This is also the case with respect to restoration of fluorescence from dark epimerase by means of the 5′-uridylate-glucose compound.

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Biology of Germ Cells in Mammals

The extra-gonadal origin of germ cells during gametogenesis in mammals has now been defined. However, the manner in which the germ cells migrate from the yolk sac to the genital ridges was not clear. Examination of living tissues from yolk sac, genital ridges, and gonads of mice, hamsters, and human embryos by phase microscopy has shown that the germ cells have cytologic and behavioral characteristics which clearly distinguish them from all other embryonic cells. The individual germ cells migrate from the yolk sac to the genital ridges by ameboid movement. The pattern of movement has been recorded cinematographically. The primitive oögonia continue localized movements within the differentiating ovary even after the pachytene stage of meiosis has been reached. When embryonic ovaries are grown in tissue culture the primitive oögonia will grow to a size of 100 microns or more. Occasionally a secondary membrane appears which simulates the zona pellucida. (Supported by USPHS grant GM 11396)

RICHARD J. BLANDAU
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Neurosecretion in Microphthalmic and Normal Rats

Female rats with microphthalmia reach sexual maturity from 4 to 6 weeks later than do normal controls and their breeding performance is poor. The supra-optic nucleus (SON) and the median eminence of the hypothalamus have been demonstrated repeatedly to be associated with reproductive phenomena. The purpose of the present study was to examine histological evidence for a difference, if any, in the neurosecretory activity of the SON between a normal strain and a microphthalmic strain of rats using as criteria (i) cell counts of the SON and (ii) counts of Gomori aldehyde fuchsin staining SON neural staining bodies.

Female rats with microphthalmia were found in general to have fewer neurosecretory cells in the SON than the normal colony animals. A larger number of aldehyde fuchsin staining neural staining bodies from the SON was found in sexually mature females than was true of sexually immature females, as would be expected. There was a larger number of neurosecretory cells in the SON of immature females of the normal strain when compared to the microphthalmic strain, but this difference between the two strains was reduced in sexually mature females. More than one-third of the microphthalmic eyed females had considerably fewer neurosecretory cells in the SON, and contained fewer staining bodies than the lowest count in the normal colony. Statistically there was no difference between the sexually mature animals.

Although no clear-cut consistent statistically significant difference could be demonstrated in the neurosecretory activity of the SON between normal and microphthalmic females, the evidence is interpreted to indicate that the microphthalmic females tend to have fewer cells in the SON as well as lower neurosecretory activity.

LUDVIG G. BROWMAN
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The Reactivity of a Crystalline Hemoprotein: The Reaction of Azide with a Suspension of Ferrimyoglobin Crystals

The assumption that crystalline proteins and enzymes have reactivities similar to those of the dissolved materials is basic to the application of x-ray crystal structures to the elucidation of their reactions in solution [J. Boyes-Watson *et al.*, *Proc. Roy. Soc. London Ser. A.* **191**, 83 (1947)]. Many data suggest that this assumption is valid, but definitive evidence on the point is lacking [F. M. Richards, *Ann. Rev. Biochem.* **32**, 269 (1963)]. A dual wavelength spectrophotometric technique allows the use of the rapid-flow apparatus for the measurement of the kinetics of the reaction of crystalline and dissolved ferrimyoglobin with azide at 630 and 600 mμ. The product is ferrimyoglobin azide, a compound of known structure [L. Stryer *et al.*, *J. Mol. Biol.* **8**, 96 (1964)]. Crystals of myoglobin were prepared from horse skeletal muscle [H. Theorell and Å. Åkeson, *Ann. Acad. Sci. Fennicae Ser. A* **60**, 303 (1955)]. The second-order velocity constants for the crystals and the dissolved protein at 24°C and *p*H 6.0 in 95 percent ammonium sulfate are respectively 4×10^3 and 8.5×10^4 M^{-1} $\sec^{-1}$, indicating a 21-fold slower reaction of the crystalline material. The two methods have been employed to ensure that the slower reaction is not due to a diffusion limitation in the crystals. First, crystals fractured by ultrasound from 5×30 to 2×9 μ were found to react at the same rate. Second, the energy of activation for the formation of the azide compound in the crystal suspension is 19.6 kcal, a value much too large for the diffusion-limited reaction. It appears that the conformation change due to crystallization of the protein has influenced the reactivity of the haematin iron by over 20-fold. It is possible that the hydrogen bonding of azide to the distal histidine (see Stryer *et al.*, above) is a factor that slows the reaction in the crystalline material.

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Aromatic Character in Cyclic Conjugated Systems

The fundamental factor responsible for aromatic character of cyclic conjugated systems usually is regarded to be appreciable cyclic π-electron delocalization. Attempts in the past to evaluate the degree of aromatic character have utilized physical measurements, such as bond distances, resonance energies, and so forth, that are only indirect manifestations of this fundamental factor. It has been presumed during the last few years that π-electron delocalization is reflected more directly in ring currents revealed by nuclear magnetic resonance or by diamagnetic susceptibility measurements. Proton magnetic resonance and perpendicular diamagnetic anisotropy determinations of various benzenoid and nonbenzenoid compounds (triphenylcyclopropenylium ion, fulvene, cycloheptatrienylium ion, β,β-diphenylheptafulvene, heptafulvalene, cyclooctatetraene, dibenzopentalene, azulene, heptalene, acepleiadylene), and other carbocyclic and heterocyclic systems will be considered in discussion of this presumption.

HYP J. DAUBEN, JR.
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Coding Ambiguity in Polypeptide Biosynthesis

The nature of the amino acid coding ambiguity of polyU induced by ethanol and streptomycin in the *E. coli* cell-free system has been further investigated. In the presence of these compounds, polyU directs the incorporation of leucine and isoleucine in addition to phenylalanine. In general, supplementation of the reaction mixture with sRNA freed of amino acids reduces the ambiguity. An interdependence was observed in the incorporation of the above three amino acids. The presence of leucine markedly reduced the incorporation of both phenylalanine and isoleucine. Leucine incorporation was increased by the addition of phenylalanine. Under some conditions, phenylalanine also stimulated isoleucine incorporation. Isoleucine was stimulatory to leucine incorporation but showed little effect on phenylalanine incorporation. Preliminary observations indicate the presence of C^{14}-leucyl-phenylalanine and C^{14}-phenylalanyl-leucine (as well as the labeled homologous dipeptides) in partial acid hydrolyzates of polypeptides resulting from ambiguous polyU coding. The above data suggest that under conditions of ambiguous coding a single triplet containing only U's is capable of recognizing more than one aminoacyl-sRNA, the affinity of binding of any particular aminoacyl-sRNA being dependent on the experimental conditions. It is concluded that ambiguous coding by a polynucleotide containing a single nucleotide base produces single peptide chains each containing several different amino acids.

EARL W. DAVIE, ANTERO G. SO
JAMES BODLEY, FANYELA WEINBERG
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Cytogenetic Evidence for Long-Continued Evolutionary Stability in the Genus Plantago

Plantago insularis, native to the islands, seacoast, and deserts of the southwestern United States and adjacent Mexico, and *P. ovata*, native to south central and southwestern Eurasia and to North Africa, are both annual species with the somatic chromosome number $2n=8$. They are similar in external morphology, karyotype, and the ecological conditions of their habitat. In both species, individual chromosomes can be recognized at mitotic metaphase and at prophase of mitosis and meiosis by their general form and their patterns of heterochromatic segments. In respect to one pair of chromosomes, differences between the species can be recognized in both parental and F_1 hybrid karyotypes.

At meiotic prophase in the F_1 hybrid, pairing between chromosomes derived from the two parental species is nearly complete, but asymmetrically thickened strands indicate chromomeric and segmental nonconformities. Associations of four chromosomes at prophase and M_1 of the hybrids suggest interchange differences. Although some irregularity occurs in later stages of meiosis, most microspores receive a normal complement of four chromosomes. Pollen sterility is complete, due to the male sterile condition of the *P. ovata* maternal parent. Seed setting from backcross pollination is high, indicating that gene exchange between the two species is possible. The compatibility of the parental genotypes in the hybrid indicates that genetic and chromosomal differentiation of the two species has not been accompanied by radical changes in genes controlling developmental processes.

Phytogeographical, geological, and paleontological evidence indicates that the common ancestor of *P. insularis* and *P. ovata* could not have migrated from the Old World to the New more recently than the Oligocene epoch, 25 to 35 million years ago. Consequently, these two species have been separated for many millions of generations with little or no greater evolutionary divergence than that necessary for subspecific differentiation.

ALVA DAY, G. LEDYARD STEBBINS
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Site-Site Interactions in Muscle Phosphorylase

Muscle phosphorylase contains several sites affecting its enzymatic activity: a site involved in the binding of AMP (required for the activation of phosphorylase *b*), another one phosphorylated during conversion of phosphorylase *b* to *a*, and a third one at which pyridoxal phosphate (PLP) is covalently attached. Much information is available regarding interactions involving the AMP, the seryl phosphate, and the active site, in contrast to the PLP site, chiefly because of the tightness of binding of the cofactor to the protein, and hence of its unreactivity. It has now been found (Hedrick and Shaltiel) that slight distortion of the molecule in citrate or imidazole solutions or both (which expose the PLP) followed by cysteine attack can readily remove PLP (half-life of 3 to 5 min at *p*H 7.0 and 0°C, and 50 sec at 30°). Resolution is totally blocked by addition of AMP or upon phosphorylation of the seryl site. The apoenzyme can still be converted from apophosphorylase *b* to apophosphorylase *a* by phosphorylation of the protein, and still binds AMP. However, this latter process no longer confers to the molecule the structural modifications it imparted before resolution. These interactions will be discussed in terms of regulation of enzyme activity.

EDMOND H. FISCHER
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An Electronic Particle Separator with Potential Biological Application

An electronic device has been developed that is capable of separating particles (suspended in a conducting liquid) on the basis of volume. Particle volume is sensed by a Coulter aperture. The suspension is ejected from a nozzle as a fluid jet and uniformly broken into droplets by the action of a piezoelectric crystal. An interval of 250 μsec is required for a particle to travel from volume-sensing point to jet separation point, where it is caught in a forming droplet. At the time of formation, droplets are charged according to volume of the contained particles. The charged droplets pass through an electrostatic field and are deflected in proportion to their charge. Mixtures of human and mouse red blood cells (which differ in volume by a factor of two) have been separated quantitatively. Volume fractions of mouse lymphoma cells randomly growing in suspension culture have been separated. Tests with Chinese hamster ovary cells showed 96 percent viability and normal growth rate after passing through the device. Application to exfoliative and bone-marrow cytology is in progress. The separation principle, which has been proved with polystyrene microspheres and various biological cells, may be extensible to separation of particles on the basis of any electronically measurable property such as optical density or fluorescence. Work performed under the auspices of the USAEC.

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Invariant Properties of the Iso-sensitivity Function and Their Relations to Utility Measurement

Four experiments were performed in which human observers reported whether or not they could detect the difference in amplitude between a pair of acoustic stimuli presented on each of several thousands of trials. In one experiment the probability that the pair differed was systematically varied. In another experiment the relative value of reporting the differences was systematically varied. In a third experiment the instructions to the subject were systematically varied, and in a fourth, the monetary value of reporting a difference was altered by multiplying the rewards by a positive constant.

In the first three experiments the iso-sensitivity function, the function that describes the relation between correct reports of a difference and false reports of a difference, was unchanged under all conditions. In the fourth experiment the absolute response probabilities of correct detections and false reports were reproduced even though fivefold changes in value were made. This last result lends additional empirical support to the accumulating evidence that the utility function of money is a power function.

EUGENE GALANTER
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Observations on the Repression of Alkaline Phosphatase Synthesis in Escherichia coli

Sadler and Novick (1965) and Gallant and Stapleton (1964) have used the relationship between differential rate and regulator gene dosage to approximate the relationship between differential rate and repressor concentration. This approach depends on the assumption that regulator gene products are in substantial excess over their sites of action (presumably operator loci). We have verified this assumption in the case of the phosphatase system and the R1 regulator gene, through the use of merodiploids heterozygous for an R1 allele. Under conditions of repression, heterogenotes showed a differential rate about twice that of homozygous $R1^+$ strains, in experiments where the noise level of spontaneous constitutive recombinants was very low. This is the result expected if the R1 gene product is in substantial excess over the number of presumptive phosphatase operators. If the number of R1 gene products was very low, the heterogenotes would show a much higher differential rate.

Alkaline phosphatase synthesis is derepressed at low extracellular concentrations of orthophosphate. We have measured the *intracellular* level of orthophosphate during derepression, in order to find out whether orthophosphate is the true corepressor. We found only a slight decrease in intracellular orthophosphate during derepression. Furthermore, under conditions of partial repression in a temperature-sensitive constitutive mutant, an inhibitor of oxidative phosphorylation (dinitrophenol) produced derepression without any decrease in intracellular orthophosphate. These results suggest that the corepressor is not intracellular orthophosphate, but rather some high energy phosphate compound.

