National Academy of Sciences

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Further Studies on Memory Formation in the Goldfish

We previously reported that goldfish readily learn to swim over a barrier in a shuttle box upon a light signal to avoid a mild electric shock and that for weeks they show memory of a single training session. Various agents were administered before or after a training session, and memory was tested at various times following training. Initial experiments were performed with intracranial injections of puromycin, an antibiotic known to block RNA-mediated protein synthesis selectively. Memory fixation, or the formation of long-term memory, was blocked if puromycin was injected within 1 hour after training, but not at later times. Acquisition of the task was not affected by pretrial injection of the antibiotic, but the associated (short-term) memory decayed over the next 2 days. Another antibiotic inhibitor of protein synthesis, acetoxycycloheximide, blocked both long-term memory formation and protein synthesis in the goldfish brain at concentrations about 10^{-3} (weight per volume) that of puromycin. Electroconvulsive shock after trial also blocked long-term memory formation.

Recent studies with an automated apparatus have substantiated and extended these observations. Acetoxycycloheximide blocks long-term memory formation when injected within 3 hours following training, and short-term memory decays over the next 2 days. While electroconvulsive shock or intracranial injection of KCl can block long-term memory formation in the goldfish, these agents may also impair protein synthesis. Further experiments were performed to investigate how specific the inhibition of protein synthesis was for the observed block of long-term memory formation. Intracranial injection of a sublethal dose (2 μg) of actinomycin D causes a rapid depression in brain RNA synthesis. Under these conditions, acquisition of the training task is unimpaired, but long-term memory formation is blocked.

While selective inhibitors of protein synthesis block long-term memory formation in the goldfish, we have found that an agent that blocks RNA synthesis selectively also blocks long-term memory formation. The possible complex effects of such blocks over the entire brain preclude a simple interpretation of the results. At the subcellular level, however, these experiments suggest that both unimpaired transcription of RNA in the nucleus and translation into protein in the cytoplasm are required during the critical post-trial period of memory formation.

BERNARD W. AGRANOFF
ROGER E. DAVIS
University of Michigan

Proton-Proton Elastic Scattering and Structure within the Proton

The differential cross section for proton-proton elastic scattering at 90°C in the center of mass was measured at laboratory momenta ranging from 5.0 to 13.4 Gev/c. Fifty-one measurements were made at momentum intervals of 100 or 200 Mev/c. The extracted proton beam of the ZGS impinged upon a CH_2 target. The two scattered protons were detected by two spectrometers consisting of magnets and scintillation-counter telescopes in coincidence. The incident beam flux was measured by radiochemical analysis of the CH_2 targets. The experiment showed no evidence for any $S = 0$, $T = 1$ dibaryon resonances in the 3300 to 5200 Mev mass range. It also yielded some information about the validity of the statistical model and the analyticity of the scattering amplitude. The most interesting result of the experiment was a sharp break in the fixed-angle cross section. This may be evidence for the existence of two inner regions of the proton with radii 0.51 ± 0.02f and 0.34 ± 0.02f.

Supported by a research grant from AEC and by a summer grant from Argonne National Laboratory.

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Genetic Homologies and Drift within Population of DNA Molecules

The technique of nucleic acid hybridization, involving DNA molecules, has been applied to questions of genetic homology within and between many diverse organisms. We have attempted to assess the degree of DNA homology within *Tetrahymena pyriformis* with the agar method of McCarthy and Bolton. Strains of varying degrees of genetic relationship were employed. By competitive hybridization, more homology was found within a breeding group (syngen) than between syngens and related protozoa. Discrimination between closely related genotypes was more stringent at higher temperatures of incubation. At 69°C, the order of homology with respect to homozygous strain 7, syngen 1, was as follows: syngen 1, homozygous strains 8, 17, and 21, 60 percent; syngen 9, TC 89, 40 percent; syngen 10, En-10/11, 37 percent; *Paramecium aurelia*, 22 percent; *P. bursaria*, 6 percent. The closest degree of homology was found within a homozygous strain (for example, strain 7) but detectable differences of the order of 4 to 5 percent were found between clones of strain 7 and the hybrid progeny derived from strains 7 and 8 and backcrossed five times to strain 7. From these hybrid

clones, genes specific to each parental strain could be selected by reincubation. The success of these experiments may have depended on the use of multiple incubations and the discriminate choice of the temperature of incubation. The significance of these intersyngenic, intrasyngenic, and interclonal differences will be discussed in light of the behavior of the macronucleus.

SALLY ALLEN
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Implications of Curvilinearity in the Relationship of Efficiency of Performance to Strength of Motivation for Studies of Individual Differences in Achievement-Related Motives

The final strength of the tendency which motivates performance of a task (Tfin), as presently conceived, is a summation of separate component tendencies. Each of these depends on the strength of an individual's motive for a certain kind of goal (personality) and the incentive provided in a given situation. Typically, in achievement-oriented behavior, Tfin = $T_s - T_{-f} -$ Text, where T_s is tendency to achieve success, t_{-f} is tendency to avoid failure (that is, resistance to achievement-oriented activity), and Text represents the combined influence of various sources of extrinsic motivation (for example, social approval or monetary reward). This conception, combined with an assumption that efficiency of performance is a positive function of Tfin, yields the hypotheses that, normally, level of performance will be correlated positively with independent measures of individual differences in strength of achievement motive and negatively with measures of motive to avoid failure (anxiety). An alternative assumption (Yerkes-Dodson law), that there is an optimum level of motivation which depends upon requirements of the task, seems now to provide a more compelling guide in light of the inconsistent findings among earlier studies guided by the simpler assumption which neglected the effect of differences in nature of the task and provided inadequate control of other possible determinants of Tfin. Given the assumption of curvilinearity between efficiency and Tfin, the correlation between performance and individual differences in strength of achievement-related motives may be positive, zero, or negative depending upon the overall intensity of motivation in a given situation and the nature of the task.

JOHN W. ATKINSON
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Selective Autolysis of Nuclei as a Source of DNA Precursors for Other Nuclei within the Same Cell

After the end of conjugation, *Paramecium aurelia* contains two developing new macronuclei and approximately 35 fragments of the old macronucleus which broke down during conjugation. If the exconjugants are fed, the fragments persist and are segregated within the expanding clone; but if the cells are starved, an exponential decrease in the number of macronuclear fragments is observed, although no cell division occurs. Thus, by the time that 4 days of starvation have elapsed, the cells contain on the average only 2 to 3 fragments. During this time, the new macronuclei increase in volume exponentially, while the remaining fragments increase in volume only slightly.

When cells containing macronuclei with tritium-labeled DNA were starved following conjugation, more than 84 percent of the total activity was conserved throughout the 4-day starvation period. At the start of the starvation period, the new macronuclei contained about 4 percent of the total activity; by the 4th day, they contained over 90 percent. The activity per unit volume of the new nuclei increased exponentially, while the activity per unit volume of the remaining fragments increased linearly.

Thus, it appears that under starvation conditions macronuclear fragments are selectively autolyzed, with their DNA supplying precursor material for DNA synthesis in the remaining fragments as well as in the new macronuclei. The autolysis of macronuclear fragments is remarkable because there is decrease only in fragment number, not in fragment volume or in activity per unit volume of the fragments.

Supported by contract COO-235-43 from AEC to T. M. Sonneborn.

JAMES D. BERGER
Indiana University

Recent Developments in Thermoelectricity

The thermoelectric power has been referred to as the most sensitive transport property of a metal. Until about a decade ago, however, this phenomenon was not well understood, and the available data were confusing and viewed with some skepticism. Since then, the recognition of the role of phonon-drag and, more recently, magnon-drag has shed new light on thermoelectricity to the point where it is now possible to use this effect as a sensitive tool in the study of electronic structure and of electron-lattice interaction. For example, the thermopower of Mg, Zn, and Cd single crystals, which display marked anisotropies, clearly demonstrate the close relationship between the shape of the Fermi surface and the electron-phonon interaction. Also, gross changes in the Fermi surface due to alloying may be studied by thermopower measurements. Recently, measurements of the thermopower and of the Peltier effect of single crystals in a magnetic field have revealed the anticipated de Haas-van Alphen oscillations. These experiments promise to be particularly useful in Fermi surface studies because the amplitudes of the oscillations are unusually large, often an order of magnitude greater than the monotonic background, and the harmonic content of the oscillatory pattern is intrinsically enhanced over the more conventional de Haas-van Alphen experiments. Moreover, measurement of the Peltier effect permits precise determination of the absolute magnitudes of the oscillation amplitudes, with the result that one can compare theory and experiment in considerable detail.

FRANK J. BLATT
Michigan State University

Mutational Evidence for Controlled Genic Activity as a Mechanism of Differentiation

Changes in biological function through time (for example, embryonic development) and differing environments (for example, enzyme induction) encourage investigations into mechanisms whereby certain specific traits within a genically restricted spectrum are expressed in lieu of other traits. In *Paramecium aurelia*, any one of

three alternative classes of clones may arise after conjugation in wild-type stocks: clones of the odd-numbered mating type, clones of the complementary even-numbered mating type, and clones containing both mating types. Because clones of identical genotype may differ phenotypically in these three ways, it has been suggested that the basis for the differences rests in repression or derepression of specific portions of the genome.

Three genic mutations that restrict clones to expression of the odd-numbered mating type have been reported in two different syngens or biological species. With the selection system devised by Taub [*Genetics* **48**, 815 (1963)], at least one and possibly as many as six more odd-restricted mutants have been found in a third syngen after treatment with *N*-methyl-N-nitroso-*N'*-nitroguanidine. A mutation of a different class was also found. It regularly causes the production of both mating types within a single clone.

Thus, two of the three classes of clones producible by wild type have become sole possibilities because of single gene mutations. If each of these mutant genes is in some way functionally defective, the parallelism of each mutant phenotype to those occurring as normal alternative phenotypes in wild type strengthens the hypothesis that mating-type differentiation is a result of differential genic activity.