JONATHAN GALLANT
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Psychological Properties of the Disconnected Hemispheres in Man

The development of cerebral dominance and lateralization of speech, handedness, and other specialized asymmetries in the brain of man raises problems regarding the right-left apportionment of mental functions in the two hemispheres. These and related questions have been studied recently in several patients in whom the cerebral cross-connections have been surgically divided for treatment of seizures [Bogen and Vogel, *Bull. Los Angeles Neurol. Soc.* **27**, 169 (1962)]. In general, the personality, temperament, and intelligence have remained largely unchanged as seen in ordinary daily behavior and in generalized testing with both hemispheres operating freely. When each hemisphere is tested separately, striking differences are seen between right and left sides, particularly in linguistic ability. The disconnected left hemisphere perceives, comprehends, and expresses itself both in speech and in writing at a level hardly distinguishable from that of the normal intact brain. Only in execution of drawings involving spatial relation, and certain spatial design tasks, has the left hemisphere so far been shown to be inferior to the right.

The complete inability of the right half brain to communicate either by speech or by writing makes detection and assessment of its latent mental capacities devious and difficult. By employing nonverbal tests designed for simple manual readout, the mute minor hemisphere can be shown to perceive, think, emote, learn, and remember at a level characteristically human [Gazzaniga *et al.*, *Brain* **88**, 221 (1965)]. Comprehension even for printed and spoken words and numbers is demonstrable and proceeds outside the cognitive awareness of the left hemisphere. Emotional effects, however, triggered by the presentation of "pin-up" pictures to the minor hemisphere, reach also the speaking hemisphere affecting the tone of speech, causing giggling, blushing, and so on, but leave the unstimulated, speaking hemisphere incognizant of what generated the feeling. With both hemispheres working together, reaction-time studies involving simultaneous performance of two separate discrimination tasks presented one to each hemisphere show that split-brain subjects, unlike normals, can do the two reactions as fast as one.

M. S. GAZZANIGA
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Fiber Tensor Products

In analogy with the definition of fiber bundles, there is given a definition of "fiber tensor products." Just as there are circumstances in which a topological space is locally a Cartesian product, so there are circumstances in which the tensor product of two C-modules, A and B, with respect to C, is locally the tensor product of modules A_j' and B with respect to a fourth ring D over which A, B, and C are modules.

In particular, when A, B, C are group algebras over locally compact abelian groups G, H, K, related by homomorphisms $\theta_G : K \rightarrow G$, $\theta_H : K \rightarrow H$ with Im (θ_G) and Im (θ_H) closed and where $D = C$, then $A\ 0_C\ B$ is locally $A_j'\ 0_\gamma\ B$ if the orbit space of $G \times H$ under the action of K $(k\ (g,h) = (\theta_G\ (k)\ g, \theta_H\ (k^{-1})\ h)$ is a fiber bundle. In this situation A_j' is $L_1\ (V_j)$ where V_j is an element of the base covering that is associated with the fiber bundle.

Bernard R. Gelbaum
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Photochemical Studies of Tobacco Mosaic Virus and Its Ribonucleic Acid

The action spectrum of the U1 strain of tobacco mosaic virus (U1-TMV) suggests that the protein of this strain is involved in biological inactivation by ultraviolet light. The comparatively small sensitivity of the U1 strain indicates a protective role for the protein. The sensitivity of the U2 strain at 253.7 mμ is virtually identical to that of the RNA and suggests that the U2 protein has little protective effect at this wavelength. It has been found that when U2-RNA is reconstituted with U1 protein, the sensitivity of the hybrid particles towards 253.7 mμ radiation is virtually identical to the sensitivity of U1 virus. The sensitivity of the hybrid particles, U2 protein-U1-RNA, on the other hand, is like that of native U2-TMV. A direct alteration of U1 protein by 253.7 mμ irradiation was demonstrated by the finding that for each biological hit undergone by U1-TMV, an average of one protein subunit was bound to the RNA. In studies with free RNA it has been found that the photoreactivable sector of U1-RNA increases with increasing wavelength. The increase is especially large at wavelengths greater than 280 mμ. The photochemical lesions produced by radiation of long wavelengths may undergo dark repair under suitable conditions.

Milton P. Gordon
Judy Goddard
Virginia Merriam
David Streeter
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The Selective Origin of Incompatibility Barriers in the Plant Genus Gilia

Nine intersterile species of annual *Gilia* in Pacific North America and South America, belonging to the same section (leafy-stemmed *Gilia*), fall into two classes with regard to geographical distribution. Five species in the California foothills and valleys are sympatric; four maritime species are completely allopatric in relation to one another and either completely or mainly allopatric in relation to the foothill-and-valley species. The sympatric foothill species are isolated by very strong incompatibility barriers, whereas the allopatric maritime species can be crossed *inter se* with the greatest of ease. The observed correlation between crossability and geographical distribution is interpreted as evidence in favor of the hypothesis of a selective origin of hybridization-preventing mechanisms in annual plants under conditions of sympatry.

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Effect of Heart Beat and Respiration on Flow Patterns in the Cavae, Pulmonary Artery, Pulmonary Vein, and Aorta in Intact Dogs

Ultrasonic flow transducers were implanted in dogs, and the animals were allowed to recover completely. Vena caval flow reversed with atrial systole, and the two forward flow waves were coincident with descent of the A-V valve with ventricular contraction and with the rapid-filling phase of ventricular diastole. Pulmonary vein flow was remarkably similar, and in the absence of transmitted pulmonary artery pressure pulses, the venous flow patterns are attributed to *vis a fronte*.

Right ventricular ejection begins slightly before and ends shortly after left ventricular ejection and has a more rounded contour. There is a momentary reversal of flow in the root of the aorta and pulmonary artery in early diastole, with greater prominence in the former.

Inspiration produces two primary changes in flow patterns, and an increased heart rate shortens the filling time for the ventricles, which tends to reduce the stroke volume unless compensated by the enhanced venous return produced by inspiration. The transmission time of this inspiratory surge to the left heart is usually very brief, less than that of two heart beats. The pulmonary vein demonstrates a net increase in flow during inspiration in the majority of instances, and the summed aortic flow is similarly increased during inspiration in more than half the subjects, in spite of a slight reduction in aortic stroke volume due to the increased heart rate.

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School of Medicine

Expression of the Tryptophan Genes in Escherichia coli Heterogenotes

The temperate coliphage ϕ80 specifically transduces the genes of the tryptophan cluster. Independently isolated nondefective transducing phages (ϕ80pt phages) vary in their content of tryptophan genetic material, some carrying the genes from the tryptophan synthetase side of the cluster (T'sase$^+$ A'sase^{del}), others those from the anthranilate synthetase side (T'sase^{del} A'sase$^+$), presumably containing the tryptophan operator. Lysogenization of a T'sase A mutant bacterium by a T'sase$^+$ A'sase^{del} pt phage yields transductants which are unstable heterogenotes. In all cases the A protein level in these heterogenotes is under normal repression-derepression control by tryptophan. Tryp$^-$ mutant 5927, isolated by R. Somerville, is a T'sase$^+$ A'sase$^-$ point mutant in which expression of the tryptophan operon has been drastically reduced (extreme polar mutant). Heterogenotes of 5927 lysogenized by a T'sase^{del} A'sase$^+$ pt phage show restoration of the expression and repressibility of the T'sase A gene. These observations and others suggest that ϕ80pt may be inserted into the *Escherichia coli* chromosome in heterogenotes by pairing and crossing-over between the bacterial tryp genes and those of the phage.

Benjamin D. Hall, Koji Okamoto
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The 12-Hourly and 24-Hourly Atmospheric Oscillations

Both the 24-hourly and the 12-hourly oscillations of atmospheric pressure are caused by the daily solar heating. But in the daily temperature curve the 24-hourly component has an amplitude about three times larger than that of the

12-hourly component, while the 12-hourly pressure oscillation is larger and much more uniformly distributed over the earth than the 24-hourly oscillation. The hypothesis of a strong resonance magnification of the 12-hourly wave had to be abandoned after the upper-air observations had shown that the structure of the atmosphere is not capable of producing this magnification. Since the 12-hourly temperature wave is large enough to explain the magnitude of the 12-hourly pressure oscillation, especially if the large daily temperature variation in the ozone layer is taken into consideration, the problem is to account for the smallness of the 24-hourly pressure oscillation despite the large 24-hourly temperature oscillation.

The main term in the expression for the diurnal pressure oscillation, obtained by a spherical-harmonic analysis of the available observational data, is found to be in reasonably good agreement with theoretical estimates. The theory shows that the 24-hourly pressure wave is suppressed largely because its global distribution on the rotating earth has to be very different from that of the 24-hourly temperature wave. For the 12-hourly oscillation the geographical distributions of the temperature and pressure waves are much more similar, resulting in the observed better development of this oscillation.

BERNHARD HAURWITZ

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Search for Optical Activity in the Orgueil Meteorite

Nagy's report of an optical rotation of $-0.023°$ to $-0.084°$ in the Orgueil meteorite has not been confirmed. The highest rotation found in several fatty-acid and hydrocarbon fractions isolated from the meteorite was $-0.002°$. The apparent rotations observed by Nagy may have been caused by several effects described by Rouy and Carroll: scattered depolarized light from colloidal particles, reduced instrument sensitivity due to low transmittance of the solutions, and a systematic levo-bias of the polarimeter. Comparable "rotations" were indeed observed in various optically inactive controls matching meteorite extracts in transmittance and light scattering properties.

RYOICHI HAYATSU

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Singular Measures with Absolute Continuous Convolution Squares

Let G be a locally compact nondiscrete abelian group. Then there is a continuous singular probability measure μ on G such that $\mu * \mu$ is not only absolutely continuous with respect to Haar measure but also has its derivative in ℓ_2 (G). The support of μ can be made to contain any preassigned σ-compact subset of G.

EDWIN HEWITT
H. S. ZUCKERMAN

University of Washington

Effect of Methyl Groups upon the Solvent Power of Aliphatic Liquids

Cyclopentane, cyclohexane, *cis*- and *trans*-decalin belong to a group of 14 liquids that dissolve iodine to give violet solutions in which its solubility conforms closely with the equation

$$RT \ln (a_2{}^s/x_2) = v_2\Phi_1{}^2(\delta_2-\delta_1)^2$$

where $a_2{}^s$ is the activity of solid iodine referred to liquid as standard state; x_2 is its measured mole-fraction; v_2 is the extrapolated molal volume of liquid iodine; Φ_1 is the volume-fraction of the solvent; the δ's are the "solubility parameters of the components" defined as the square roots of the energy of vaporization per cubic centimeter, $(\Delta E^{vap}/v)^{1/2}$.

Solubilities of iodine in methyl- and ethyl-cyclohexane deviate from this equation by approximately 300 cal, dimethyl cyclohexane by approximately 400 cal. In normal pentane it deviates by 700 cal, in four higher normal paraffins by approximately 420 cal. In 2,2- and 2,3-$(CH_3)_2C_4H_8$, 2,2,3-$(CH_3)_2C_4H_7$, and 2,2,4-$(CH_3)_3C_5H_9$, it deviates by approximately 800 cal.

The term $(\delta_2-\delta_1)^2$ is twice the excess of the arithmetic mean of $\Delta E_1{}^{vap}/v_1$ and $\Delta E_2{}^{vap}/v_2$ over their geometric mean, which latter is a relation, like the geometric mean of the van der Waals *a*'s, much used in attempts to deal quantitatively with binary mixtures of fluids. It is clear that the attractive potential between molecules of iodine and aliphatic hydrocarbons exceeds the geometric mean relation by large amounts related to the ratio of methyl to methylene hydrogen.

JOEL H. HILDEBRAND
JOHN DYMOND

University of California, Berkeley

Aspects of Posthypnotic Amnesia

Posthypnotic amnesia has long been accepted as one of the criteria of hypnosis and was originally the defining characteristic of the deeper hypnotic state that has been called somnambulism. It is of interest to know how "spontaneous" such posthypnotic amnesia is, or instead, if it is the result of subtle expectations on the part of the subject, due, for example, to the metaphor that is commonly used that hypnosis is like sleep.

Suggested posthypnotic amnesia results in amnesia for hypnotic experiences far more frequently than such amnesia when no suggestions are given; in fact, in our laboratory sample, there is little evidence for spontaneous amnesia at all.

Among several kinds of posthypnotic amnesia, an interesting variety is source amnesia, that form in which facts learned under hypnosis are remembered but the subject forgets that he learned them under hypnosis. In a laboratory test of spontaneous versus suggested source amnesia, under circumstances in which general posthypnotic amnesia was suggested, it was possible to demonstrate spontaneous source amnesia, but again at lower frequency than when it was suggested. All manifestations of amnesia are correlated with degree of hypnotic susceptibility as measured in other ways.

Because of the higher frequency of posthypnotic amnesia among children than among adults, some speculations are offered as to the developmental history of posthypnotic amnesia.

ERNEST R. HILGARD

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The Location of Star Formation in Galaxies

Narrow-band filter photography of 75 nearby galaxies provides information on the location of ionized gas in the galaxies. Since ionized gas is found in regions where star formation is going on or has recently occurred, the photographs allow a study of the location of stellar genesis in galaxies of various types. Among the spiral galaxies, those with tightly wound arms have stars forming only in a narrow concentric ring near the outer limits of the galaxy. Those with wide, loose arms have stars forming throughout the galaxy. Ionized gas clouds are concentrated to the spiral arms, but, contrary to expectation, not more so than the general stellar population. A few gas clouds lie between arms. Many remarkable asymmetries exist, with examples having been found of galaxies with ionized gas on one side only, galaxies with arms and no gas, and galaxies with gas and no arms. For the few objects for which the neutral-hydrogen distribution is known, there is a clear correlation between the observed location of ionized gas clouds and the concentration of neutral hydrogen.