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BRUCE C. BYRNE
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Possible Triradiate Background of Some Echinoderms and Its Bearing on Phylogeny

Early Paleozoic echinoderms were multiple palimpsests of superimposed symmetries and correlated adaptive forms. Nowhere is this better seen than in the aberrant classes of "carpoids," Homostelea, Homoistelea, and Stylophora, which are unique among echinoderms in exhibiting no recognized five-part symmetry. Despite the fractionation of the old-class Carpoidea into three classes, there seems to be a common phylogenetic bond between them, setting them apart, possibly as a subphylum, from other echinoderms. Like all echinoderms, "carpoids" are bilaterians. This bespeaks vagility (the eleutherozoic condition). Fixation seems to have ensued (the pelmatozoic condition), perhaps in their pre-Phanerozoic period. Some degree of radial symmetry then followed, modifying the initial bilateral plan. It is the present contention that this attained a triradiate condition, but, contrary to all other echinoderms, did not attain a pentraradial plan. Free mobility then ensued (secondary eleutherozoic condition), with correlated superimposition of advanced bilateralization. The "tails" of "carpoid" classes all appear to derive from exothecal ambulacra and to be radial homologs of one another. They are not modified stems or columns, and, moreover, were consistently posterior in functional position. The terminal "carpoid" habitus in all three classes was essentially that of ostracoderm fishes, with many convergences (or possibly homologies) in their morphology.

KENNETH E. CASTER
University of Cincinnati

Occurrence of Similar Sets of Transport Agencies for Amino Acids in Various Cells

The first analyses for independently operating, parallel transport systems for neutral amino acids in animal cells showed two of these present: one Na^+-dependent, highly concentrative for amino acids with small or linear side chains; the second, Na^+-independent, probably not concentrative, and most reactive with amino acids with large hydrocarbon side chains. The specificity patterns overlapped enough so that most amino acids migrated by more than one route. Comparisons among cells and tissues have shown only minor variations in the properties of the latter system, whereas major differences were found in the principal Na^+-dependent system of the Ehrlich cell and several other tissues, on the one hand, and that of nucleated and reticulated red blood cells on the other. We now find that a transport system of the latter type, favoring alanine, serine, and cysteine, also operates in the Ehrlich cell, so that the two Na^+-dependent systems are present together. They may be resolved by the unreactivity of the *N*-methyl derivative of alanine or related amino acids with the alanine-serine-cysteine system. Thus, in the presence of excess *N*-methylalanine, the Na^+-dependent transport of amino acids becomes very similar among various cells. The similarity of certain Na^+-independent systems in microorganisms to those of animal cells and the general occurrence of a transport system reactive with taurine and β-alanine and of systems reactive with anionic or cationic amino acids, respectively, should be noted. Both the structural basis of the unity described and the role of genetics and differentiation in its expression call for study.

HALVOR N. CHRISTENSEN
University of Michigan

Diagnosis of Normokalemic Primary Aldosteronism, a New Form of Curable Hypertension

In 1954, we described a new clinical syndrome, primary aldosteronism, characterized by hypertension, hypokalemia, overproduction of aldosterone, and normal excretion of 17 hydroxycorticosteroids, and usually caused by a small adrenal cortical adenoma; surgical removal of this adenoma cures the entire syndrome. Within a few years, hundreds of such cases were diagnosed and cured. Hypokalemia quickly became the alerting hallmark, since, without this, there seemed no need to investigate the hypertensive patient further for primary aldosteronism.

In 1964, we found that plasma renin activity is distinctly subnormal in primary aldosteronism. This has been true in our 34 consecutive cases proved at operation whether hypokalemia had been severe or mild. This has been confirmed by many investigators. We had suspected and had published upon the probable existence of a normokalemic form of primary aldosteronism. We had indicated that without hypokalemia such patients would not be recognized as having curable hypertension and had suggested that suppression of plasma renin activity might be the key to finding this postulated condition.

During intensive studies, not yet completed, upon 184 hypertensives with persistently normal serum potassium values, we have detected the existence of normokalemic primary aldosteronism in 14 and have proved it at sur-

gery. Preoperatively, all exhibited the combination of hypertension, suppressed plasma renin activity, overproduction of aldosterone, and normal urinary 17 hydroxycorticosteroids. They differ from the more advanced form only in that they maintain normokalemia. To date, nine of the 14 are cured of their hypertension, and four more are greatly improved. We believe that many hypertensive patients will fall into this curable group, but the actual prevalence of this disorder has not been determined.

JEROME W. CONN
University of Michigan

Infection Experiments with a Fungus (Coelomomyces) Which Kills Malarial Mosquitoes

Greenhouse infection of *Anopheles quadrimaculatus* with *Coelomomyces punctatus* has been maintained continuously since January 1966, except during 2 months of excessively hot weather in the summer of 1966. Soils from locations where parasitized larvae occurred gave a high rate of infection but were unnecessary, since soils from locations where there was no inoculum gave infection. Successful infection seems to depend on putting germinating sporangia and hatching eggs together for about 24 hours and then transferring the larvae to an environment favorable for the best development of the larvae. One of our main tasks now is to find the best conditions for the production of the maximum amount of infection in the greenhouse. The parasite appears to enter the larva during the first instar, since germinating sporangia, put with 2nd, 3rd, and 4th instars produced no infection. As soon as we can find out for certain when infection occurs in *A. quadrimaculatus*, we propose to try to find out how the parasite enters the host. Germination of thick-walled sporangia in *Coelomomyces indicus* and *C. anophelesicus*, from Indian species of *Anopheles* which transmit malaria and dengue fever, has been done, but we have not been able to correlate this with the hatching of the eggs.

J. N. COUCH
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Pathophysiological Consequences of Increased Gastric Mucosal Permeability to Acid

The normal mammalian gastric mucosa does not digest itself, because it is relatively impermeable to H^+ secreted by its own oxyntic cells. A model of the pathophysiological consequences of abnormally high permeability has been developed through studies of chronically prepared pouches of the oxyntic glandular mucosa of the dog's stomach. Permeability of the mucosa to H^+ is greatly increased when the mucosa is exposed to fatty acids (acetic, propionic, butyric), to salicylates (aspirin, salicylic acid) in acid but not in neutral solution, to natural and synthetic detergents (conjugated bile salts, decyl sulfate), to ethanol at concentrations above 8 percent (weight by volume), and to urea. Back diffusion of H^+ into the mucosa causes a large output of modified interstitial fluid containing Na^+, glucose, and plasma proteins. Fluid output probably results from intramucosal release of histamine with consequent changes in capillary permeability and hemodynamics. Entry of H^+ into the mucosa also stimulates motility, probably by activating reflex pathways within the intramural plexuses, and it also appears to stimulate secretion of HCl and pepsinogen. Rapid back diffusion of H^+, especially when combined with cholinergic stimulation, frequently causes bleeding which may be severe and prolonged.

HORACE W. DAVENPORT
University of Michigan

Regulatory Variants in Eucaryotes

Because regulatory variants are not easily recovered as induced mutants in eucaryotic organisms, interpopulation crosses of the mold *Neurospora* were studied. It was felt that genetic divergence between various isolates of a species or genus would make components of regulatory systems incompatible in some hybrids. The specific activity of an enzyme of arginine synthesis, ornithine transcarbamylase (OTC), was measured in natural isolates of *Neurospora* spp. This activity was remarkably constant in many exotic isolates of the genus, and various genes (including the structural gene) which affect activity appeared, in hybrids, to be interchangeable [Grindle and Davis, *Genetics* **54**, 337 (1966)]. One exotic isolate of *N. crassa*, however, was slightly elevated in its activity for this enzyme, and the high activity was not "repressed" by the addition of arginine, despite normal expansion of the intracellular pool of this compound. Crosses of this isolate to strains of the standard laboratory background, followed by backcrosses to the latter, yielded a prototrophic recombinant carrying a gene allowing arginine uptake, but severely limiting the intracellular arginine pool size, whether or not arginine was present in the medium. This behavior was correlated with an unconditional sevenfold to tenfold elevation of OTC activity. Thus, interpopulation crosses may be used to detect and isolate biosynthetic regulatory variants. The data suggest that certain variants of the constitutive type, whatever their origin, may represent altered size or distribution of metabolite pools rather than mutations of the primary sensing elements of the regulatory system.

ROWLAND H. DAVIS
University of Michigan

Complementary Functioning of Two Components Required for the Reduction of N_2 from Four Nitrogen-Fixing Bacteria

Two components required for nitrogenase activity from each of four nitrogen-fixing bacteria [*Clostridium pasteurianum* (*C.p.*) strain W-5, *Bacillus polymyxa* (*B.p.*) strain Hino, *Klebsiella pneumoniae* (*K.p.*) strain M5A1, and *Azotobacter vinelandii* (*A.v.*) strain OP] have been shown to have complementary functioning for reduction of N_2.

The crude extracts from each organism were fractionated on DEAE-

Table 1.

0.3*M* NaCl fractions	0.5*M* NaCl fractions of			
	A.v.	*K.p.*	*B.p.*	*C.p.*
A.v.	+	+	+	
K.p.	+	+	+	
B.p.		+	+	+
C.p.				+

+, Stimulation of N_2 reduction.

cellulose with increasing amounts of NaCl (0.175, 0.3, and 0.5*M*). The 0.3*M* NaCl eluate (dark brown) and the 0.5*M* NaCl eluate (yellow) contained little or no activity separately, but, when mixed (1 : 1), yielded a substantial increase in activity. The results of the cross-reactions are shown in Table 1.

Six of the twelve possible cross-reactions have resulted in stimulation of N_2 reduction. Homologous crosses were generally higher in activity than heterologous crosses. The facultatively anaerobic bacteria (*K. pneumoniae* and *B. polymyxa*) possessed the widest range of complementarity, whereas the aerobic bacterium (*A. vinelandii*) and the anaerobic bacterium (*C. pasteurianum*) possessed more restricted ranges of complementarity.