PAUL W. HODGE
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Sense-Antisense Relations in the DNA of Bacteriophage λ

Call the sequence of bases in messenger RNA the sense sequence. For a given gene, one of the DNA strands contains the sense sequence and the other contains a sequence that can be termed antisense. In a chromosome consisting of one DNA molecule, we wish to determine the sense-antisense distribution between the strands for each of several genes in the chromosome. The chromosome used here is that of bacteriophage λ.

Two methods for determination of the sense-antisense distribution are being developed. Both depend upon the isolation of each strand, intact and free of the other strand; this has been accomplished [W. Doerfler and D. S. Hogness, *Fed. Proc.* **24**, 226, (1965)]. In the more highly developed of the two methods, we (with W. Doerfler) form each of two heteroduplex DNA molecules in which one strand is wild-type and the other contains a mutation in the gene of interest. In the case of certain genes, comparison of the biological activity of the two heteroduplex molecules allows determination of the sense-antisense distribution for the gene. This has been done for one of the λ genes (gene *N*). In the second method, we (with J. B. Egan) depend upon the isolation of small specific fragments of the λ chromosome and the determination of which strand will form DNA-RNA hybrids with messenger RNA specific to the fragment. At present we are at the initial stage of isolating and characterizing such fragments.

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Mechanism of Action of B_{12} Coenzymes

The enzymatic conversion of vitamin B_{12} or B_{12a} to adenosyl-B_{12} requires the participation of oxido-reduction cofactors (dihydrolipoic acid or reduced diphosphopyridine nucleotide plus flavin adenine dinucleotide) and adenosine triphosphate plus a divalent metal ion (Mn^{++} or Mg^{++}). Extracts of *Clostridium tetanomorphum* have been separated into two fractions: (i) a B_{12a} reductase which converts B_{12a} (in which the cobalt atom is in the +3 oxidation state) to B_{12s} (Co = +1); and (ii) an adenosylating enzyme which transforms B_{12s} to adenosyl-B_{12}. The latter enzyme has been obtained in highly purified form and its properties documented. B_{12a} reductase is more difficult to purify, possibly because it involves a multicomponent enzyme system.

The above reactions focus attention upon the ligand attached to the "top" side of the B_{12} molecule. In methionine biosynthesis (5-methyl tetrahydrofolate + homocysteine $\rightarrow$ tetrahydrofolate + methionine) adenosyl methionine is required as a cofactor for the crude enzyme from mammalian liver while methyl-B_{12} (or methyl cobinamide) is required for the purified enzyme. The methyl group of methyl-B_{12} does not appear to migrate during methionine synthesis via the above equation. This is consistent with a mechanism in which methyl tetrahydrofolate is complexed to the "bottom" side of the methyl-B_{12}, thereby facilitating release of the methyl group and transfer to homocysteine.

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Studies of Thyroxine Biosynthesis

The steps in the biosynthesis of thyroxine by the thyroid gland after trapping of iodide are: (i) the oxidation of iodide to some reactive form; (ii) the attack of this reactive iodine on tyrosine; and (iii) the coupling of iodinated tyrosine molecules to make the hormones triiodothyronine and thyroxine.

The first of these steps appears to be the peroxidation of iodide since the reaction is blocked in the gland by catalase. A peroxidase which will catalyze the oxidation of iodide has been solubilized from the particulate portion of the thyroid gland and partially purified. This enzyme is competitively inhibited with respect to iodide by the goitrogens methimazole and propylthiouricil. Reducing agents such as ascorbic acid and thiourea do not inhibit the enzyme but do reduce the reactive product, iodine.

The second step, the iodination of tyrosine, appears to be catalyzed by an enzyme in the particulate fraction of the thyroid. This enzyme is different in its reaction to inhibitors from iodide peroxidase and would seem to be a separate enzyme.

The final reaction in the production of thyroxine, the coupling of iodotyrosines, may involve a number of steps. Hillman [*Z. Naturforsch.* **116**, 424 (1956)] and others have shown that the α-keto derivative of diiodotyrosine, 4-hydroxy-3,5-diiodophenyl pyruvic acid, will react with diiodotyrosine to form thyroxine. The first step in the coupling reaction then may be the production of the reactive α-keto derivatives of iodinated tyrosine. An α-ketoglurate dependent transaminase has been partially purified from beef thyroid tissue which will transaminate tyrosine and monoiodotyrosine. This enzyme is immunologically different from beef liver tyrosine-α-ketoglutarate transaminase and has a different *p*H optimum. The role of this enzyme in the biosynthesis of thyroxine is under investigation.

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Calcium Uptake by Fragments of Sarcoplasmic Membrane

Fragments of sarcoplasmic membrane were obtained by differential centrifugation from homogenates of rabbit skeletal muscle and used for the determination of calcium uptake and enzymatic hydrolysis of adenosine triphosphate (ATP). Uncoupling of these two activities was obtained by adding diethylether to the reaction mixture in concentrations as low as 2.5 to 5.0 percent by volume (0.35 to 0.70*M*). Calcium accumulation by microsomes was measured at calcium concentrations varying from $2.5 \times 10^{-5}M$ to $1.0 \times 10^{-4}M$. In the absence of oxalate, treatment with diethylether markedly decreased calcium uptake. In the presence of oxalate, large volumes of diethylether (25.0 percent by volume) inhibited calcium uptake, while lower concentrations (2.5 to 5.0 percent by volume) increased calcium accumulation by microsomes. This latter effect was much more pronounced at low concentrations of oxalate. Under the same conditions, the rate of ATP hydrolysis was increased or unchanged after diethylether treatment. In contrast, Salyrgan, an SH group reagent, simultaneously inhibited calcium binding and adenosine triphosphatase activity.

Diethylether uncoupling appears to differ from deoxycholate uncoupling insofar as it does not cause solubilization of phospholipids and protein, and, at low concentrations, does not prevent microsomes from retaining calcium in the presence of oxalate. Assuming that the formation of a high-affinity calcium-membrane complex is a step in the process of calcium uptake, it is proposed that the presence of diethylether disturbs the reciprocal relation of charged groups belonging to the lipid or to the protein layers of the membrane or both. This hypothesis is discussed in relation to the interaction of calcium with lipids and deacylated phospholipid derivatives.

G. Inesi
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X-Ray Structural Study of a Bacterial Ferredoxin

Bacterial ferredoxin is a nonheme iron-containing protein having a molecular weight of about 6000 and approximately seven Fe atoms in the molecule.

An x-ray investigation of the structure of ferredoxin isolated from *Micrococcus aerogenes* has been initiated. Single crystals satisfactory for collecting x-ray diffraction data were grown from aqueous solution at *p*H 7.3 by salting out with $(NH_4)_2SO_4$. The crystals are orthorhombic with dimensions $a = 20.58$ Å, $b = 37.86$ Å, $c = 39.29$ Å. The space group is $P2_12_12_1$.

Two sets of data to a resolution of 5 Å have been collected from the same crystal with an interval of about 2 weeks. The crystal gradually deteriorated on standing at room temperature and lost approximately half its diffracting power during the 2-week period. However, the Patterson syntheses (vector maps) from the two sets of 5-Å data have essentially the same features, indicating the structures are essentially the same. Neither synthesis indicates the Fe atoms to be arranged in a single linear array. The vector map based on the 3-Å data shows the same general features as those based on the 5-Å data and, in addition, a great deal more detail. It is reasonable to assume that most of the prominent peaks are due to Fe-Fe interactions. There are a total of 28 × 27 = 756 such peaks (in addition to the origin peak) although the problem is complicated by overlap and coincidence. However, an attempt is being made to locate the Fe atoms by direct interpretation of the Patterson function.

L. H. Jensen
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The Structure of the High-Pressure Forms of Ice

The structures of several of the high-pressure forms of ice have been determined by the x-ray diffraction method. In every form each water molecule is bonded to four neighbors by hydrogen bonds, with increasing deviation of the bond angles from the tetrahedral value, permitting non-hydrogen-bonded contacts between molecules to form. There is proton disorder in all of the forms of ice except ice II. A discussion of the properties of the high-pressure forms of ice in relation to structure will be given.

Barclay Kamb
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Evidence Regarding Amino Acids and Sulfur Isotopes in Meteorites

A number of meteorites have been analyzed and amino acids found in all chondrites. The aliphatic compounds were always found to predominate. Contamination of the meteorite, from either handling or dust, could not be eliminated entirely. The finding of some amino acids in chondrules, carefully separated from the matrix of the meteorite, suggests that part of the sequence detected is authigenic.

Sulfur isotope studies (S^{34}/S^{32} and S^{33}/S^{32} ratios) by mass spectrometry of separated components show that small isotope effects have occurred. The pattern of fractionation, however, gives no evidence for biogenic processes having been involved in the transformation of the sulfur compounds isolated.

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Insulin Generation by Recombination of Synthetic and Natural A- and B-Chains

Great advances in peptide chemistry in the last decade have paved the way for undertaking the synthesis of polypeptide chains approaching the size of naturally occurring proteins. On the assumption that if chemically synthesized A- and B-chains of insulin were available it might be possible to obtain insulin by oxidation of a mixture of the sulfhydryl forms of the two chains, we undertook the synthesis of insulin. As a result of these studies, the synthesis of the A- and B-chains of sheep insulin and their isolation in the *s*-sulfonate form was accomplished. Combination experiments carried out between the synthetic A- and natural B-chain, the synthetic B- and natural A-chain, and between the synthetic A- and B-chains generated insulin activity. The comparative efficiency of insulin generation from these combination experiments was similar to that obtained by the combination of natural chains prepared by sodium in liquid ammonia treatment of the fully benzylated chains. The yield of insulin generation in the latter case is 10 to 20 percent of that produced by the combination of natural chains prepared by sulfitolysis of insulin. From the sulfitolytically prepared natural chains, recombination yields (based on native insulin) ranging from 1 to 5 percent are usually obtained, and more recently a Chinese group claims a yield of 40 percent.

Recent purification efforts enabled us to obtain synthetic A-chain which, upon combination with natural B-chain, generates insulin in a yield of 1.4 to 2 percent (percentage based on natural insulin). In the course of this investigation, a method was developed for the cleavage of insulin and the isolation of its individual chains in highly purified form and on a preparative scale. The natural chains purified by this method can be recombined readily to produce crystalline insulin in yields ranging from 4 to 7 percent.

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Three-Dimensional Ultrastructure of Adhesion Sites in Developing Amphibian Epidermis

Epidermis from larval newts (*Taricha torosa*) has been examined by election microscopy, utilizing stereographic techniques and ruthenium red staining in addition to more conventional procedures. Particular attention was paid to the architecture of large desmosomes, which presumably are strong attachment sites between adjacent cells, and hemidesmosomes which seem to provide similar adhesion between basal epidermal cells and underlying adepidermal connective-tissue components. An acid mucopolysaccharide material, within or lying upon the external cell unit membrane leaflet, can be demonstrated as continuous with pillars, partitions, or strands which bridge the extracellular gap at either of these adhesion sites. Intracellular components of desmosomes and hemidesmosomes include a dense plaque anchored to and parallel with the internal cell membrane surface, and numerous tonofilaments which approach the plaque from the filamentous skeleton of the cell. A new finding is that while a few filaments approaching a desmosome plaque actually enter the plaque, the majority loop at some distance from the plaque and return toward main filament tracts of the cell skeleton. In hemidesmosomes, more numerous filaments approach each plaque and are gathered into very densely packed bundles. Within each crowded bundle looping probably occurs much more sharply than is the case in desmosomes. The orientation of filaments as they approach desmosomes or hemidesmosomes is believed to be a reflection of the relationship of a given adhesion site to overall cell supportive architecture. No evidence has been found to indicate tonofilament passage into or through cell membranes. Therefore, the cell membrane is envisioned as separating intracellular supportive structure from an extracellular adhesive mechanism.

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Electron Correlation in Molecules

The variation-perturbation treatment of electron correlation in atoms and molecules appears to be quite promising. In this treatment the Hartree-Fock Hamiltonian is generally taken as the unperturbed system, H_0. This choice undoubtedly leads to rapid convergence, but the resulting variational equations are unwieldy and, particularly for molecules, difficult to solve. Furthermore, molecular Hartree-Fock solutions are not easily obtained. It is desirable, therefore, to find a more convenient starting point with satisfactory convergence properties. What we want is to have the perturbation treatment determine a first order wavefunction that gives an energy, that is $E \cong E_0 + E_1 + E_2 + E_3$, differing from the exact value by less than, say, 5 kcal/mole. One obvious choice for H_0 is the true Hamiltonian minus all electron repulsion terms. A calculation of electron correlation in H_2 has been carried out on this basis and we find that the above standard is easily met. Some results on four electron systems are given and the advisability of accounting for screening by inner shells in the first approximation is discussed.