R. W. DETROY, D. F. WITZ
R. A. PAREJKO, P. W. WILSON
University of Wisconsin

How Ciliary Basal Bodies Develop

In spite of near universality of fine structure of centrioles and basal bodies of cilia and flagella, little is known about their origin and development. Previous workers have concluded that they arise in association with a dense plaque of apparently unorganized material, but the earliest identifiable stage shows the complete mature structural pattern: a more or less hollow cylinder, the wall of which consists of 27 microtubules arranged as nine equally spaced triplets. Electron microscopic observations on *Paramecium aurelia* revealed the following sequence of developmental stages. (i) There is a circular plaque of dense fibrous material showing no microtubules. (ii) The plaque includes two to nine single microtubules usually connected by a single fiber; they are equally spaced on a circle when nine are present, and on an arc when there are fewer than nine. (iii) The plaque contains two to nine adjacent doublets; when there are less than nine doublets, singlets bring the total to nine. (iv) The plaque contains nine sets of microtubules, some doublets and some triplets. (v) There is a complete ring of nine triplets. In serial sections, stages 1 and 2 are confined to a single section. Stages 2, 3, and 4 are characterized by a progressively more hollowed-out plaque, suggesting that plaque material may be assembled into microtubules. The variations in stage 2 suggest that the ninefold symmetry is established by the tubules and their fibrous "linkers," as a short arc develops into a complete ring.

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RUTH V. DIPPELL
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First-Stage Electrical Effects in Vision

Following a flash of light, all oriented biological pigment molecules appear to generate millisecond electrical potentials. After an initial flash which initiates the bleaching of rhodopsin in the retina, a second flash generates further photovoltages from the intermediates formed in the bleaching process; and the half-lives for the thermal decay of intermediates A, B, and C (meta I, meta II, and P465) have been measured in the eye of the albino rat in this way, over a range of temperatures. The half-lives of these intermediates at 37°C are 40 microseconds, 140 seconds, and 1100 seconds, respectively. Only the decay of intermediate A is rapid enough at physiological temperatures possibly to be involved in visual excitation. The neural pulse represents an amplification of a threshold retinal quantum absorption by some 10^5 to 10^7 times, with a delay of some tens of milliseconds. The questions ahead are what solid-state, biochemical, or membrane amplification processes are active during this delay interval and how they may be detected and studied.

T. G. EBREY
J. R. PLATT
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Influenza Virus Envelope Protein: Biological Activity as a Function of Reassociation

Envelope protein, a major structural element of the influenza virus surface envelope, is recovered in an active state following solubilization of lipid-extracted virus concentrates in 67 percent acetic acid. Intermediate forms of the protein, a molecule of the dimensions of a polypeptide (2*S*) in concentrated acetic acid and a reassociated 4*S* component in dilute acid can be demonstrated by physical techniques. At neutral *p*H, further association occurs, and virus surface-specific antigenicity and hemagglutinating activity are recovered.

The process of self-assembly in vitro of the protein controlled by adjustments in *p*H leads to a family of macromolecules. On the basis of sedimentation rates in sucrose gradients and differences in biological properties, several distinct components can be defined. All the forms have one attribute in common—antigenicity similar to that of the surface of the intact virus particle—while they differ in their interaction with erythrocytes. The simplest is a uniform 12*S* protein which accounts for the bulk of the antibody-binding capacity and protein nitrogen of envelope protein preparations but which does not bind to or agglutinate red blood cells. Slightly larger is a component which absorbs to erythrocytes but does not agglutinate them. Hemagglutinating activity is limited to the larger heterogeneous protein assemblages with a sharply defined minimal size (30*S*).

It is proposed that the several structural forms represent alternative stable states of isolated envelope protein and that the functional characteristics of each component are dependent on its degree of association.

EDWARD A. ECKERT
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An Evaluation of the Role of Carnitine in Regulating Fatty Acid Oxidation and Gluconeogenesis

Carnitine catalyzes an increase in long-chain fatty acid oxidation by slices, whole homogenates, and mitochondrial preparations from several tissues. This effect is intimately associated with the formation of fatty acylcarnitine, mediated by the carnitine palmityltransferase reaction. Carnitine enhances fatty acid oxidation by facilitating translocation of long-chain fatty acyl groups across an interface which functionally separates the site of fatty acid activation from the site of oxidation [*Advances Lipid Res.* **1**, 286 (1963)]. The use of (+)-palmitylcarnitine, a competitive inhibitor of the

transferase, has permitted the conclusion that in heart mitochondria the rate of formation of (−)-palmitylcarnitine is the rate-controlling step for long-chain fatty acid oxidation [*Proc. Nat. Acad. Sci. U.S.* **54**, 1226 (1965)]. In liver mitochondria and slices, however, the dependence on carnitine for long-chain fatty acid oxidation is not absolute, and the role of different thiokinases and intramitochondrial compartments is currently being evaluated. The control of fatty acid oxidation by liver is of considerable physiological importance because of the close interrelations between rates of gluconeogenesis and fatty acid oxidation in this organ. We have recently reported that (+)-palmitylcarnitine decreases gluconeogenesis by pigeon liver homogenates and that the inhibition is relieved by (−)-carnitine but not by (+)-carnitine [*Proc. Nat. Acad. Sci. U.S.*, in press]. (−)-Palmityl- or decanylcarnitine stimulates gluconeogenesis, presumably as a consequence of the increased rate of fatty acid oxidation. Extensions of these findings to different types of preparations will be presented, and the interrelations between fatty acyl CoA and acylcarnitine derivatives on intermediary metabolism will be discussed.

IRVING B. FRITZ
University of Michigan

Organization of DNA in Chromosomes of Eucaryotes

Several types of experimental evidence favor the hypothesis that the DNA in chromatids of mitotically active cells of higher organisms is distributed along two or more longitudinally oriented parallel strands.

Measurements of amounts of DNA by Feulgen microspectrophotometry show that the diploid prophase, metaphase, and anaphase chromosome complements in dividing neuroblasts of *Drosophila melanogaster* larvae ($2n = 8$) fall into two classes, which can now be specified as 4C and 8C, in comparison with the 1C haploid value of the spermatozoon. Doubling in the amount of DNA without an increase in chromosome number suggests a doubling in number of chromosomal stands.

Radioautographic studies of mitotic chromosomes in root-tip cells of *Haplopappus gracilis* ($n = 2$) during the two mitotic cycles after incorporation of tritiated thymidine provide information about the frequencies of "isolabeling" (about 25 percent) and of different types of chromosomal exchanges. The data obtained do not fit predictions based on models of single-stranded chromosomes with subunits having different "polarities." (Evidence leading to a similar conclusion has been obtained from the analysis of mosaic salivary glands of *Drosophila melanogaster* induced by x-ray irradiation of spermatids.)

Counts of silver grains over identical segments of sister chromatids of *Vicia faba* at metaphase of the first division after pulse labeling of root-tip cells with H^3-thymidine show significant inequalities. Such asynchronous DNA replication is most readily explained in terms of a polynemic chromosome. Models of chromosomes consonant with these and other lines of experimental evidence will be discussed.

HELEN GAY
University of Michigan

Molecular Basis of Carcinogenesis by Human Adenoviruses

The 31 human adenoviruses (Ad) form three groups based on several properties of viral DNA and oncogenicity of purified virus: (i) closely related Ad 12, 18, and 31 with 48 to 49 percent G + C, all "highly oncogenic"; (ii) closely related Ad 3, 7, 11, 14, 16, and 21 with 50 to 52 percent G + C, all but Ad 11 "weakly oncogenic"; and (iii) the remaining adenoviruses with 55 to 61 percent G + C, all "nononcogenic."

We detected viral-specific mRNA in the nucleus and polyribosomes of virus-free tumor cells induced by seven different human adenoviruses. These findings strongly suggest that the integration and transcription of at least part of the viral genome are obligatory steps in adenovirus carcinogenesis.

Hybridization of purified adenovirus mRNA's with different adenovirus DNA's did not detect ribonucleotide sequences common to the mRNA's of highly and weakly oncogenic adenoviruses. Thus, different viral-coded information appears to be involved in carcinogenesis by these two groups of human adenoviruses.

Base compositions of purified Ad 12, 18, 7, and 16 mRNA's from tumor cells are distinctly different from those of ribosomal, transfer, and nuclear RNA. The average G + C content of Ad 12 and 18 mRNA's (47 to 48 percent) and DNA's are very similar, but that of Ad 7 and 16 RNA's (47 to 48 percent) and DNA's differ by 2 to 5 percent. These results imply that viral DNA regions with an average G + C content of 47 to 48 percent are integrated or preferentially transcribed in adenovirus-induced tumor cells.

MAURICE GREEN
MAGDALENA PIÑA
KEI FUJINAGA
Saint Louis University School of Medicine

Neutron Activation Studies of the Source of Prehistoric Hopewellian Obsidian Implements from the Middle West

For 120 years, American archaeologists have been attempting to identify the source, or sources, of obsidian artifacts found in prehistoric Hopewellian culture sites of about the time of Christ in the Middle West. Conjectures included Alaska, Yellowstone, the Southwest, and Mexico.

Neutron activation analyses have been made on 23 specimens from five sites in Ohio, on 20 samples from ten sites in Illinois. About 90 percent of these specimens can be confidently identified with source specimens from Obsidian Cliff in Yellowstone, while the rest of the Midwestern specimens apparently can be identified with secondary Yellowstone sources.

Source material from 23 obsidian deposits in Alaska, the United States, and Mexico have been tested, and only the Yellowstone deposits can be identified with the prehistoric finds in the Middle West.