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The d-Step Conjecture of Polyhedra of Dimension $d<6$

A *d-polyhedron* is a *d*-dimensional set P which is the intersection of a finite number of closed halfspaces in a real vector space; the *diameter* of P is the smallest number k such that any two vertices of P can be joined by a path formed from k or fewer edges of P. Two functions Δ and Δ_b are studied, where $\Delta(d,n)$ is the maximum diameter of *d*-polyhedra with $n(d-1)$-faces and $\Delta_b(d,n)$ is similarly defined for bounded polyhedra. The well-known *Hirsch conjecture* of linear programming asserts $\Delta(d,n) \leq n-d$, and the *d-step conjecture* asserts $\Delta(d,2d) \leq d$. The Hirsch conjecture was previously proved for $d \leq 3$, the bounded *d*-step conjecture (concerning Δ_b) for $d \leq 4$. Here it is shown the bounded 5-step conjecture is true ($\Delta_b(5,10) = 5$) but the general 4-step conjecture is false ($\Delta(4,8) = 5$). It is also shown $\Delta_b(4,9) = 5$. Other new values of Δ and Δ_b are obtained from two reduction theorems, $\Delta(d+k, 2d+k) = \Delta(d,2d)$ and $\Delta_b(d+k, 2d+k) = \Delta_b(d,2d)$, which justify emphasis on the *d*-step conjecture. Some rough bounds for Δ and Δ_b are established; in particular, $\Delta(d,2d) \geq d + [d/4]$, so the excess over the value conjectured by Hirsch tends to infinity with d. Also proved is the equivalence (though not on a dimension-for-dimension basis) of the bounded Hirsch conjecture, the bounded *d*-step conjecture, and the assertion that in a bounded *d*-polyhedron in which each vertex is of valence d, any two vertices can be joined by a path which does not revisit any $(d-1)$ face.

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Permeability of Suberized Roots to Water and Salt

Most investigators assume that water and salt are absorbed only through young, unsuberized roots, but not through older, suberized, bark-enclosed roots. This assumption is incorrect because it has been shown that absorption of both water and salt occurs through suberized roots of several woody species. Measurable water absorption occurred through attached, suberized roots of pine to which potometers were attached. When the pull of transpiration was simulated by applying pressure to the medium in which the roots were immersed, water moved in freely through the surfaces of suberized roots of loblolly and shortleaf pine, dogwood, grape, and yellow poplar. The permeability of suberized roots varies widely, ranging from about one-half to one-sixth of the permeability of unsuberized roots grown under similar conditions. Salt also moved in through suberized roots under pressure. Furthermore, root pressure exudation was observed from grape root systems bearing no unsuberized surface and from segments of completely suberized roots of loblolly and white pine. Suberized roots therefore resemble unsuberized roots in containing an ion barrier and a mechanism for active transport of salt into the xylem. As suberized roots usually constitute over 95 percent of root surface under forest stands, the larger fraction of water and salt must be absorbed through them. In view of this situation more attention ought to be given to research on the mechanism and pathway of water and salt absorption through suberized roots.

PAUL J. KRAMER
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Some Crystal-Structure Studies of Salicylaldimine Chelates

Crystal-structure studies of chelates of substituted salicylaldimines are of interest both because of the use of these compounds in analytical chemistry and as a subject for the investigation of a number of aspects of the theory of bonding in molecules. A number of published structures and recent unpublished structures will be discussed in terms of the information they present regarding several topics in molecular structure: bond lengths in π-electron systems; planarity of chelate rings; variability of the "bite" of chelating agents; and the effects of steric interactions on the conformation of molecules.

Bond lengths in two recent, accurate structure determinations, bis(*N*-phenylsalicylaldiminato) nickel and bis(*N*-isopropyl-3-methylsalicylaldiminato) nickel, are in very good agreement with quantum mechanical bond order calculations. Comparison of the deviations from planarity of a number of salicylaldimine chelates suggests that these molecules are quite flexible and that the deviations are caused by steric effects. The "bite" of the salicylaldiminate ion, when chelating to nickel (II), increases as the coordination configuration changes from planar to tetrahedral to octahedral. Examination of the hydrogen atom contacts in bis(*N*-isopropyl-3-methylsalicylaldiminato) nickel shows the steric interactions which cause many N-substituted salicylaldimine nickel chelates to assume a tetrahedral configuration, although it is still not apparent why the 3-methyl compound itself is planar.

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Partial Fate of Warfarin in the Rat

When the anticoagulant, *rac*-warfarin sodium [(3-α-acetonylbenzyl)-4-hydroxycoumarin sodium salt], labeled in the No. 4 carbon atom with C^{14} is injected intraperitoneally into rats, approximately 90 percent of the radioactivity is excreted during the succeeding 14 days. About one-half of the radioactivity is excreted in the urine and the remainder in the feces. No $C^{14}O_2$ has been observed in the expired air.

The 4-C^{14}-warfarin sodium or its metabolites apparently accumulate in the kidney, liver, and pancreas. These organs had respectively 3, 12, and 15 times the concentration of radioactivity found in the blood 96 hours after the administration of the labeled warfarin sodium.

Isotopic dilutions of an ether extract of the urine with synthetic 6-, 7-, and 8-hydroxywarfarins showed that these compounds are metabolic products of the labeled warfarin sodium. These metabolites accounted for approximately 15.4, 35 and 9.5 percent, respectively, of the total radioactivity in the ether extract. Unchanged warfarin accounted for about 7 percent of the total radioactivity. These results were further confirmed by paper and thin-layer chromatographic techniques.

If the structural similarities between vitamin K and the coumarin anticoagulants are considered essential for anticoagulant activity, a change in the structure, such as in the hydroxywarfarins, would be expected to destroy the activity. The hydroxywarfarins showed no hypoprothrombinemia inducing activity in the rat.

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Protein Synthesis with Single-Stranded DNA as a Template

Although one of the strands has a base sequence identical to that of messenger RNA, denatured DNA is normally inactive as a template for the direction of protein synthesis in vitro. The addition of antibiotics of the streptomycin family does, however, permit template activity of high efficiency by single-stranded DNA from any source. The synthesis of RNA molecules as intermediates in information transfer is not demanded. By using DNA of different base compositions and apurinic acid, it can be demonstrated that the directed incorporation of amino acids is in accordance with accepted code word assignments. The molecular weight of the product is similar to that of normal proteins. Possible applications of this highly efficient variant of cell-free polypeptide synthesis will be discussed.

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Specific Purification of Enzymes by Chromatography on Biochemical Derivatives of Cellulose

Chromatographic methods which utilize the specific, reversible complexing of some biological materials have found occasional use in their purification. A few instances have been reported in which enzymes were enriched by passage through columns of an insoluble matrix to which substrate is appended. This general technique now has been extended to include the syntheses of several cellulose derivatives which can be used to purify certain enzymes and other proteins with specific binding sites.

Flavokinase has been extensively purified by chromatographing on flavin-celluloses. These compounds were synthesized by reacting an aminoriboflavin substrate and an aminolumiflavin competitive inhibitor with chlorocarbonylmethyl cellulose prepared from carboxymethyl cellulose. Reduced nicotinamide adenine dinucleotide cytochrome *c* reductase, pyridoxamine phosphate oxidase, and glycolate oxidase have been purified by chromatographing the apoenzymes on flavin mononucleotide-celluloses. The compounds were synthesized by reacting riboflavin dichlorophosphate with cellulose phosphate and diethylaminoethyl cellulose and by reacting the riboflavin phosphate coenzyme and dicyclohexylcarbodiimide with cellulose. Avidin has been obtained in essentially pure form by chromatographing on biotin-cellulose. This compound was synthesized by converting biotin to its acid chloride and reacting the latter with cellulose.

The use of biochemically specific absorbents to selectively purify biological materials which are not readily amenable to other fractionation procedures is beginning to be of considerable importance. Though not always successful, the relative ease of synthesis of many such absorbents, the small number of reactive sites generally needed, and the usual simplicity of the overall method are real advantages which should lead to further developments.

DONALD B. MCCORMICK
CHARALAMPOS ARSENIS
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Heterosaccharide Moiety of the Tamm and Horsfall Urinary Glycoprotein

The urinary glycoprotein of Tamm and Horsfall temporarily inhibits hemagglutination by the myxoviruses influenza, mumps, Newcastle disease, and so forth. The enzyme neuraminidase, found in these viruses, splits the glycosidic bond between the sialic acid and galactosamine of the receptor substance which is found in the red blood cell, the host cell, and the glycoprotein.

To elucidate the nature of the carbohydrate moiety of the glycoprotein which contains the receptor group, the glycoprotein has been digested by the proteolytic enzymes papain and pronase. The remaining heterosaccharide has been isolated and purified by differential centrifugation and Sephadex chromatography. The heterosaccharide has been studied in the ultracentrifuge and by carbohydrate analyses. It is homogeneous in the ultracentrifuge and has a molecular weight of approximately 4×10^3. This implies that there are two heterosaccharide units per glycoprotein minimum molecular weight of 28×10^3. The glycoprotein is polymeric with molecular weight 7×10^6.

Experiments are underway to determine the amino acids which remain attached to the heterosaccharide and therefore the amino acids by which the heterosaccharide is attached to the protein moiety.

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Alkanes in Fragments of the Holbrook, Homestead, and Waconda Meteorites

The benzene-methanol extracts of fragments of the Holbrook, Homestead, and Waconda chondrites contain fractions of C_{16} to C_{28} alkanes which are apparently almost identical in composition. Gas-liquid chromatograms of these fractions of chondritic alkanes do not appear to vary more than do gas-liquid chromatograms of a specific fraction of alkanes. Furthermore, significant compositional variations are not detected either in the alkanes obtained from different fragments of the Holbrook and Homestead meteorites or in successive extracts of intact or pulverized and hydrofluoric-acid-disintegrated fragments of the Holbrook and Waconda chondrites, respectively. The quantities of C_{16} to C_{28} alkanes in the successive extracts of the intact or pulverized and hydrofluoric–acid–disintegrated Holbrook and Waconda samples show that the major portion of the alkanes were retained within the interiors rather than on the surfaces of these samples. Gas-liquid chromatographic and mass spectrometric analyses indicate that pristane and phytanes are constituents of the chondritic alkanes. Pristane and phytanes are isoprenoid-type hydrocarbons which are structurally related to the phytol moiety in the chlorophyll molecule.

W. G. MEINSCHEIN
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An Explanation of the Unusual Optical Specificity of Glutamine Synthetase

Purified glutamine synthetase from sheep brain converts both the L- and D-isomers of glutamic acid to the respective isomers of glutamine according to the following equation:

$$\text{Glutamic acid} + NH_3 + ATP \rightleftharpoons \text{Glutamine} + ADP + P_i$$

However, the enzyme acts stereospecifically on α-methyl-DL-glutamic acid, utilizing only the L-isomer [*Biochemistry* **4**, 1063 (1965)]. On the other hand, of the four isomers of β-methylglutamic acid, the enzyme is active only toward one of the D-isomers, and of the four isomers of γ-methylglutamic acid, it is active only toward one of the L-isomers. The results of studies on the four β-hydroxyglutamic acids and the four γ-hydroxyglutamic acids are consistent with the experiments on the methyl-substituted glutamic acids. These findings and the observation that the enzyme converts β-glutamic acid only to a D-isomer [E. Khedouri and A. Meister, *J. Biol. Chem.* **240**, 3357 (1965)] can be explained by a hypothesis concerning the conformation of the enzymatically active substrates and the steric relationship of substrate to the enzyme. The differences in reactivity of the L- and D- substrates with ammonia and hydroxylamine seem to reflect differences in the spatial relationships between the γ-acyl phosphate moieties of the activated amino acids and the binding site of ammonia on the enzyme.

ALTON MEISTER
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Hypsometry of Oceanic Physiographic Provinces

The frequency distribution of depths in ocean basins has been determined using the most recent American and Russian oceanographic charts as sources, and a computer for data processing. Data have been compiled for individual ocean basins and marginal seas, and also for nine different physiographic provinces including, for examples, "ocean basin," "rise and ridge," and "continental shelf and slope." The summary result differs little from a hyposometric curve drawn in 1912. Considering both land and sea, by far the most common levels are approximately at sea level and at about 5 km, the general depth of ocean floor. The compilation by physiographic provinces permits a new understanding of the hypsometric curve even though it does not change the shape. The distribution of depths in the "ocean basin" province is singly peaked and symmetrical. The distribution in the "rise and ridge" province is similar but the mean depth is about 1 km less. This can be interpreted as indicating that one province is merely the elevated equivalent of the other, a conclusion which generalizes the field observation that some localized oceanic rises and ridges are formed by ephemeral bulges in the mantle under a normal oceanic crust. Most occurrences of depths between sea level and the deep sea floor have been produced by the formation of rises and ridges rather than by the deposition of sediment derived from continents. The development and collapse of rises and ridges may have caused substantial fluctuations in sea level during geological time.

H. W. MENARD
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Low-Lying Electronic Energy Levels of NO-Type Molecules

Molecules having the same number of valence electrons are expected to have similar electronic energy levels, but only very little is known about the relative position of such levels within a group. Since NO is one of the best studied molecules, we have begun a systematic survey of 11 electron diatomics, using modifications and combinations of three classical experimental methods in optical spectroscopy:

1) Low-resolution studies of molecules enriched with newly available isotopes for the identification of species and electronic transition energies during exploratory work, when molecular constants are not yet known.

2) High-dispersion ($\leqq 0.2$ Å/mm) spectroscopy for fine structure analysis of heavy molecules and the deduction of precise molecular parameters; and

3) Selective excitation of energy levels with active nitrogen and other quantized energy sources for the production of simple spectra in complex systems, and when a molecule cannot be easily prepared in pure form.

NS, NSe, and AsO will be used as examples for the three methods, and the newly discovered energy levels will be used in the correlation of 11 electron molecules.

BEAT MEYER
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Experiments on Myosin and Its Adenosine Triphosphatase

The molecular weight of myosin is measured anew; osmometry gives $\overline{M}_n = 4.7 \times 10^5$ g, and equilibrium sedimentation gives $\overline{M}_w = 4.5$ to 5.1×10^5 g. Reagents of widely differing chemical reactivity, and agencies like decreased temperature and substrate alteration all "modify" Ca^{2+}-stimulated nucleoside triphosphatase activity. These observations suggest that the reagents and agencies cause a conformational change in the myosin molecule; this change is partly "elastic," partly "plastic." The interpretation that such a change occurs is affirmatively tested by showing that an ordered series of structure-disrupting anions inhibit differentially the enzymatic activities of native and modified myosin. When myosin collaborates with actin in the simplest contractile system the myosin molecules are not all equivalent. Those "touching" actin constitute a fast, Mg^{2+}-activated adenosine triphosphatase, and those not touching actin constitute a slow, K^+-activated adenosine triphosphatase. For the fast enzyme, adenosine triphosphate (ATP) and protein yet-to-be-converted-structurally are the substrates, and either can be rate-limiting. Experiments with inhibition by hydrostatic pressure show that the fast enzyme rate has a large molar volume of activation [about 34 to 50 ml (mole ATP)$^{-1}$], while the slow enzyme rate is unaffected by pressure. The rate of structure conversion or of structurally coupled ATP hydrolysis is activated about twofold by "physiological" concentrations of ouabain and other cardioactive glycosides.

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Evidence Regarding Optical Activity in Meteorites

Using a high-sensitivity recording spectropolarimeter, the optical rotatory dispersion and various spurious rotation effects had been studied in solvent extracts of the Orgueil meteorite, in a number of soils as well as in dark-colored solutions containing a synthetic dye and sulfur, and other solutions containing suspended particulate matter and so forth. The readings obtained in the Orgueil and soil extracts were found to be dissimilar to the readings of the various controls. It appears that optically active organic compounds are present in Orgueil, but one cannot yet say with full certainty if these readings are not caused by terrestrial contaminations. The various instrumental conditions that may give false rotation readings can be reasonably estimated.

BARTHOLOMEW NAGY
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Review of Earlier Work on Carbonaceous Material and Organized Elements in Meteorites

In 1834, Berzelius isolated organic substances from a carbonaceous meteorite and raised the question whether this matter may be similar to humus on earth. In the 19th century, carbonaceous matter from meteorites was studied by Wöhler and Berthelot.

During the past four years, the interest in studying the organic constituents of carbonaceous meteorites has been revived and the use of modern ultramicrochemical analytical methods yielded interesting results. The meaning of these results cannot yet be evaluated with certainty. Saturated and aromatic hydrocarbons, fatty and aromatic acids, nitrogenous and sulfur-containing organic compounds have been reported in solvent extracts of carbonaceous meteorites; however, the majority of the meteorite organic matter, which appears to be a type of random polymer, has not yet been studied in detail. Polymer degradation methods followed up by chromatographic and spectroscopic analyses show promise to evaluate the nature of the insoluble meteorite organic matter.

Mineralized microstructures, containing seemingly organic residues (that is, the organized elements), have been reported in carbonaceous meteorites. These structures resemble microfossils, although some of them, particularly the ones which are not mineralized, appear to be terrestrial biological contaminations. The parent-body environment or carbonaceous meteorites had been studied by mineralogical and petrographic methods and by the considerations of phase-equilibria diagrams. A low-temperature, aqueous environment appears to have been present at one time on the parent body (or bodies) of the carbonaceous meteorites.

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Paraffinic Hydrocarbons in Carbonaceous Meteorites

Small samples of 20 carbonaceous meteorites (Wiik Types I, II, III, and others) were extracted with organic solvents and the extract was fractionated by silica gel chromatography. The paraffinic-hydrocarbon fractions eluted from the silica gel column by *n*-heptane were analyzed by gas chromatography and mass spectrometry. Normal and isomeric alkanes from C_{15} to C_{30} were found in all meteorites in varying amounts and distributions. Pristane and phytane, two saturated polyisoprenoid hydrocarbons, considered to be degradation products of chlorophyll, were found in all meteorites but one, although in several (mainly Wiik Type I) the amounts were very small. Essentially all meteorites of Type II gave a very similar hydrocarbon chromatographic pattern indicating a common origin for the hydrocarbons in this group of meteorites. The distribution of paraffinic hydrocarbons, including pristane and phytane, in some of these meteorites was found similar to that of microfossil-bearing Precambrian rocks 2×10^9 years old or older. The Orgueil (Type I) was similar to the Gunflint chert, and Type II meteorites and some of Type III (Mokoia) were similar to the Soudan rock. It remains to be seen whether these hydrocarbons were formed (i) on the Earth-Moon system, as suggested by Urey, (ii) on the asteroids, (iii) in the solar nebula, or (iv) whether they may be the result of a unique terrestrial contamination process. (Work supported in part by NASA grant NsG–257–62.)

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Inheritance of Biochemical Characteristics in Interspecific F_1 and F_2 Hybrids of Tragopogon

Species of *Tragopogon* have been found to differ in the distribution of a number of secondary substances tentatively identified as flavonoids. In the F_1 and corresponding tetraploids, the presence of parental compounds was found in general to be additive, with the hybrids containing most of the characteristic substances of both parents. From this it may be inferred that the biosynthetic pathways leading to the formation of these substances are controlled by dominant genes, and that each genome controls its own characteristic syntheses. In the F_2, recombination occurs, but the ratios obtained are not clearly Mendelian, perhaps because of linkage with lethal factors expressed as high sterility in the F_1. Sometimes in the F_1, a nonparental compound "hybrid substance" will be synthesized consistently or a parental compound consistently will not appear. In the F_2, synthesis of the hybrid substance segregates like that of the parental compounds. This apparent interaction between an enzyme present in one species and a precursor present in another may be of wide significance in the origin of new biosynthetic pathways of potential evolutionary value.

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The Close-Packed-Spheron Theory of the Structure of Nuclei and the Mechanism of Nuclear Fission

The assumption that atomic nuclei consist of closely packed spherons (aggregates of neutrons and protons in localized 1*s* orbitals—mainly helions and tritons) in concentric layers leads to a simple derivation of a subsubshell occupancy diagram for nucleons and a simple explanation of magic numbers. The correlation with the shell model is that occupied subshells with only the value 1 for the principal quantum number contribute only to the outer layer of spherons (the mantle), those with values 1 and 2 to the mantle and next inner layer, and so on. The theory leads to simple explanations of prolate ellipsoidal deformation of nuclei and of asymmetric fission.

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Expansion of General Relativity about the First-Order Schwarzschild Metric

Recent astronomical observations have generated considerable interest in the broad category of relativistic astrophysics. In particular, there is increasing probability that astronomical bodies may be found whose description requires the full framework of general relativity. Since the equations of general relativity have exact solutions only for over-simplified physical systems, one is forced to consider some approximation procedure if realistic physical models are to be considered. However, in approximation methods formulated so far, the equations are expanded about flat space-time solutions to the problem, the curvature of space-time and gravitational effects being ignored to lowest order. For the case of gravitationally collapsed stars, where the curvature is large, and cosmological models, where space cannot be assumed asymptotically flat, these expansions do not converge.

We consider, therefore, an approximation method in which the lowest-order approximation is taken to be some appropriately chosen exact solution of the field equations of general relativity. The physically realistic solution is then found by expanding the metric (the "gravitational potentials") about the lowest-order metric, and solving for the deviation of the metric in terms of the perturbing matter. This has been carried out explicitly for the case of a point mass (Schwarzschild metric) where the perturbing matter is not close to the Schwarzschild singularity, the position of infinite gravitational red shift. The techniques used to solve the expanded equations are based on the physical effects of light signals in curved space, rather than on a formal mathematical prescription.

P. C. PETERS
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New Methods for Exploring the Solar System

Until about 30 years ago, all of our information regarding the solar system was obtained from telescopes and other instruments on the surface of the earth. These observations were limited by atmospheric turbulence and opacity. In order to carry instruments above the sensible atmosphere, balloons and rockets have been used. Now, technology has advanced to a point where satellites and space probes are being flown.

This paper will discuss the capabilities and limitations of each of these methods, with particular emphasis on the deep space probes of the United States. The flight of Mariner IV to Mars will be used to illustrate the present state of development of remote-controlled instrumented spacecraft.

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Immunogenetic Studies with Cultured Rabbit Fibroblasts

This investigation was intended to explore the possibility of using surface isoantigens as selective markers for studies of genetic variation in diploid mammalian cell strains. Although there are many technical disadvantages to the use of diploid strains, diploidy is highly advantageous for purposes of genetic analysis.

Cell strains derived from rabbit skin were found to remain diploid for approximately 40 generations in culture. To obtain selective markers for these cell strains, antiserum was produced (presumably to surface isoantigens) by injecting cultured skin fibroblasts of a Flemish rabbit into New Zealand rabbits. Treatment of suspended cells of the injected strain with these antiserums rendered the cells incapable of attaching to culture vessels or of forming colonies. These antiserums were also reactive against cell strains derived from some, but not all, New Zealand rabbits, indicating sharing of isoantigens or isoantigenic determinants with cells of the injected strain. For a given antiserum, reactivity as measured by cell killing was greater with the injected strain than with other reactive strains. Mixing experiments involving an antiserum-sensitive and an antiserum-resistant strain demonstrated that minority populations of resistant cells could be selectively isolated from the majority population of sensitives, but there was a limit to the size of the sensitive population which could be selected against.

It is concluded that isoantigenic markers are potentially useful for studies of genetic variation in cultured diploid mammalian cells.

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The Catalytic Versatility of Erythrocyte Carbonic Anhydrase

Studies in these laboratories have shown that the catalytic effect of erythrocyte carbonic anhydrase (CA) is not limited to CO_2 hydration but the enzyme very powerfully and reversibly catalyzes the hydration of acetaldehyde and many other related carbonyl systems. Experiments designed to test and to scrutinize the spatial characteristics of the active site of CA show that the catalytic efficiency of this enzyme becomes progressively smaller as the number of methyl substituents in the aldehyde increases. We have also shown that CA catalyzes the hydrolysis of certain esters. The various CA-catalyzed hydrations and hydrolyses obey Michaelis-Menten kinetics, show a point of inflection at about *p*H 7.0, and are subject to noncompetitive inhibition by acetazolamide and by anions.

CA contains one gram atom of firmly bound zinc per mole of protein, and the enzyme conforms to the operational definition of a metalloenzyme. We have independently established that although catalysis by imidazole is moderate and that of zinc ions in a variety of buffers is mild, enhanced catalysis is observed when zinc ions are introduced into imidazole buffers.

The totality of these and related observations will be analyzed in terms of our suggestion (i) that hydrated CA acts as both a general acid-base and a nucleophilic catalyst towards carbonyl and *related* systems, and (ii) that the underlying mechanisms (including the active site) for CA-catalyzed hydration of CO_2 of aldehydes as well as the hydrolysis of esters are essentially very similar.

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Microtubules and Intracellular Motility

It is now established that structures referred to as microtubules appear very commonly in the fine structure of both plant and animal cells. These units appear as dense-walled cylinders, about 250 Å in diameter and several microns in length. They tend to be remarkably straight over their entire length. The cylinder is constructed of 13 subunits, arranged in a bundle around a center of lower density. In this and other respects these structures are similar to the filaments of the 9 + 2 complex of cilia and flagella,

A survey of the distribution and orientation of microtubules in biological systems makes it apparent that they are associated with the intracellular migration of cytoplasmic components, involved in some instances in the development of pronounced asymmetries in cell shapes. Thus assemblages of them are found in association with streaming patterns in plant cells, parallel and peripheral to the channels of pigment migration in melanophores, and as the fibers in the mitotic spindle. They are prominent as neurotubules in nerve fibers. The highly anisometric arms of the suctorians and heliozoans have parallel and ordered arrays of microtubules as axial structures. In the latter location the microtubules have been shown to break down in response to low temperatures, colchicine, and high hydrostatic pressures with a concomitant withering of the tentacles or axopods. Recent evidence also relates microtubules to the morphogenesis of thick filaments in the myofibril.

These observations and others are used to support the view that microtubules serve as elastic cytoskeletal elements and that energy released at their surfaces is translated by some as yet unknown mechanism into cytoplasmic movements.

Keith R. Porter
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The Strong Collision Requirement in Unimolecular Reactions

The earlier, simpler, and more practical formulations of thermal unimolecular reactions, which include the Rice-Ramsperger-Kassel theory, the Slater theory, and the Marcus-Rice theory, have all employed the assumption of strong collisions for the description of the activation-deactivation process. This feature appears in the assertion that all collisions of activated molecules (that is, molecules whose active energies exceed the critical reaction threshold, E_0) with normal species bring about deactivation. Alternatively, the assumption appears in the requirement that the Maxwell-Boltzmann distribution is preserved in a reacting gas at all energies below the critical, even in the low-pressure regime.

One extreme model of collisional redistribution of energy, the random statistical model, is known to fulfill the strong condition. The assumption is, of course, far from universally valid, and rate theories have been formulated which formally reject the assumption. A collisional model which fails the strong conditions is the Landau-Teller model and its derivatives. This model is also an extreme one for highly excited complex collision partners. Apart from extremes, the conditions for failure or success of the assumption have never been delineated.