First, the analysis procedure involved determination of the sodium and manganese contents of the obsidian specimens. On the basis of a correspondence of these elements, the possible geologic sites were narrowed to a relatively small number. Further analyses, based on over ten additional elements, permitted distinguishing between those geologic obsidian sites that have sodium and manganese contents identical with the obsidian artifact.

JAMES B. GRIFFIN
A. A. GORDUS
University of Michigan

Excretion of Ketosteroids and Proneness to Breast Cancer

Certain women develop breast cancer, while others living in comparable environments never do. The roots of the innate resistance possessed by certain individuals must be found before there is hope of conferring this resistance upon those who are prone.

These roots are probably to be found in the biochemical individuality of the persons concerned. This encompasses distinctiveness in a great variety of ways, involving enzyme and hormone levels and other compositional differences in tissues and all body fluids.

In the present investigation, it was found that, in a group of premenopausal women who had been operated on for breast cancer, the urinary excretion levels of four of six ketosteroids were significantly different from those in other women of the same age who had no breast cancer: etiocholanolone lower (in cancer group) ($P < .05$); 11-hydroxyandrosterone higher ($P = .001$); 11-hydroxyetiocholanolone lower ($P < .01$); 11-ketoetiocholanolone lower ($P < .05$). Multiple discriminant analysis showed that the excretion values for six ketosteroids, taken as a group, showed significant differences ($P < .002$).

It cannot be stated with certainty that these differences in excretion patterns antedated the onset of cancer, but there is a strong presumption that they did, in view of the fact that well individuals—even infants—always possess highly distinctive patterns which constitute their biochemical "fingerprints."

This investigation appears to point the way toward spotting individuals who are prone to have breast cancer, *before* the disease attacks. Propetology, the study of proneness, is a first step toward the prevention of cancer.

ROSE MARY GUTIERREZ
ROGER J. WILLIAMS
The University of Texas

Hormones in Reproduction and Teratology in Guinea Pigs

Teratology may be defined as that division of embryology and pathology which deals with abnormal development and congenital malformations. Until the thalidomide disaster of 1961, few people were interested in birth defects, and even fewer were actively involved in an investigation of them.

However, the importance of birth defects in the human population is readily apparent. Various studies indicate that from five to seven children of every 100 born alive will require medical attention before age 2 because of a birth defect.

As acute as is the problem of congenital malformations in human beings, the problem of which species to employ in any investigation of the etiological factors concerned is of even greater importance. Comparative studies have shown that the reaction to given teratogenic substances may vary according to species and strain; therefore, great care must be taken to avoid casual transference to man of generalizations obtained from the interpretation of animal studies.

The importance of the guinea pig as the species in which to investigate the teratogenic effects of hormones responsible for the maintenance of pregnancy is determined by the fact that its hormonal control of pregnancy is similar to that of the human female. Evidence will be presented which (i) justifies this statement, (ii) indicates that both hypo- and hyperadrenal activity interferes with the maintenance of pregnancy in guinea pigs and produces birth defects, (iii) demonstrates that the effects of hypothyroidism depend upon the time of thyroidectomy, and (iv) suggests an interrelation between the thyroid and adrenal during reproduction.

RICHARD M. HOAR
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Pyridine Nucleotide in Mung Bean Mitochondria

Freshly prepared mitochondria from dark-grown mung bean hypocotyls contain 4 to 6 mμmoles of diphosphopyridine nucleotide per milligram of protein. There is no detectable triphosphopyridine nucleotide in these mitochondria, nor is there any detectable pyridine nucleotide transhydrogenase. In a phosphorylating reaction medium, steady-state changes of pyridine nucleotide, corresponding to respiratory states 1, 3, 4, and 5, are demonstrated with fluorescence techniques.

In a nonphosphorylating reaction medium, the addition of succinate to the mitochondria reduces intramitochondrial pyridine nucleotide slowly and to a limited extent. The rate of reduction is greatly increased by adenine diphosphate (ADP), adenine triphosphate (ATP), or oligomycin, and to some extent enhanced by magnesium ion at low concentrations. Inorganic phosphate biphasically stimulates and inhibits the reduction. Uncouplers of oxidative phosphorylation, such as carbonyl cyanide *m*-chlorophenylhydrazone, or inhibitors of electron transport such as amytal, rotenone, malonate, antimycin A, and KCN inhibit the reduction.

The reduction of intramitochondrial pyridine nucleotide by succinate thus follows in part a pattern predicted from the work on animal mitochondria or particles isolated therefrom, while several differences are noted in the mode of succinate-linked reduction of pyridine nucleotide between plant and animal mitochondria. An important difference lies in the finding that oligomycin does not inhibit the ATP-dependent reduction of pyridine nucleotide, whereas it is capable of inhibiting ADP-stimulated uptake of oxygen in the phosphorylating medium. It appears, therefore, that the energy-transfer system in mung bean mitochondria differs from that in animal mitochondria.

Supported by grant NSF-GB4342.

HIROSHI IKUMA
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Estrogen-Binding Substances of Target Tissues

Estrogen-dependent tissues contain minute amounts of unique components called "estrogen receptors." These show striking affinity for tritiated estradiol, both in vivo and in vitro, which involves separate uptake and retention processes. Strong but reversible interaction of hormone with receptor, without chemical transformation of the steroid, is an early step in uterotrophic action.

Two different radioactive hormone-receptor complexes are detected in uterine homogenates. In the supernatant fraction, Toft and Gorski demonstrated a complex sedimenting at 9.5*S*; in KCl extracts of the nuclear fraction, we observed a 5*S* complex. In uteri exposed to estradiol in vivo or at 38°C in vitro, the hormone is mainly associated with nuclear receptors, even though binding capacity of 9.5*S* receptors is far from saturated;

after brief exposure at 2°C, the 9.5*S* complex predominates. In both receptors, sulfhydryl groups are essential for binding ability. Both substances are sensitive to pronase but not to ribonuclease or deoxyribonuclease, suggesting that they are proteins; one is not a simple aggregate of the other. The 9.5*S* complex can be formed by adding estradiol directly to the supernatant fraction; the 5*S* complex requires exposure of tissue to estradiol before nuclei are extracted.

Estrone, which shows little affinity for target tissues in vivo and uptake but not retention in vitro, forms a stable 9.5*S* but unstable 5*S* complex. With hormone-dependent rat mammary tumor, estradiol yields a 5*S* but no 9.5*S* complex. These observations suggest that, in uterus, extranuclear 9.5*S* receptors take up estradiol which then is retained by nuclear 5*S* receptors to stimulate growth processes.

E. V. JENSEN, E. R. DESOMBRE
T. KAWASHIMA, T. SUZUKI
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University of Chicago

A Cosmic Ray Program for the Study of Strong Interaction Physics in the Range of 100 to 1000 Gev

A major cosmic ray research facility has been designed for the summit of Mt. Evans, Colorado to study the strong interactions of elementary particles over the energy range from 100 to 1000 Gev. Employing high-resolution spark chambers and magnetic analysis of the incident and emergent particles, this facility would accumulate about 10^5 interactions per year in a liquid hydrogen target at energies beyond those presently accessible with particle accelerators. Over the past 2 years, a program of feasibility studies, coupled with specific cosmic-ray physics experiments at mountain altitudes, has been carried out.

In a recently completed experiment, a new upper limit to the flux of massive, elementary particles (for example, "quarks") was established at about 4×10^{-10} cm^{-2} sr^{-1} sec^{-1} independent of charge. Currently under construction is an experiment to measure the proton-proton total cross section in a liquid hydrogen target at energies above 100 Gev.

LAWRENCE W. JONES
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Cinephotomicrography of Starch Contents of Tomato Tissue Culture Cells in Microculture

Tomato cells (*Lycopersicum esculentum* Mill. var. Bonny Best) grown in tissue culture contained many starch granules. The amount of starch decreased with increasing time in vitro. Tomato cells exposed to the crown gall tumor-inducing bacteria furthermore showed increased starch dissolution over that of normal cells. The present study with the microculture method investigated the relative amounts of starch in healthy tomato cells after varying lengths of time in culture.

Starch granules in tomato cells were identified by polarized light and I_2KI. Cells were observed through the phase-contrast microscope and photographed both by still and cinephotomicrography. Cells in liquid shake culture lost their starch more rapidly than cells grown in microculture. The starch content of living tomato cells as viewed in microculture was rated on an increasing scale of 0 to 4. Young, healthy, tomato cells had dense cytoplasm and numerous cytoplasmic strands. Abundant large starch granules (type 4) surrounded and obscured the nucleus. Starch granules became smaller and least numerous (type 0) in aging cells. The relative amounts of starch within these normal cells may be useful further to characterize differences between healthy cells and those exposed to crown gall tumor-inducing bacteria.

MILLICENT KALIL
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Genetically Controlled Diploidy in a Higher Fungus

The life cycle of *Schizophyllum commune* consists of haploid dikaryotic, and diploid phases. The haploid and dikaryotic phases are propagated indefinitely in vegetative mycelia; the diploid phase is restricted to a single nuclear generation in the sexual process in the basidium and is immediately followed by meiosis to yield the haploid spores. Recent studies of the interactions between certain fully compatible haploid strains have demonstrated that the stability of the dikaryotic phase is controlled by a single dominant gene. In the absence of this gene, the dikaryon is converted into a morphologically distinct, vegetative, diploid phase. Somewhat surprisingly in view of previous reports, the compatible diploid lacks clamp connections, the distinguishing morphological feature of the dikaryon. In vegetative growth, the diploid is not entirely stable, as occasional hyphae or entire sectors display dikaryotic morphology. In matings with haploid strains, the diploid is quite unstable and appears often to undergo spontaneous haploidization. Individual fruiting bodies have been obtained from such pairings, however, from which segregated all known genetic markers of both diploid components as well as those of the haploid mate.