This paper presents an examination of the features of various collisional transition probability functions. An iteration matrix calculation has been made of the relative steady-state energy-level populations and rates for various real and model unimolecular reactions, in the low-pressure limit. The conditions of completeness and detailed balance were observed. The effects of various parameters, such as temperature, critical energy level, molecular complexity, and form of the transition probability functions, are delineated. The calculational results are applied to experimental inert gas molecule effects. Measured inefficiencies may be translated into various statements of the average amount of energy transferred per effective collision. With the use of results derived earlier from related chemical activation experiments, it may be concluded that the strong collision assumption is obeyed by many complex substrates, as exemplified by much of the existing literature.

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Studies on the Enzymic Activity of Lysozyme

Lysozyme catalyzes the hydrolysis of β(1-4)-linked polymers of *N*-acetylglucosamine. The interaction of the enzyme with these saccharides is of particular interest, owing to the recent determination by Phillips and coworkers of the crystal structure of lysozyme and certain lysozyme-saccharide complexes.

The strength of the complexes formed between lysozyme and the oligosaccharides of *N*-acetylglucosamine increases with chain size through the trimer, but further lengthening of the chain does not strengthen the binding. A comparison of the association behavior of various sugars shows that the region of the protein which interacts most strongly with a saccharide unit (the monosaccharide binding site) requires an amide group in the molecule bound, but that otherwise great latitude in saccharide structure is permitted. Binding of the trisaccharide alters the ionization of two groups, of pK 4.3 and 6.4, but only the latter group is affected by binding of the monosaccharide. Both ionizations are reflected in the pH-rate profile for trimer hydrolysis.

Lysozyme catalyzes the transfer of glycosyl units from *N*-acetylglucosamine polymers to saccharide acceptors. Transfer from the trisaccharide to *N*-acetylglucosamine-C^{14} showed that cleavage occurred at the bond furthest from the reducing end of the sugar. In contrast, hydrolysis in H_2O^{18} indicated that both C_1-O bonds of the trimer were cleaved with approximately equal frequency. Since the enzyme-catalyzed exchange reaction is slow, these results are in apparent conflict, but can be understood if several trisaccharide-enzyme complexes of different stability and reactivity are formed.

The relationship of the foregoing results to the crystallographic data and the enzymic mechanism will be discussed.

JOHN A. RUPLEY
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Nondestructive Testing of Human Cardiovascular Function in Health and Disease

Nondestructive techniques for comprehensive descriptions of cardiovascular function in health and disease are required for validation of physiological concepts and for scientific classification of pathological conditions. Rapid advances in technology promise new approaches for extracting information from internal organs without pain, hazard, or damage to the skin. A most promising approach to the development of new nondestructive techniques takes the form of directing external energy into the body and analyzing the alterations in this energy when it emerges. For example, ultrasonic beams directed into the body undergo reflection from acoustic interfaces to give information about size, position, and movement of organs. In recent years, an ultrasonic flowmeter based on the Doppler-shift principle has been developed for use on the surface of the skin. The many different applications of an externally applied flowmeter can be used to indicate the potential advantages of nondestructive techniques for evaluating cardiovascular function in normal subjects and in patients with various vascular diseases.

The Doppler principle is generally familiar. If a beam of 5-megacycle ultrasound is directed through the skin and tissues, a small portion of the ultrasonic energy is backscattered from acoustic interfaces and particles in the blood. Backscattered sound from motionless structures has the same frequency as the incident sound and is excluded from the output. Ultrasound returning to the receiver crystal from particles in motion is shifted in frequency in proportion to their velocity as predicted by the Doppler-shift equations. The information can be detected by directly listening to fluctuating audible frequencies or by a readout circuit for inscription on recording paper.

The major incentive for developing an external flowmeter was investigation of cardiovascular responses which should be studied in man (that is, postural responses, adaptations to temperature, the origins of Korotkoff sounds, muscle pumping mechanisms in the extremities, and so forth). The device has been incorporated into an integrated facility in which information from several flow and dimensional recordings are employed simultaneously for peripheral vascular research. By combining the quantitative accuracy of one technique with the rapid sampling capabilities of another, multiple recordings of the same variables provide a more comprehensive understanding of physiological functions. As an extra dividend, external flow detection has demonstrated usefulness in clinical medicine, particularly in obstetrics and arterial occlusive disease. In future, we can anticipate evolution of new generations of techniques for obtaining objective physical measurements from inside the body of man.

ROBERT F. RUSHMER
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Ontogeny of Specific Proteins during Pancreatic Development

The accumulation of specific proteins in the developing mouse pancreas occurs primarily 13 to 18 days after fertilization. Ten- to 12-day pancreatic rudiments exhibit low amylase activity (comparable to the level in other embryonic tissues). Between 13 and 18 days, however, the specific amylase activity increases approximately 50,000-fold. The pattern of accumulation of lipase, chymotrypsin, and insulin is qualitatively similar to that of amylase but not necessarily coordinate. Increased enzyme activity and morphogenesis (zymogen granule formation) is also observed when 11-day pancreatic epithelia are cultured with mesenchymal tissue or a sedimentable (particle) fraction obtained from embryo extracts [Rutter *et al., Nat. Cancer Inst. Monograph* **13**, 51 (1964)]. Both mesenchymal tissue and embryo particle fraction stimulate epithelial growth and produce morphogenesis and increased specific enzyme activity.

The increased amylase activity in cultures is found solely in the epithelial cells; the pattern of accumulation corresponds temporally and in magnitude to that observed in the intact rudiments. The accumulation of amylase is blocked by puromycin at any stage. Actinomycin D, on the other hand, is more effective in the cryptic period prior to the rise in amylase activity. Culture of rudiments of different embryological age in the same system does not result in a temporal modulation of the anticipated response of either tissue. Internal control is therefore presumed.

The active principle supporting differentiation has been found only in embryonic extracts. A variety of cell fractions and purified factors active in other systems have all proved inactive.

WILLIAM J. RUTTER
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A Multivariable Control System: Determinants of the Stroke Volume of the Heart

This paper reports a mathematical analysis of a multivariable biological system in unanesthetized animals. Procedures have been applied to several variables, but only one example—regulation of stroke output of the heart—is presented. Analysis is based on a model where stroke volume (S) is determined by diastolic filling pressure of the heart (PF), upstream pressure (PA), and interval between beats (I). Data is collected, edited, and computed on a digital computer. In a correlation matrix, S is strongly correlated with the other variables. Other variables are also strongly intercorrelated. To eliminate effects of interaction, a set of orthogonal variables is constructed using the Gram-Schmidt procedure. This produces a second matrix of original versus orthogonal variables. From this matrix we can determine relationships between variables and see how well we can account for stroke volume. After computation, stroke volume is virtually completely accounted for by combined contributions of I, PF, and PA, usually in that order of importance. We can also use the last matrix to determine maximal and minimal contributions of each variable to stroke volume. Each of the listed variables has its own unique effect. At times, the range of possible effects of a particular variable is high due to the strong interaction and to "noise." In literature, controversy has arisen about the contribution of individual variables to stroke volume. Our results make it appear that many claims which were made about particular relationships were right, and that most of them were only partly right.

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Reverse Osmosis and Sap Pressure in Vascular Plants

A method has been developed which permits measurements of sap pressure in the xylem of vascular plants. As long predicted, these are normally negative, ranging from −4 to −5 atmospheres in a damp forest to −80 atmospheres in a desert. Mangroves and other halophytes maintain at all times a sap pressure of −35 to −60 atmospheres. Diurnal cycles of 10 to 20 atmospheres are common. In tall conifers there is a hydrostatic pressure gradient that closely corresponds to the height of the tree. Sap extruded from the xylem by gas pressure on the leaves is practically pure water. At zero turgor this procedure gives a linear relation between the intracellular concentration and the tension of the xylem. From this pressure-volume curve one may extrapolate the intracellular water volume. The osmotic equivalent of the intracellular freezing point matches closely the xylem pressure when the turgor is zero. The sap pressure in mangroves, balanced by a similar osmotic potential in root and leaf cells, is low enough (−35 to −50 atmospheres) to diffuse fresh water out of the sea and into the root by simple reverse osmosis. The semipermeable membrane separating the micellar wall from the cytoplasm is subjected to a very high (up to 80 atmospheres) hydrostatic pressure difference.

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Terminal Nucleotide Sequence of Yeast Transfer RNA's Specific for Serine, Tyrosine, Glycine, Threonine, Phenylalanine, and Alanine

Unfractionated transfer RNA was prepared from baker's yeast by phenol extraction and chromatography on columns of diethylaminoethyl-cellulose. The RNA was charged with radioactive amino acid using a cell-free extract of yeast. The amino acyl RNA was digested with T_1 ribonuclease at *p*H 5.4. Amino-acyl oligonucleotides were isolated directly from the digest by chromatography and electrophoresis.

For each of the amino acids mentioned above only one amino acid oligonucleotide could be detected. The nucleotide sequences of the oligonucleotide components were determined by alkaline hydrolysis, digestion with snake-venom phosphodiesterase, digestion with pancreatic ribonuclease, and by sequential degradation using periodate oxidation, amine-catalysed cleavage, and phosphomonoesterase digestion. From the structures of these oligonucleotides, the terminal nucleotide sequences at the acceptor ends of the corresponding chains of yeast transfer RNA have been deduced. These are as follows: serine, . . .GpCpCpA; tyrosine, . . .GpApCpCpA; glycine, . . .GpCpApCpCpA; threonine, . . .GpCpApCpCpA; phenylalanine, . . .GpCp(ApCp,Cp)A; alanine, . . .GpUp(Cp,Cp,Ap)CpCpA. The terminal sequences found for alanine and serine acceptor RNA are in agreement with the structures determined by Holley (*1*) and Zachau (*2*) using a different method from that described here.

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Resistance of Oligonucleotide-Oligolysine Complexes to Ribonuclease Digestion

Polylysines react on a molar basis with RNA to form insoluble complexes at low salt concentrations and neutral *p*H. Soluble complexes are formed, however, at low polylysine-to-RNA ratios. The soluble complexes are susceptible to hydrolytic degradation by specific and nonspecific ribonucleases with release of soluble nucleotides. Digestion continues until the formation of a precipitate with a nucleotide-to-lysine ratio of one. After dissociation of the precipitated complex and separation of its components, the "protected" nucleotide chain is again susceptible to cleavage by the original enzyme. Oligolysines smaller than the heptamer do not form enzyme-resistant complexes; the protected nucleotide sequence is shorter than the original RNA and is directly related to the size of the polylysine used to form the complex. Present evidence indicates that the nucleotide composition of the protected sequence may be markedly different from that of the parent molecule and that overlapping nucleotide sequences may be obtained by this procedure.

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Effects of Univalent Cations on Immunoelectrophoretic Behavior of Pyruvic Kinase

An investigation into the effect of different univalent cations on the immunoelectrophoretic behavior of pyruvic kinase has been conducted. When univalent cations known to activate the enzyme were utilized in the environment, one pattern of immunoelectrophoresis ("K pattern") was obtained. When univalent cations that failed to activate the enzyme were present in the environment, another pattern ("Tris pattern") was observed. These differences in the immunoelectrophoretic behavior of pyruvic kinase must reflect differences in the physical organization of the enzyme. These differences are too subtle, however, to be detected by sucrose-density-gradient centrifugation. Therefore they are probably not due to changes in the state of aggregation of subunits. Manipulation of the univalent cationic environment results in the reversible interconversion of the immunoelectrophoretic patterns caused by tris$^+$ and by K$^+$. These results are consistent with the reported reversible activation and deactivation of the enzyme by certain univalent cation species.

Catalase activity is not affected differentially by different species of univalent cations, and the immunoelectrophoretic behavior of catalase was not influenced differentially by the different species of univalent cations studied. Effects of univalent cations on immunoelectrophoretic patterns therefore are apparently not a general phenomenon.

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Oregon State University

The Replication of Viral RNA with a Purified Enzyme

We have purified two different RNA "replicases" (RNA-dependent RNA polymerases) induced in *Escherichia coli* by serologically distinct RNA bacteriophages. The protein preparations are free of detectable levels of ribonuclease, phosphorylase, and DNA-dependent RNA polymerase. The enzymes show a mandatory requirement for RNA and can mediate prolonged and extensive net synthesis of polyribonucleotide. The two "replicases" exhibit a unique discriminating selectivity in their response to added RNA. Under optimal ionic conditions, both are virtually inactive with heterologous RNA, including ribosomal and sRNA of the host. Neither "replicase" can function with the other's RNA. Each recognizes the RNA of its origin and requires it as a template for its synthetic activity.

Experiments will be described with the Qβ "replicase" which established the following central features of the reaction: (i) the replicase cannot function properly with fragments of homologous RNA and is, therefore, designed to avoid the replication of either foreign sequences or incomplete copies of its own genome; (ii) the enzyme generates a polynucleotide of the same molecular weight (1×10^6) as the viral RNA; (iii) reactions initiated with templates at concentrations below saturation of enzyme exhibit autocatalytic synthesis of RNA; (iv) the purified product can serve as a template with an efficiency equal to that of viral RNA; (v) the RNA produced by the enzyme is biologically active, as demonstrated by infectivity tests. It is fully as competent as the original RNA to program the synthesis of complete virus particles.

The data obtained show that the self-propagation of complete viral genomes is being observed in a simple system composed of purified "replicase," template, riboside triphosphates, and magnesium. Every step and component necessary to complete the replication cycle must be represented in the reaction mixture. The unambiguous analysis of the molecular details of a replicating mechanism would appear now to be possible.

S. SPIEGELMAN
I. HARUNA
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Genetic Control of Uptake of Aromatic Amino Acids in Neurospora

A system has been developed for the selection of both forward mutation and reversion of genes controlling the uptake of aromatic amino acids. Forward mutants (loss of the wild-type uptake system) can be obtained by selecting resistant colonies on inhibiting levels of the analogs 4-methyltryptophan and *p*-fluorophenylalanine, and these mutants map at a single genetic locus in linkage group IV which has been designated *mt*. To select revertants (restored uptake) the mutant is crossed to a tryptophan-requiring strain to obtain the double mutant. This strain is unable to grow on a low concentration of tryptophan, a level sufficient to support the growth of tryphophan mutants with normal uptake. Seven revertants were selected on this medium and all are alike in the following characteristics: they have recovered nearly normal uptake rates for the aromatic amino acids; they have become partially sensitive to the analogs. The reversion events are controlled by suppressor genes unlinked to *mt*; all seven suppressors may be at the same locus in linkage group I. They are nonspecific: when a single suppressor is put into combinations with several different *mt* mutant alleles, it restores uptake to them all. Comparisons of the uptake of tryptophan by wild-type and by a suppressed mutant revealed one clear difference. The presence in the medium of any of three other amino acids (arginine, lysine, and histidine) blocks the uptake of tryptophan by the suppressed mutant but does not interfere with tryptophan uptake by wild-type. It is suggested that the suppressor mutation has modified a second uptake system, expanding its range of substrates to include the aromatic amino acids.

DAVID R. STADLER
University of Washington

Possible Fossils from Orgueil and Other Meteorites

Organized elements in carbonaceous chondrites are interpreted as fossils by Claus and Nagy, van Landingham, Palik, Staplin, Timofeyev, and others. The basis for this opinion is mostly morphological. The same elements are considered by others to be mineral substances, contaminants, and abiotic organic structures. Some of the elements are illustrated and discussed in this paper. While many can be rejected, a number cannot be explained at this time, particularly among those shown to be indigenous to the chondrites by independent investigators.

There is little basis for presuming that extraterrestrial taxa should resemble life forms on earth, either fossil or recent. It is noteworthy, therefore, that many of the described elements superficially resemble various "lower" taxa, mostly algal and fungal. Some are almost indistinguishable from the simple vesicles of Precambrian microplankton, as might be expected if the chondrites are displaced terrestrial fragments of lunar fragments contaminated by the earth.

Cooperation, including free exchange of material, is urged upon all investigators. This will aid in the rejection of recognizable contaminants and artifacts and enable us to concentrate efforts towards characterization of those elements that remain.

FRANK L. STAPLIN
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Differential Synthesis of Nucleic Acids Associated with Cellular Differentiation in the Leaf Sheath Epidermis of Barley

The abaxial epidermis of the upper culm leaves of barley contains, in addition to the usual elongated epidermal cells, specialized cells of four types: guard cells and subsidiary cells of stomatal complexes and pairs of siliceous and suberous short cells which alternate with long cells in all of the cell rows except for those which bear stomata. Since the two guard cells of a stoma are formed by the division of a single initial, and the members of a siliceous-suberous pair are likewise formed by a mitotic division which occurs late in development, the epidermis in an intermediate stage of development consists entirely of cell rows in which the shorter initials of the pairs of specialized cells alternate with longer cells which will normally not divide again. The shorter initials are formed by differential mitoses at a late stage of meristematic activity, in which each mitotic division produces distally a short initial and proximally a longer epidermal cell initial.

Application of H^3-thymidine followed by autoradiography showed that nuclei of longer cells became heavily labeled when exposed immediately after telophase of the mitosis which forms them but not at late interphase stages. Nuclei of both guard-cell mother cells and suberous-siliceous mother cells did not become labeled until late interphase, at a stage immediately preceding prophase of the division which forms the specialized guard cells or siliceous-suberous cells. These results indicate that in the cells which enlarge greatly but differentiate relatively little, the G_1 period of RNA-protein synthesis is short or lacking, while G_2 is much prolonged. On the other hand, in the initials which form specialized cells, G_1 is very long and G_2 is short or lacking. Autoradiography following treatment with H^3-uridine gave opposite results, and thus provided evidence in favor of this hypothesis. The results are discussed with reference to current theories regarding differential gene action.

G. LEDYARD STEBBINS, PETER JURA
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Stepwise Degradation of Tobacco-Mosaic-Virus RNA

Stepwise degradation of 3′-unphosphorylated oligonucleotides can be brought about by repeated cycles of the three steps: periodate oxidation of the terminal glycol group, β-elimination of the terminal aldehyde derivative, and enzymatic release of the new terminal phosphate from the chain. Known conditions to achieve the second step were not sufficiently mild to permit the repeated application of the procedure to large RNA molecules, such as tobacco-mosaic-virus RNA.

We now wish to report that aniline, when used as a catalyst with model compounds or RNA, causes quantitative elimination at *p*H 5 in 3 hours at 25°C. All steps in the reaction with viral RNA were monitored by analyzing for the appearance and loss of aldehyde groups by the C^{14} semicarazide binding capability of the RNA.

The identification of the bases released in the degradative cycle was achieved by using C^{14}-labeled viral RNA. Five cycles of degradation have yielded the tentative sequence G-C-C-C-A, with decreasing assurance from right to left.

The infectivity of the RNA appeared to be lost when the first base was split off. The discrepancy of this and part of the sequence data with results obtained upon exonuclease degradation of tobacco-mosaic-virus RNA will be discussed.

A. STEINSCHNEIDER
H. FRAENKEL-CONRAT
University of California, Berkeley

Sexual Differentiation in Intersexes of Drosophila

In *Drosophila melanogaster* the coloration of the abdomen is different in the two sexes. In the female the dorsal surface shows a series of six narrow black pigmented bands corresponding to the posterior margins of the first six segments. In the male only the four anterior segments carry such bands of pigment, while the fifth and sixth segments are totally pigmented. Triploid intersexes (2 × 3A) vary in their abdominal pigmentation as well as in other sexual traits over the range from typical female to typical male phenotype. A remarkable fact is that the abdomina of intersexes often are mosaics of male and female pigmented areas. Experiments with genetically marked chromosomes show that this mosaicism is not the result of X-chromosome loss or of somatic crossing over between the sex chromosomes. Rather, sexual differentiation in triploid intersexes seems to be decided as either female or male independently in different parts of the developing individual. The findings are supported by earlier observations of Redfield concerning female-male mosaicism for sexually different eye-color mutants of *Drosophila*. A similar mosaicism in triploid intersexes has been worked out in detail by Seiler for the moth *Solenobia*. Diploid intersexes in *Drosophila* show intermediate expression of sexually alternative traits.

CURT STERN
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Organic Compounds in Carbonaceous Chondrites and Their Origin

Trapped volatiles in the carbonaceous chondrites Orgueil, Murray, and Cold Bokkeveld were identified by time-of-flight mass spectrometry. The meteorites contain a surprisingly limited assortment of compounds: H_2, CH_4, CO, CO_2, NO, N_2, SO_2, CS_2 as well as benzene, alkylbenzenes, naphthalene, anthracene, and other aromatics; sulfonic acid esters, and chlorinated hydrocarbons. Ethane and its homologs are present at $<10^{-3}$, the abundance of methane. Ammonia is likewise undetectable. Neither Miller-Urey reactions nor biological processes seem capable of accounting for this distribution, particularly the high methane/ethane ratio and the preponderance of aromatic hydrocarbons. However, the observed distribution agrees remarkably well with a distribution calculated by Dayhoff *et al.* for equilibrium conditions in a hydrogen-poor C-H-O-N mixture at 500°K. Apparently the bulk of the organic compounds in carbonaceous chondrites formed by spontaneous, quasi-equilibrium reactions among hot gases in the solar nebula, after the loss of much of the hydrogen. No external energy sources (such as radiation) seem to be required.

Laboratory experiments on simple gas mixtures tend to support Dayhoff's calculations. These experiments have yielded significant clues to the formation conditions of the organic compounds in meteorites.

MARTIN H. STUDIER
Argonne National Laboratory
RYOICHI HAYATSU, EDWARD ANDERS
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The Origin of Quasars

It is highly probable that the quasistellar radiosources and the more recently discussed quasistellar galaxies are indeed superluminous objects at extremely great distances. The analogy between these light sources will be finally established if appropriate variations in the emission of quasistellar galaxies will be found. The rather rapid variation of the visible light and the short-wave radar emission of quasistellar radiosources is most easily explained by assuming spatially separated events which in the course of a few years liberate an energy approximately equal to C^2 times the mass of the sun.

A specific explanation of the great energy production might be obtained by postulating collisions between galaxies and antigalaxies. In such a collision, only a small part of the available energy would be released. The observed phenomena may be produced when a star from one galaxy penetrates a protostar from the other galaxy. The fluctuations may be due to the statistical occurrence of such encounters. The hypothesis of matter-antimatter annihilation could be best decided by observing the characteristic gamma radiation emitted in such a process.

The superluminous objects are visible at distances nearly as great as the radius of the universe. Hence, one can expect to find relevant information concerning the origin of the universe. The interpretation of this information is, however, dependent on the theory of the luminous objects. According to different theories, the probability of occurrence and the absolute luminosity will depend in a different manner on cosmic age.

EDWARD TELLER
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Properties of the Genetic Code Deduced from the Study of Proflavine-Induced Mutations

The alteration in the primary structure of bacteriophage T4 lysozyme resulting from a particular pair of proflavine-induced mutations in the lysozyme genetic region has been studied. It was found that a block of five amino acids are changed. The amino acid sequence in the changed region of the double mutant can be related to the amino acid sequence in the corresponding section of the wild-type lysozyme with the aid of the current codon assignments of Nirenberg *et al.* These observations provide direct evidence supporting: (i) the general nature of the genetic code that has been proposed by Crick *et al.;* (ii) the associated hypothesis that proflavine-induced mutations consist of the insertion or deletion of base pairs in DNA; (iii) the 5′ to 3′ direction of translation of a messenger RNA molecule into a protein molecule; (iv) six of the codons that have been proposed on the basis of direct evidence from studies in vitro; and (v) four of the codon assignments that have been proposed on the basis of indirect arguments.

E. TERZAGHI, Y. OKADA
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Neuron Population Approach to the Study of Cerebral Physiology

When inquiring into the functional organization of a circumscribed region of cerebral tissue, an adequate sample of the evoked neuronal activity can only be obtained by lumping data gathered from many animals under identical experimental conditions. When assembled, such data yields a picture of the total neuronal discharge in time following an afferent input. The assembled population can be fractionated into functional subsets according to specific response properties, including modality specificity, receptive field, response latency, number of spikes per discharge, threshold stimulus strength, and ability to follow iterative stimulation. At four "forepaw" sites in cat somatosensory cortex, neurons have been classified as *s* or *m* depending upon whether their excitatory receptive field was or was not confined to the contralateral forelimb. The two subsets thus obtained show markedly different response properties, cluster at different levels in the cortex, and occur in different relative proportions at the four cortical sites. Modality sensitivity is not uniform in the two sets. The activity of *m* neurons, which include most corticofugal neurons, can be blocked with barbiturates. The population response patterns show that these two subsets consist of basically different neurons having developmental affinities for different "kinds" of afferent fibers. Though they reside intermingled in the same tissue, *s* and *m* neurons are members of separable neuronal networks and fail to show any strong interaction. Thus, the population approach reveals a hitherto unsuspected refinement in cortical organization; pending association with structure, it holds out the promise for eventual anatomical and biochemical dissection of the most complexly organized matter in existence.

ARNOLD L. TOWE
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School of Medicine

Magnetoacoustic Effects in Metals

The propagation of sound waves in a metal is strongly influenced by the properties of the valence electrons and by their direct interaction with the coherent lattice vibrations. This interaction is responsible for most of the sound absorption at low temperatures and for a major portion of the stiffness contributing to sound velocity at all temperatures. Both of these effects are altered in the presence of a magnetic field and altered in ways which depend sensitively on the electron distribution and the electron relaxation time; that is, on properties of the Fermi surface. The sound absorption and velocity may either increase or decrease with application of a magnetic field depending on the values of critical parameters and on the type of sound wave. Of special importance are two kinds of superimposed oscillations: (i) the so-called geometric resonances coming from near coincidence of electron orbit sizes and the wavelength of sound; and (ii) the de Haas-van Alphen oscillations coming from quantization of the electron orbits. The emphasis up to the present time has been on the study of sound absorption. In recent measurements (A. G. Beattie and E. A. Uehling, "Observations on the magnetoacoustic effects in aluminum," in preparation) the geometric resonances in the velocity of sound as well as in the absorption have been observed. For reasons which are not yet understood, the velocity of sound appears to be a more sensitive indicator of these resonances than the absorption. Some interpretation of both the absorption data and the velocity-of-sound data in terms of the electron distribution and relaxation time will be described.

EDWIN A. UEHLING
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Organic Material in Meteorites: A Review

That chemical compounds of biological origin exist in meteorites is established beyond question by the work of Nagy *et al.* on hydrocarbons, fatty acids, and optical activity, of Meinschein and Frondel and of Oró *et al.* on hydrocarbons, and of Hodgson and Baker on porphyrins; moreover, this work shows that the hydrocarbons certainly are those produced by terrestrial type organisms. Hayatsu's work indicates that the interior of large specimens may not contain such materials. The problem is to determine the origin of these compounds. It is likely that they are due to contamination of the meteorites after they arrived on earth, and the difficult problem is to prove their indigenous character by eliminating all possible sources of contamination. Efforts have been made to do this, but it is difficult to arrive at a conclusion that they are definitely due to contamination. The porphyrin studies indicate that vanadyl porphyrin is present and chlorins are not, and hence that the Orgueil meteorite resembles old sediments and not recent terrestrial material. Fossil forms have been reported by Staplin and Timofeyev as well as by Nagy *et al.* Some of these are certainly modern terrestrial forms and some are very likely artifacts, but also some are mineralized and are very similar to terrestrial fossil forms of the dinoflagellate type such as found in ancient terrestrial rocks.