YIGAL KOLTIN
JOHN R. RAPER
Harvard University

Hydrogen Bonding of C — H • • • O = C in Proteins

It is well established that the N — H • • • O = C hydrogen bond is a major determinant of the three-dimensional structure of polypeptides and proteins. Several proposals have been made that C — H • • • O = C hydrogen bonds can also occur in such structures. Because they were based upon model building or fiber x-ray diffraction methods, these studies could not provide definitive demonstrations of the existence of such bonds. Recent analyses [S. Krimm, K. Kuroiwa, T. Rebane, *Conformation of Biopolymers*, G. N. Ramachandran, Ed. (Academic Press, 1967); S. Krimm and K. Kuroiwa, to be published] of the infrared spectrum of polyglycine II, however, furnish more direct evidence of the presence of C — H • • • O = C hydrogen bonds in the structure of this polypeptide. For example, although the spectrum of polyglycine I shows two unperturbed CH_2 stretching frequencies at 2850 and 2923 cm^{-1} (C — H • • • O = C hydrogen bonding being impossible in its structure), the spectrum of polyglycine II exhibits four CH_2 frequencies, two near the above values and two displaced lower and higher by about 50 cm^{-1}. At −170°C, the latter bands shift in a manner which indicates that these perturbed frequencies arise from an attractive rather than a repulsive interaction. The presence of unperturbed modes is consistent with the antiparallel chain

structure of polyglycine II [S. Krimm, *Nature* **212**, 1482 (1966)]. An examination of the structure of myoglobin (S. Krimm and H. C. Watson, to be published) now indicates that C — H • • • O = C hydrogen bonds are present in its α-helical segments. Therefore, although weaker than the N — H • • • O = C bond, the possibility of an attractive C — H • • • O = C interaction should now be incorporated into the principles underlying the structure of polypeptides and proteins.

S. KRIMM

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A Temperature-Sensitive Mutant Affecting Phage DNA Synthesis and Lysogenization

Infection of *Salmonella typhimurium* LT2 with high multiplicities (20) of wild-type phage P22 results in almost 100 percent lysogenization of infected clones. Phage DNA synthesis reaches a peak at 6 to 7 minutes, followed by complete repression as the cells survive the infection with consequent integration of the phage genome (as prophage) into the bacterial chromosome. A temperature-sensitive mutant which fails to synthesize phage DNA at 37°C has been isolated. Cells infected with the mutant at 25°C show high frequency of lysogenization. At 37°C, no lysogeny is observed; the cells survive as sensitive bacteria, that is, integration of the prophage does not occur. Once established at 25°C, lysogeny is stable at high temperature. Revertant phages regain both the ability to synthesize phage DNA and to lysogenize at high temperature.

Temperature-shift experiments indicate that the effects on phage DNA synthesis and prophage integration are temporally distinct. The time of onset of the function necessary for phage DNA synthesis is within the first few minutes of the latent period, while that for integration of the prophage is initiated at about 60 minutes. A shift to the nonpermissive temperature at any time before 60 minutes prevents lysogenization. The data are consistent with the interpretation that a phage-controlled function necessary for the synthesis of phage DNA is also involved in integration of the prophage into the host chromosome.

MYRON LEVINE

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Antigenic Behavior of Cytochrome c

Eukaryotic cytochromes *c* afford a set of homologous proteins of known primary structures which presents a unique opportunity for mapping surface conformations and investigating immunological questions relating to globular proteins in general. Antibodies elicited in rabbits to the cytochromes *c* from several vertebrates cross-react to varying extents with the proteins of all 26 species examined. Competitive binding and complement-fixation studies with cytochromes *c* varying by one or a few residues permit the identification of antigenic determinant sites. In general, only a limited number of such determinants are present. Characteristically, in those cases which have so far been defined, they occur when a large hydrophobic residue, such as isoleucine, occupies a position which is commonly filled by a hydrophilic residue in the majority of cytochromes *c* from other species. Appropriate antibody populations can distinguish between the presence or absence of a single methyl group if it is placed at or near the binding site. Similarly, some antiserums differentiate between ferri- and ferrocytochromes *c*, indicating a change in antigenic structure which probably reflects the well-known difference in tertiary structure between the two oxidation states of the protein. The effect of oxidoreduction on the surface conformation of cytochrome *c* is nevertheless not universally identical in extent. Thus, chicken protein appears to be immunologically indistinguishable in the ferric and ferrous forms. Correspondingly, crystals of chicken ferricytochrome *c* can be reduced without macroscopic disruption, in contrast to all other cytochrome *c* crystals which have been examined.

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Chemical Ecology: Fungus-Growing Ants

The attine ants of the New World tropics culture a fungus in their nests and utilize this fungus as their food source. These fungi appear to be restricted to attine nests. The chemical basis and competitive advantage of this complex symbiosis will be discussed.

The fungus cultured by *Atta colombica tonsipes* has been found to be an effective cellulose decomposer. In turn, the mycelia are a rich source of nutritive carbohydrates for the ants. By culturing and feeding on such a fungus, the ants have succeeded in utilizing as a nutrient source the vast reserves of cellulosic materials in their environment.

In culturing the fungus, the ants apply salivary and fecal material to their gardens. The possible involvement of antibiotic and growth-stimulating materials in this culturing operation has been investigated, and the results will be presented. The presence of a growth-promoting substance in ant extracts has been demonstrated. Its significance and its origin will be discussed.

M. M. MARTIN

RAYMOND M. CARMAN

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Neural Coordination in a Simple Ganglion

Motor neurons in the stomatogastric ganglion of decapod Crustacea interact with one another by means of collaterals within the ganglion neuropil. Such interaction is often specific between individual neurons and may involve among others either electrotonic or inhibitory junctions. Characteristic, repetitive activity patterns in about one-third of the 30 to 35 neurons of the ganglion in Australian mangrove crabs can be shown to depend upon a wiring configuration employing several kinds of interneuron junctions. Four neurons with mutual electrotonic connections form a positive feedback network whose output apparently builds up periodically to saturation and then cuts off. Modification of the excitability or resting potential of any one member of the network affects the output of the entire system. These four neurons (type A) form a pacemaker subsystem which in turn controls, by inhibition, two other neuron types. The collaterals of one of these (type B) inhibit some of the A-neurons by direct postsynaptic inhibition. The other (type C) inhibits the type B neuron, also by direct postsynaptic inhibition. The resultant output is a repetitive, complex pattern in which the A-neurons first dis-

charge at exponentially increasing frequencies, reach a peak, and stop; the B-neuron then discharges repetitively for a brief period and in turn is followed by C-neuron activity before the A-elements begin again. Such sequential, alternating discharge patterns are of widespread occurrence in a variety of phyla and particularly in locomotory and respiratory systems. The stomatogastric ganglion is unusually favorable for study as a model system, for it is relatively simple and permits direct and exhaustive examination of the detailed cellular mechanisms underlying such patterning.

DONALD M. MAYNARD
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Metagons for Kappa?

The metagon hypothesis (Gibson and Beale, 1964) attributes stability and infectivity to the mRNA of gene M, required for permanent maintenance of a bacterium-like symbiont, mu, by *Paramecium aurelia*. Gibson and Sonneborn (1964) reported that this mRNA multiplies like a virus when introduced into *Didinium nasutum*. Mu loss by mm paramecia is attributed to metagon loss. Sonneborn [*Amer. Nat.* **99**, 279 (1955)] pointed out parallels to the gene K-kappa system and showed that it might be used to verify the hypothesis. We have tested four points presented as evidence for the hypothesis. (i) As to the kinetics of kappa loss (detected by sensitivity to kappa killing) when Kk kappa-bearers become kk, we occasionally find metagon-type kinetics (progressive loss between generations 8 and 15), but kappa may disappear in as few as five generations or be retained by all cells for up to 65 generations. Beale and McPhail [*Genet. Res.* **9**, 369 (1967)] also report deviations from metagon-type kinetics. (ii) In regard to retention of symbionts by at least one descendant of each kk cell when most have lost them, we find often that all descendants lose kappa. (iii) We have never found symbiont loss following ribonuclease treatment of mu- or kappa-bearers. (iv) In testing the requirement of metagons from paramecia for symbiont maintenance by *Didinium*, we have had didinia acquire and maintain kappa when they could not have acquired from paramecia anything with metagon properties. Thus, we have no evidence supporting the metagon hypothesis and much evidence against applying it to the kappa system.

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Mixed Function Oxidase Reactions in Biological Oxidations: Human Placenta

The biosynthesis of steroid hormones by endocrine tissues and the metabolism of many drugs by the liver are catalyzed by a variety of enzyme systems which require, in addition to oxygen, a reducing cofactor and are therefore classified as mixed function oxidases. Recent studies of others, utilizing carbon monoxide as a specific inhibitor, have established that a new hemoprotein, cytochrome P_{450}, participates in a number of steroid hydroxylations and drug oxidations carried out by the adrenal cortex and liver; they have suggested that this cytochrome may play a role in additional mixed-function oxidase systems of this type.

The human placenta also carries out mixed-function oxidase reactions on steroids. The side chain of cholesterol is removed by placental mitochondria in the production of progesterone, and androgens are aromatized to estrogens by placental microsomes. Both of these conversions require oxygen and reduced triphosphopyridine nucleotide, and both proceed via hydroxylated intermediates. The presence of cytochrome P_{450} has been demonstrated in both placental microsomes and mitochondria but steroid aromatization is not inhibited by carbon monoxide under conditions which are effective for demonstrating cytochrome P_{450} involvement in other mixed function oxidase reactions. Cyanide and azide, which do not interfere with most mixed function oxidase reactions, are effective inhibitors of aromatization.

These findings suggest that cytochrome P_{450} plays no part in steroid aromatization or that, in placental microsomes, it possesses properties distinctly different from those displayed in adrenal cortex and liver.

ROBERT A. MEIGS
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Repetition and Retrieval from Memory

The correlation of repetition and recall is poorly understood. Experiments on the immediate unordered recall of common nouns after one presentation of a list examine several hypotheses about the mediation of the repetition effect.

In all experiments some words occur twice in a list with interpolations of 0, 2, 4, 8, 20, or 40 other words between occurrences. Under these conditions, the probability of recall of a repeated word increases with the distance between its occurrences. The rate of presentation of the words (1.3, 2.3, and 4.3 seconds per word) affects the recall probability but not the relationship to distance between occurrences.

The subjects indicated recognition of repetition by saying "old" or "new" to each word during list presentation. Recognition of repetition decreases as distance between occurrences increases and cannot be a necessary condition for the effect of repetition on recall. However, words recognized as repeated are better recalled than those not recognized, and single-occurrence words that are falsely recognized as repeated are more poorly recalled than those recognized as "new."

Recall protocols provide evidence of contextual coding of words during acquisition. This coding varies, as overall recall varies, with distance between occurrences of repeated words. Separation of repetitions appears to have an effect on recall to the extent that it allows two independent contextual codings of the word.

A model of the effect of repetition on recall requires at least three variables: total presentation time, recognition of repetition, and opportunities for independent contextual coding.

ARTHUR W. MELTON
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Tubule-Bearing Vesicles Associated with Slime-Body Formation in Differentiating Cells of Bean Root Tips

Vesicles of a previously unreported type have been observed during a fine structural investigation of cellular differentiation in root tips of bean (*Phaseolus vulgaris* L.). The vesicles are restricted to differentiating procambial cells of the phloem and pericycle bor-

dering on the protophloem sieve elements in the phloem strands. They are approximately 40 to 150 mμ in diameter and bear numerous radiating projections spaced about 150 angstroms apart at their points of origin. The projections are tubular, with an electron opaque cortex and transparent core. The tubules are approximately 320 to 330 angstroms long and 80 angstroms thick.

These vesicles are also distinguished by occurrence in clusters ranging from a few to several hundred individuals. They appear to arise from dictyosomes, and at first they are surrounded by an indistinct layer of fine fibrous material. Tubules are differentiated from the surface material after the vesicles move away from the dictyosome. The vesicles appear to be held together by embedment in a viscous matrix.

The procambial cells that form vesicles with tubular projections, including pericyclic cells adjacent to protophloem elements, also produce proteinaceous slime bodies. The vesicles appear before and during slime-body formation and disappear as the bodies enlarge. Since the vesicles are also frequently observed at the periphery of slime bodies, it is tentatively assumed that they contribute to slime-body formation.

These unusual vesicles thus may play a unique role in the cytoplasmic differentiation of certain cell types. In this laboratory, similar vesicles have been seen in differentiating cells of the phloem strands of *Dianthus* roots and *Coleus* petioles.

ELDON H. NEWCOMB
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Neuron Correlates of Motivational Processes

Animals with several fine-wire electrodes implanted chronically in the brain for single unit recordings were pretrained to immobilize themselves for brief intervals in order to obtain food or water. Unit responses were recorded during these periods of immobilization, during other quiet awake intervals, and during sleep. Recordings were made simultaneously from four probes in each animal. Groups of seven to 22 probes were studied in each of the following areas: hippocampal, preoptic, ventral reticular, lateral hypothalamic, ventral tegmental, thalamic, and dorsal reticular. Relatively large and stable changes occurred within the course of the brief waiting periods. These were consistent with probes in dorsal reticular formation, thalamus, and ventral tegmentum; here they were regularly in the positive direction, with neurons firing more rapidly as the reinforcing stimulus became more immanent. With probes in hippocampus, preoptic area, ventral reticular formation, and hypothalamus, there were stable augmentations and diminutions, but the average changes for the group were not significant. There were gross food and water differences: dorsal reticular formation, thalamus, and hippocampus fired more rapidly while animals awaited food; preoptic area, lateral hypothalamus, and ventral reticular formation favored water. These were attributed to gross differences between the food and water behaviors. There were individual cases where neurons seemed especially related to either food or water behavior to the extent that they might be involved in the anticipatory representation of the incentives; these were particularly frequent in the hippocampal and preoptic groups.

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Studies on the Mode of Action of Vitamin K

It has been shown that puromycin and actinomycin D antagonize the effect of vitamin K in stimulating prothrombin synthesis in chicks deficient in vitamin K [R. E. Olson, *Can. J. Biochem.* **43**, 1565 (1965)]. Parenteral vitamin K_1 increases the prothrombin content of the blood linearly from 5 to 90 percent in 6 hours. This effect of a single dose of the vitamin (26 μg per 100 g of body weight) is maintained for 24 hours. Experiments were conducted to determine the effect of vitamin K_1 upon the biosynthesis of nuclear, ribosomal, and messenger RNA in chicks deficient in vitamin K. The $NaPH^{32}O_4$ and H^3-orotic acid in separate experiments were administered parenterally to deficient and vitamin K_1-treated chicks 6 and 20 hours after receiving the vitamin. The chicks were killed at various times after administration of the isotopic precursor.

Polysomes and nuclei were prepared from pooled livers of each group by sucrose fractionation. The RNA derived from polysomes and from nuclei was subjected to further sucrose-gradient centrifugation in order to determine the distribution of radioactivity among the various species of RNA. The results indicate that vitamin K-deficient chicks show a more rapid incorporation of P^{32} and orotic acid-H^3 into all fractions than chicks treated with vitamin K_1 show for the first 3 hours. In later periods, the chicks treated with vitamin K_1 show relatively better incorporation. The data suggest that the biosynthesis of nuclear and derivative cytoplasmic forms of RNA is increased in vitamin K deficiency and reduced by the administration of vitamin K_1. The magnitude of the effect is such as to suspect a hitherto unrecognized effect of vitamin K on RNA metabolism.

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Fusion of SV40 Tumor Cells and Indicator Cells

Infectious simian virus 40 (SV40) is not generally found in cells from tumors induced by this DNA virus; however, these cells do contain virus-related antigens suggesting that at least part of the SV40 genome persists. If the complete viral genome is present, but only part of it is expressed, then production of infectious SV40 might be induced by fusion of the tumor cells with indicator cells in which this virus normally undergoes productive replication.

Cell culture lines derived from two hamster tumors induced by SV40 were examined. These cells contained virus specific T (tumor) antigen, but not V (virion) antigen. Infectious SV40 has not been detected in cell-free preparations of their cultures. When the tumor cells were cocultured with indicator cells (strain BS-C-1), some of the cultures yielded infectious SV40 or produced V antigen indicative of virus synthesis. The proportion of cultures yielding infectious SV40 increased markedly when tumor cells were mixed with indicator cells and then exposed

to ultraviolet-inactivated para-influenza virus, type 3. This virus produced cell fusion among the different cell types resulting in large numbers of multinucleated giant cells.

Heterokaryons (containing nuclei of both indicator and tumor cells) were occasionally found in cocultured cells that had not been treated with para-influenza virus. In fused cocultures, about 50 percent of the multinucleated cells were heterokaryons. Twenty-four hours after fusion, heterokaryons frequently contained nuclei with and nuclei without T antigen, but with time they contained T antigen in all their nuclei. The V antigen, indicative of SV40 production, was seen in a few cells at 24 hours after fusion. These cells were frequently heterokaryons. Two to four days later with the second cycle of virus replication, V antigen was most commonly present in mononucleated indicator cells. The majority of the heterokaryons did not develop V antigen during 6 days after fusion, the period of observation.

The observations suggest that at least some of the tumor cells contained the whole SV40 genome, only a portion of which was expressed. Further, these experiments demonstrate the potential usefulness of virus-induced heterokaryons for detecting viral genome in malignant cells.

FRANCIS E. PAYNE
LUCAS DE VRIES
University of Michigan

Quantization of Circulation and Torque in Superfluid Helium

A torque should exist on a cylindrically symmetric, uniformly heated body immersed in superfluid helium if and only if there is trapped circulation about the body. The torque (per unit power input) will be quantized if the circulation is. For a heat input of 10 milliwatts, a single quantum of circulation should produce a torque of 5×10^{-7} dyne/cm, which is of measurable magnitude. The proposed experiment is a superfluid analog of the Deaver-Fairbank and Doll-Näbauer experiments, which demonstrated flux quantization in superconducting rings.

R. PENNEY
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Solar Abundance of Lead

Most determinations of the abundances of elements in the sun have depended on measurements of the total intensities of appropriate spectral lines (that is, the total energy subtracted from the continuous spectrum expressed in equivalent angstroms). If the spectral lines are weak and formed in crowded regions of the solar spectrum, blending can become very important and must be taken into account. Often an approximate procedure suffices, but if the element is important and is represented by only a few lines, it is preferable to use a method of spectrum synthesis in which the intensity distribution in the solar spectrum in the neighborhood of the line is reproduced by taking into account absorption produced by all relevant lines. One requires that the shape, as well as the total intensity of the line in question, be reproduced. In this way, otherwise unsuspected blends may be recognized.

The observational material consists of traces of the solar spectrum obtained with the vacuum spectrograph of the McMath Hulbert Observatory of the University of Michigan. Applications to the 3639 and 3683 lines of lead are illustrated. The procedure is tedious because of the large number of overlapping lines that must be taken into account. The 3639 line gives an unsatisfactory result in that it requires a line with an inadmissibly large damping constant. Presumably, this line is blended with an unknown contributor. The best result is given by λ 3683 which gives

$$\log N(\mathrm{Pb}) = 1.57$$
$$[\log N(\mathrm{hydrogen}) = 12.00]$$

with Helliwell's experimental f-values.

Supported by NASA.