Critical evaluation is justified and possibly all soluble components observed so far are due to terrestrial material. The problem has very great importance, and further diligent study on the question of indigenous biological material is justified.

HAROLD C. UREY
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Carboxypeptidase Catalysis

We have employed carboxypeptidase A as a model for mechanisms of enzyme action. Since metalloenzymes exhibit characteristics which are uniquely determined by the constituent metal atom, carboxypeptidase allows experimental approaches, inapplicable to enzymes of purely organic composition, such as (i) the use of chelating agents as site specific reagents; (ii) replacement of the native zinc with other metal ions, some of which enhance the esterase and abolish the peptidase activity, curtailing the enzyme's dual specificity; (iii) binding of substrates and inhibitors to the apo- and metalloenzymes; and (iv) for studying the metal-induced changes in specificity and mechanism of action of the enzyme, either kinetically or at equilibrium.

The metal-dependent change in specificity is qualitatively similar to that resulting from acylation, iodination, photooxidation, alkylation, or coupling with diazonium compounds. The enzymatic consequences of such *organic* modifications can be prevented, however, by inhibitors and substrates, known to interact at or near the metal site. In this regard, the metal serves a new function, identifying the locus of such organic modifications. Thereby, two tyrosyl residues have been shown to be involved in the catalytic mechanism. More recently, limited proteolysis by subtilisin has been found to alter catalytic behavior markedly, but without demonstrable changes in amino-acid composition.

Kinetic studies are of limited usefulness in interpreting activities of these derivatives. The separate contributions of the catalytic and substrate binding steps as well as both substrate and product inhibition and activation are not easily weighted in terms of Michaelis-Menten kinetics. A quite different avenue, using isotopically labeled substrates, however, gives promise of providing further insight into the mechanism. Based on all these considerations an integrated approach to the mode of action of carboxypeptidase will be discussed.

BERT L. VALLEE
JAMES F. RIORDAN
MORDECHAI SOKOLOVSKY
Harvard Medical School

Displacements of the Visible Absorption Band of Iodine by Solvents of Different Classes

Walkley, Glew, and Hildebrand, in 1960, reported that π-donor and N-donor solvents give different lines on a plot of their ionization potentials against the wavelengths of the maxima of the visible band of iodine. We now find that the shifts from the 520-mμ wavelength in the vapor to solutions in a wide variety of solvents separate the solvents into distinct groups: aromatics, alkylhalides, saturated oxygen compounds, sulfur compounds, and nitrogen compounds, each of which gives points on a straight line.

It is apparent that the electronic excitation of iodine is sensitive to the solvent ionization potential not merely in a general manner, but that it distinguishes the type of electronic orbital from which the electron ionizes. This requires intimate contact between solvent and solute molecules.

Assuming charges-transfer type interactions between iodine and solvent molecules, the observed groupings may be explained qualitively, even including a number of solvents which seem to behave irregularly.

Solutions of iodine in saturated hydrocarbons will also be discussed.

EVA M. VOIGT
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Mechanism of Fatty-Acid Biosynthesis

The various acyl intermediates in the biosynthesis of long-chain fatty acids are bound in thioester linkage to a heat-stable protein referred to as acyl carrier protein (ACP). Homogeneous preparations of ACP prepared from extracts of *Escherichia coli* have a molecular weight of 9100 and contain 81 amino acid residues plus one residue each of 2-mercaptethylamine and β-alanine. The latter two moieties are integral components of 4′-phosphopantotheine which is the prosthetic group of ACP and is the source of the single thiol group of ACP, onto which the various acyl groups are bound. The 4′-phosphopantotheine is linked by a phosphodiester bond to a serine residue of ACP. Aspartic acid and leucine flank the serine residue on the amino and carboxyl sides, respectively. The sequence of amino acids in ACP is under investigation.

The intermediate reactions of fatty-acid biosynthesis and the various enzymes involved have been investigated. From crude extracts of *E. coli*, eight different enzymes have been isolated and characterized that participate in the conversion of acetyl CoA and malonyl CoA to palmitate and *cis*-vaccenate, with the intermediate formation of β-hydroxydecanoyl ACP. The latter intermediate appears to be the point of divergence in the synthesis of saturated and unsaturated long-chain fatty acids, in *E. coli.*

SALIH J. WAKIL
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Spectroscopic Studies of Gaseous Nebulae with the Lallemand Electronic Camera

A gaseous nebula of the "planetary" type (so called because of its appearance), consists of a highly attenuated plasma at a gas kinetic temperature of about 10,000°K. Its spectrum consists primarily of bright lines, although there is also a weak continuous background. The bright lines of hydrogen and helium and certain lines of carbon, oxygen, and other elements are produced by photoionization followed by recombination. Many lines, including the strongest ones, represent "forbidden" transitions of ions of oxygen, neon, sulfur, nitrogen, and so forth. The physical mechanisms of line excitation are believed well understood; the necessary atomic parameters, collisional cross sections and atomic transition probabilities are being calculated.

Thus in order to assess the physical state, that is electron temperature and density, and chemical composition of a planetary nebula, it is necessary to measure the intensities of lines of as many different ions as possible. The problem is further complicated by stratification and filamentary structure in the nebula.

The necessary observational data are

difficult to get. Besides the normal limitations imposed by the earth's atmosphere which extinguishes all radiation at wavelengths below 2900 Å and permits views through only portions of the infrared, we are severely handicapped by the extreme faintness of nebulae. Although the photographic plate used at the prime focus of a large telescope is satisfactory for the blue spectral regions of many nebulae, the yellow, red, and infrared require employment of techniques such as an electronic camera whereby one can observe strategic lines such as those of [Cl III], [N I], [Fe III], [Fe VI], and C IV.

MERLE F. WALKER
L. H. ALLER
University of California, Mount Hamilton and Los Angeles

A Search for Deuterium in Stellar Spectra

Deuterium could be observable in stellar atmosphere if the atmospheric material has not been mixed to temperatures as great as 10^6 deg K, where deuterium would be consumed by proton capture. In addition, it is necessary that either there had been deuterium in the interstellar material out of which the star formed or that deuterium production by surface nuclear reactions had occurred.

Calculations by Bodenheimer at Berkeley have shown that only stars of mass greater than three solar masses can preserve any original deuterium during contraction to the main sequence. We have searched for the deuterium component of H_α in normal A and F stars of luminosity class II and Ib, since they presumably have a sufficiently large mass and have lower opacity than main sequence stars of the same mass. No deuterium feature could be found and limits as low as 1/5 of the terrestrial ratio of D/H could be set. Thus we conclude that unless there occurred some sort of fractionation during the formation of the earth the terrestrial deuterium was formed after the separation of the solar system material from the general interstellar medium.

A search for deuterium in nine stars showing a surface magnetic field and various abundance peculiarities was also fruitless. Upper limits on the ratio of D/H from 7×10^{-4} to 4×10^{-5} could be set. We conclude that either there is no production of deuterium on the surfaces of magnetic stars or that any deuterium produced is consumed sufficiently rapidly to prevent the buildup of a detectable concentration.

GEORGE WALLERSTEIN
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MANUEL PEIMBERT
University of California, Berkeley

Specific Contact Destruction of Target Cells by Immune Macrophages in Homologous and Heterologous Systems

Our previous observation, that immune macrophages from C57Bl/Ks mice deposited at loci on monolayers of homograft target fibroblasts from A/Jax mice adhere specifically and produce mutual death of the cells with resulting "plaques of clearing," has been confirmed by the reverse procedure of placing immune macrophages from A/Jax mice on monolayers of homograft target fibroblasts from C57Bl/Ks mice. The observations that treatment of immune macrophages with the metabolic inhibitors actinomycin D and chloremphenicol prevents cell destruction but not cell adherence, that specific antibody can be eluted from immune macrophages by heat, and that their plaquing activity can be destroyed by trypsin treatment indicate that cell adherence is due to cell-bound antibody but is only a passive first step in the mutually destructive interaction of immune macrophages and target cells. The nature of subsequent steps is unknown but apparently demands the biosynthetic activities of the immune cell. No evidence that complement participates in the reaction was obtained.

Efforts to produce plaques by placing peritoneal macrophages of rabbits immunized with mouse tissues on target cells of mouse fibroblasts have not given definitive results to date.

RUSSELL S. WEISER
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Factors Influencing Activity and Polymerization of Threonine Deaminase from Clostridium tetanomorphum

Adenosine diphosphate (ADP) acts as an allosteric activator of threonine deaminase in *Clostridium tetanomorphum* (Hayaishi, Gefter, and Weissbach, 1963). The present investigation has shown that the nucleotide is bound by the purified enzyme and that it protects the enzyme against inactivation by heat or dilution. On aging or upon exposure to urea, sodium lauryl sulfate, or an alkaline *p*H, the enzyme dissociates to subunits. This dissociation is prevented by ADP, by threonine, by high concentrations of phosphate buffer, and by divalent cations. Association of the subunits, on the other hand, takes place only in the presence of ADP or threonine.

The catalytic properties, binding of ADP, heat inactivation, and selective inactivation of the catalytic and allosteric sites of the subunits have been studied. The relationship of these findings to the degradation of threonine by *C. tetanomorphum* will be discussed.

HELEN WHITELEY
University of Washington School of Medicine

The Covalent Linkage of Mercury to Protein as a Route to Isomorphous Replacements

The determination of the structure of proteins by x-ray crystallography is hampered by the difficulties of obtaining suitable isomorphous crystals which contain heavy-atom replacements at regular locations in the unit cell. Isomorphous replacements are required for the determination of the phases of reflections used to construct the electron density map by Fourier synthesis. Experiments will be described in which derivatives of chymotrypsinogen and chymotrypsin are prepared with reagents which contain covalently bonded Hg. The most promising approach is to use a reagent which reacts with the active site of the enzyme, for example, *p*-nitrophenyl-α-chloromercuri-β-methoxy-dehydrocinnamate or *p*-chloromercuri-benzenesulfonyl fluoride. Crystals of δ-chymotrypsin substituted with the latter reagent have been obtained, but further investigation has shown that during purification and crystallization, the Hg content slowly decreases to 0.6 gram-atoms per mole. A study of other reagents, *p*-chloromercuribenzene diazonium chloride which couples to tyrosine and *p*-chlorobenzenesulfonyl chloride which couples to lysine in the protein, also shows a slow loss of Hg under certain conditions, especially in the presence of chloride and ammonium ions. These observations are consistent with the known properties of the Hg-C bond. Conditions which would be suitable for obtaining stable derivatives will be discussed. Two methods have been found to be particularly useful in following the Hg content of small amounts of protein crystal preparations, x-ray fluorescence spectrometry and atomic absorption spectrometry. Crystallographic studies associated with this work have been carried out by Joseph Kraut, University of California at San Diego, La Jolla.

PHILIP E. WILCOX
ELISABETH SURBECK
WILLIAM A. COBAIN
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CO_2 Transients, Active Membrane H^+ Transport, and the Body's Acid-Base Status

Estimation of the acid-base status of the body from arterial *p*H and P_{CO_2} is hampered because the kinetics of CO_2 system transients is poorly understood and the time elapsed since the onset of the disturbance is usually unknown. Considerable confusion has arisen from a failure to realize that the duration of the CO_2 distribution transient following a step change in P_{aCO_2} may be hours and that blood protein buffers are diluted by the extracellular fluid. There are three classes of H^+ transients: (i) CO_2 mixing. The minimum time constant of tissue CO_2 equilibration is tissue volume over tissue blood flow; a few minutes for most tissues but varying from a few minutes up to an hour in muscle, depending on activity. (ii) Transmembrane H^+ transfer. A fall in extracellular *p*H causes a transport, probably active, of H^+ into cells. The equilibration time is of the order of many hours. The available data can be summarized by supposing that H^+ competes with Na^+ for the outward leg of the membrane Na^+-K^+ pump and with K^+ for the inward leg. This is also suggested by the equality: $[H^+]_o/[H^+]_i \simeq [K^+]_o/[Na^+]_i \simeq 1/3$. (iii) Renal H^+ balance compensatory processes require days to weeks. Plotting the CO_2 titration curve of arterial blood in vivo on the *p*H-$[HCO_3^-]$ diagram clearly shows these H^+ movements through the variations in the slope of the curve with time following a sudden change in P_{aCO_2}.

J. WALTER WOODBURY
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Nonuniformity of Pulmonary Mechanics

Ventilation of the lungs is usually described in relation to a linear model with one or two compartments. Each compartment is specified by a functional residual volume and a ventilation. The results reported herein using multiple small casters for sampling in human lungs indicate that this model is highly oversimplified in at least two respects. By analysis of the gas concentrations from the individual catheters it appears that regions as small as lobes may require at least a five-compartment model to explain the data obtained at a particular time during the breath. Further comparison of the time course of the gas concentrations in different regions of lung during a single breath shows that the different regions do not empty uniformly and that each component of the lung must be described by a nonlinear component.

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