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University of Michigan

Experiments with Charged Quantized Vortex Rings in Superfluid Liquid Helium

Experiments on charged, quantized vortex rings in superfluid liquid helium have now yielded information on the velocity and size of the rings as functions of energy, on the deflection of rings in external force fields, and on the interaction of the rings with vortex lines in rotating liquid helium and with other vortex rings. All data are consistent with the idea that the ring behaves like a classical ring in a perfect fluid, with circulation equal to Planck's constant divided by the mass of a helium atom and core radius about 1 Å. The binding energy of the charges to the rings is also known and reasonably well explained by hydrodynamic arguments. The presence of the charge allows easy detection of the rings, but does not seem to disturb their properties appreciably, except possibly for very small rings. One of the remaining puzzles concerns the processes involved when a charged particle creates a ring. We shall describe some recent experiments in which ring-ring interactions were studied as functions of energy, temperature, and sign of charge. At the lowest temperatures (near 0.3°K) the cross section is approximately geometrical and independent of the sign of the charge. At higher temperatures, other effects, such as ion-ring interactions, are seen.

T. M. SANDERS, JR.
G. GAMOTA
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Control Systems in Insect Development

During development, two kinds of control systems appear to regulate specific syntheses in cells. We have designated these systems *firm biases*—determination—and *transitory biases*. Firm biases, found only in multicellular organisms, arise during embryonic life, and biased cells pass on biases to their offspring. Thus, firm biases are reproduced. Transitory biases, found in both unicellular and multicellular organisms, are not transmitted by cells to their offspring and depend upon the continued presence of controlling agents.

These biases may be keys to differentiation and are useful ways to view development. Their analysis in molecular terms requires genetics, and, consequently, we have forsaken conventional embryological material in favor of *Drosophila*, the best known genetically of all animals. We use a method devised by Hadorn, in which we culture fragments of imaginal disks—or-

gan anlage—in adults and examine the developmental capacities of clones derived from these fragments. These clones maintain firm biases for hundreds of cell generations. They occasionally change spontaneously, but controlled change—reprogramming—has not yet been accomplished.

We have established clones from single anlage cells and are investigating their developmental capacities. We are also investigating clones derived from a new mutant that we discovered, called *lethal malignant brain tumor* (1(2)gl[4]), which has several attributes useful in analyzing control systems. Mutant imaginal disks grow phenomenally but no longer respond to metamorphosis hormones. Mutant brain cells grow rapidly and invasively, kill their hosts, and behave like malignant tumors. These cells have been cultured for 35 transfer generations, and clones have been derived from a single cell.

HOWARD A. SCHNEIDERMAN
ELISABETH GATEFF
Case Western Reserve University

Self-Administration of Psychoactive Drugs by the Monkey: A Measure of Psychological Dependence

The establishment of chronic patterns of behavior in monkeys involving the self-administration of psychoactive drugs is equated with, and used as a measure of, the development of psychological dependence upon these drugs.

The subjects used in these experiments are rhesus monkeys in which silastic catheters are permanently implanted through the jugular vein into the right atrium. The animals are housed in open-front cubicles in which they are restrained by a jointed arm that permits relatively free movement. Drug solutions are administered through the catheters by a motor-driven syringe when the monkey presses a lever.

As we have previously reported, cocaine, amphetamine, SPA (1,1,2-diphenyl-1-dimethyl-aminoethane) and fencamfamin [2-phenyl-3-ethylaminobicyclo-2(2,2,1)-heptane] injections are capable of maintaining high rates of self-administration behavior in the monkey. With all of these drugs, day-to-day variations in 24-hour drug intake is marked when free access is given to the drug. When unit dose is varied, the monkeys adjust responding to produce a maximum 24-hour drug intake that is essentially constant over a 10- to 20-fold range. With cocaine and SPA, day-to-day variation in drug intake is decreased by limiting drug access to 4 hours each day. When unit dose is varied, responses are adjusted to maintain a relatively constant drug intake. Pickens and Thompson (1967) have observed in the rat similar uniform drug-intake patterns with cocaine and amphetamine. The remarkable consistencies with which adjustments in drug intake are made to achieve a fairly uniform drug effect suggest the existence of a homeostatic mechanism for the regulation of affective states.

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Force and Momentum for Magnetized Bodies

In absolute cgs notation, the force on a current density $\mathbf{g}$ is taken as $\mathbf{g} \times \mathbf{B}/c$ rather than as $\mathbf{g} \times \mathbf{H}/c$. The electrons carrying a microscopic current density $\mathbf{g}_\mu$ move in a spatially fluctuating field $\mathbf{B}_\mu$ and experience an average field Av. (el.)$\mathbf{B}_\mu$ that may differ significantly from the unweighted spatial average Av. (Sp.)$\mathbf{B}_\mu$ which equals the macroscopic $\mathbf{B}$. It can be proved, as proposed by D. L. Webster [*Amer. J. Phys.* **14**, 360 (1946)] that the force and moment of force produced on a body carrying a current equals the volume integral of the force density expression

$$\mathbf{f}_B = (\mathbf{g} + \mathbf{g}_M) \times \mathbf{B}/c$$

where $\mathbf{g}_M = (c/4\pi) \nabla \times (\mathbf{B} - \mathbf{H})$. The proof also shows that equal force and moment integrals result from

$$\mathbf{f}_H = \mathrm{g} \times \mathbf{H}/c + (\nabla \cdot \mathbf{H})\mathbf{H}/4\pi.$$

and from the true microscopic force density involving $\mathbf{g}_\mu$. The classic Zeleny-Page force experiments [*Phys. Rev.* **24**, 544 (1924)] cannot therefore be regarded as independent evidence for the Amperian current model versus free magnetic pole model of magnetism. The necessary mathematical lemma is that any vector field $\mathbf{F}$ such as $\mathbf{H}$ or $\mathbf{B}_\mu$ that equals $\mathbf{B}$ on the closed surface surrounding the body gives a volume integral of $(\nabla \cdot \mathbf{F})\mathbf{F} + (\nabla \times \mathbf{F}) \times \mathbf{F}$ that equals that of $(\nabla \times \mathbf{B}) \times \mathbf{B}$. A similar surface integral theorem proves that the linear momentum of the radiation and matter contained within a bounding surface (that lies in vacuum) is given by integrating the macroscopic momentum density expression $\mathbf{E} \times \mathbf{H}/4\pi c$, no matter how $\mathbf{D}$ or $\mathbf{B}$ or $\mathbf{B}_\mu$ differ from $\mathbf{E}$ and $\mathbf{H}$, provided that (i) the macroscopic current $\mathbf{g} = (c/4\pi) \nabla \times \mathbf{H} = 0$, and (ii) steady-state conditions prevail. Two postulates needed for this proof are the relativistic mass-energy relationship and the existence of a mass-energy flow density, which must be divergenceless for the case of steady-state conditions.

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Life History Studies of Physoderma (Phycomycetes, Chytridiales)

A comprehensive study of the life histories of Physodermas from 16 different hosts, made possible by effecting germination of the resting spores, has been undertaken. These obligate parasites of vascular, mostly marsh, plants are known to possess two different and distinct thalli: (i) an epibiotic, thin-walled zoosporangial stage borne on an epidermal cell and producing many zoospores, and (ii) an endobiotic, elaborate, branching, rhizoidal thallus extending through many subepidermal host cells and bearing numerous turbinate cells and dark, thick-walled resting spores. The latter in our latitudes overwinter and germinate to produce zoospores.

We find that in certain species some resting-spore zoospores give rise to epibiotic sporangia and that others give rise to the endobiotic stage. One of these species may also produce the endobiotic, as well as new epibiotic, stages from epibiotic sporangial zoospores. Two other species apparently fail to form an epibiotic stage, the resting-spore zoospores developing directly into new endobiotic systems. In still another species, resting-spore zoospores form epibiotic sporangia almost exclusively on the juvenile underwater leaves of the host; the endobiotic stage appears confined to later-formed leaves with blades.

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In Situ Studies on Nitrogen Fixation with the Acetylene Reduction Technique

A simple field method for demonstrating *in situ* nitrogen fixation, based on the original discovery [Schöllhorn and Burris, *Fed. Proc.* **25**, 710 (1966); Dilworth, *Biochim. Biophys, Acta* **127**, 285 (1966)] that the nitrogen-fixing enzyme complex reduces acetylene to ethylene, has been devised. Studies that use this method have been conducted in three ecosystems: lakes, soils, and soils with symbiotic nitrogen-fixing plants present. In lakes, acetylene reduction is associated with the presence of heterocystous blue-green algae. Hourly variations in acetylene-reduction rates by algal blooms have been followed. The rates vary throughout the day and are correlated with the abundance of nitrogen-fixing algae rather than with total algal biomass. Reduction, which is light-dependent, was characteristic of surface waters (1 to 3 meters deep) and was not detected deeper.

In soil devoid of higher plants but with a covering of gelatinous blue-green algae (mainly *Nostoc* sp.), acetylene reduction occurs rapidly and is light-dependent. In well-established turf, a low rate of light-independent reduction occurs, whereas, in soils with a light grass cover, acetylene reduction was not detected. The addition of 2 percent sucrose did not stimulate acetylene reduction in these soils over a 4-year period.

In sandy soils supporting nodulated nonleguminous angiosperms (*Alnus* and *Comptonia*), acetylene reduction was readily detected, was characteristic of the root nodules only, and increased with time for 60 minutes. Samples of *Pinus* with associated mycorrhiza and soil reduced acetylene slowly.

The method employed is simple, has minimal equipment requirements (gas chromatograph to measure ethylene), is extremely sensitive (for example, in lakes, reduction was readily detected in 30 minutes in samples containing only 4 *Gloeotrichia* colonies, and reduction by *Alnus* and *Comptonia* nodules was easily detected in 2.5 minutes), is ideal for *in situ* studies and can replace the more expensive and less sensitive ^{15}N technique for many applications. Its validity as an index of N_2 fixation has been verified by direct comparison with fixation of $^{15}N_2$.

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A Structural Gene for Trehalase in Neurospora

Two trehalases have been found in strains of *Neurospora crassa* by means of acrylamide-gel electrophoresis. The fast-moving enzyme (f) is found in strain 89601A, and the slow-moving one (or ones) in strain 4121a. The peak of f-enzyme activity is eluted from a diethyl aminoethyl column (3.5 × 45 cm) after passage of 400 ml of buffer, whereas the peak of s-enzyme activity is reached after 900 ml. A comparison between the *p*H and temperature optima, substrate specificity, and Michaelis-Menten constants of the two enzymes reveals few differences. Both enzymes can be dissociated into three other enzymic forms which migrate more slowly than the native enzyme but which retain activity. Although two of these subunits of both enzymes migrate identically, the third migrates differently.

Crosses between strains 89601A and 4121a reveal that the f and s forms of trehalase segregate as expected of a single gene which is unlinked to the "inos" locus (linkage group V) and to mating type. Thus, a structural gene for trehalase has been found, and its position is being studied.

A positive correlation between the formation of macroconidia and trehalase activity has been found in this laboratory for *Neurospora*. We determined which form of the enzyme appears in strain Y8743mA ("peach") which forms macroconidia, strain Y8743m,LA ("peach-fluffy") which forms microconidia, and strain LA ("fluffy") which is aconidial. The f-enzyme is found in *peach* and *fluffy*, but the double mutant forms both f and s enzymes sequentially in that order. Thus, a form of complementation occurs in *peach, fluffy*, whose component mutant alleles lead to the formation of the s enzyme, which does not appear in either single mutant alone. The formation of the s form of trehalase is associated with microconidiation in *peach, fluffy*, but whether a causal connection exists is unclear.

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Acquisition of Sensory Capabilities

One approach to the study of sensory and perceptual behavior is that the human being processes the input data, choosing one of a set of alternatives he feels might have led to the input. A verbal report then is a description of the alternative he chooses, rather than of the physical properties of the input. To what extent is the discrimination between input elements, patterns of which furnish the basis for choice between input elements, patterns of which furnish the basis for choice between alternatives, dependent upon innate capabilities and to what extent is it dependent upon acquired capabilities? If these capabilities are acquired, then it should be possible for observers in experiments employing elements not previously experienced to acquire new capabilities.

A set of auditory signals containing physical differences not present in the usual auditory inputs were designed for experimental purposes. Three experiments are described, one employing signals which differed only in the time dimension, one in both time and frequency, and the third employing a correlated visual input presented with a probability of 0.5 with the signals involving both time and frequency differences. In each experiment, the observer chose from among four alternatives.

In every case, chance behavior was apparent over a large number of trials, in one case for 6000. With a monetary payoff and feedback, the performance improved until there was from 85 to 95 percent accuracy. The correlated visual input led to more rapid acquisition and probably to a slightly higher level of performance.

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Input-Output Relations of the Systems Responsible for a Reflex Behavior

The myotatic reflex (stretch input to a muscle and tension output from the same muscle) is largely responsible for the maintenance of posture. It can be viewed as a feedback control system which comprises the following events: (i) generation of impulse activity by stretch receptor organs, (ii) synaptic excitation of spinal motoneurons by the receptor output, and (iii) development of muscle tension resulting from the contraction of muscle fibers innervated by the activated motoneurons. The dynamic behavior of the systems responsible for these events were studied by

means of system analysis techniques. The main findings and conclusions are as follows.

1) Linear behavior of stretch receptor (primary endings of muscle spindle) is limited to small input amplitudes (50 μ for sinusoidal stretches not much above 1 to 2 hz), the percent modulation of firing rate increasing with increasing frequency modulation. A phase lead is present.

2) The decoding by spinal motoneurons of the above information (synaptic processes) as well as the subsequent reencoding into impulse activity (motoneuron output) does not introduce any significant dynamic changes. However, the ensemble average performed by motoneuron (convergence on each motoneuron of information from several receptors) somewhat increases the linearity range.

3) The dynamic relation between motoneuron activity and muscle tension is characterized by a decrease in tension with frequency and an increase in phase lag. These changes are absorbed by the increase in gain and phase lead that occurs at the receptor and motoneuron output with the consequent stabilization of the overall reflex.

4) Multiple units (motoneuron pool) handling similar information extend the linear range of dynamic behavior for it; the overall myotatic reflex is linear for input (stretch) amplitudes far in excess of those at which linear behavior ceases for single receptor and single motoneuron.

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Test for de novo Synthesis of Enzymes

The dramatic increase in level of a particular enzyme activity in response to time or to a change in cellular environment may result from activation of an enzyme precursor or from the *de novo* synthesis of the enzyme molecule. Before a detailed study of the regulation of the activity and function of such an enzyme can be properly initiated, it is necessary to determine unequivocally whether the increased enzyme activity represents activation or synthesis *de novo*. It is known that proteins can be fractionated according to buoyant density by isopycnic equilibrium sedimentation and that stable isotopes can be used to generate a density difference between preexisting and newly synthesized molecules (Hu, Bock, and Halvorson, 1962). We have shown, by the use of $H_2{}^{18}O$ to introduce the density label, that the gibberellin-induced increases in barley endosperm of amylase (Filner and Varner, in press) and of protease (Jacobsen and Varner, in press) represent *de novo* synthesis. We have also shown by the use of D_2O to introduce the density label and in combination with gel electrophoresis, that the density labeling and equilibrium sedimentation method can be used to study the appearance of individual peroxidase isozymes in the embryos of germinating barley (Anstine, Jacobsen, Scandalios, and Varner, unpublished).

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Glycolysis, Respiration, and Enzyme Alterations in Rat Liver Neoplasia

High aerobic glycolysis is perhaps the only characteristic biochemical expression of the neoplastic state, and though its significance remains controversial, its association with neoplasia has been unquestioned. However, the recent development by H. P. Morris of a series of chemically induced, transplantable rat liver neoplasms has profoundly altered our views concerning the generality of tumor glycolysis and, in addition, has provided a tool for a new depth of understanding of tumor biochemistry.

The Morris tumors exhibit a wide range of differentiation. In general, the well-differentiated tumors grow slowly, retain many enzymic and metabolic characteristics of normal liver, have a high respiration, and exhibit very low glycolytic activity. In contrast, the poorly differentiated tumors grow rapidly, lose the "liver marker" enzymes, and have moderate to low respiration and high aerobic glycolysis. A striking inverse relationship between glycolytic activity and capability for fatty acid oxidation indicates that the energy requirements of the low glycolyzing tumors are derived from fatty acid catabolism. Two major sites of enzymatic control of glycolysis are hexokinase and pyruvate kinase. Very low levels of the former limit glucose utilization in the well-differentiated tumors, and extremely high levels of the latter promote glycolysis in the poorly differentiated tumors, probably by competing with the respiratory system for the available adenosine diphosphate. Among other striking alterations with loss of differentiation of liver neoplasms are (i) a loss in regulatory capability characteristic of certain hepatic enzymes, and (ii) a replacement of certain enzymes involved in unique hepatic functions by a set of distinctly different enzyme proteins more closely related to those of nonhepatic tissues or fetal liver.

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Prolongation of Life in Mice with Established Friend Virus Leukemia by Inoculation with Sendai Virus, Interferon, or Statolon

The virus etiology of certain murine leukemias makes it reasonable to consider treatment of these diseases, and perhaps human leukemia as well, in terms of virus inhibition. In recent years, research on virus interference has been directed toward the role of interferon, a virus-inhibitory protein produced in cells in response to virus infection.

The DBA/2 mice are 100 percent susceptible to Friend leukemia virus and die of leukemia between 26 and 76 days after virus inoculation. Sendai virus (para-influenza 1) inoculated intraperitoneally into leukemic mice 30 days after Friend virus prolongs survival by 12 days.

The mechanism by which Sendai virus prolongs the life of leukemic mice probably involves interferon, since (i) Sendai virus induced large amounts of interferon; (ii) Statolon (an extract from the mold *Penicillium stoloniferum*), when injected 28 days after Friend virus, also induced large amounts of interferon and prolonged survival by 11 days; and (iii) interferon itself injected intraperitoneally daily for 10 days commencing 31 days after Friend virus prolonged survival by an average of 9 days. However, when interferon injections were continued daily until death of mice, survival was no longer than in mice which received only ten daily injections.

This study represents a successful application of interferon as a thera-

peutic agent in a virus-induced neoplastic disease. The development in mice of a state refractory to the leukemia-inhibiting effects of interferon after 10 days' administration indicates that interferon may not be effective in the long-term treatment of virus-induced leukemia; nevertheless, interferon, because of its short-term therapeutic efficacy, is an agent worthy of further investigation.

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Novel Reaction of a Nitro Sugar with Methanol

Although vigorous methods are normally required to methylate a carbohydrate, a nitro compound obtained by the reaction of nitromethane with periodate-oxidized methyl 4,6-*O*-benzylidene-α-D-glucopyranoside formed a dimethyl ether on long standing in methanolic-solution or on refluxing with methanol. That this substance did not contain methyl alcohol of crystallization was established by elementary analysis, by infrared absorption (absence of the hydroxyl bond), and by the fact that it could be sublimed unchanged under reduced pressure. A possible explanation of this finding will be presented.

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Determination of the Number of Active Ribosomes in Muscle: Effect of Diabetes and Insulin

Ribosomes from muscle of alloxan-diabetic rats catalyze the transfer of decreased amounts of radioactivity from ^{14}C-aminoacyl-transfer RNA to protein. Administration of small amounts of insulin (4 μg per animal) increases ribosomal activity in only 5 minutes. Thus, the isolated ribosomes accurately reflect the in vivo condition.

A fundamental problem that needed to be resolved was the character of the population of diabetic ribosomes. Are all the ribosomes half as efficient as those from normal animals, or are half the ribosomes fully active while the remainder are inactive? We used puromycin to obtain an answer to the question.

We reasoned that puromycin would inhibit protein synthesis, but that each active ribosome would make one peptide bond and the nascent protein (peptidyl-puromycin) would be released. If ^{3}H-labeled puromycin were used, the nascent peptide would be radioactive. From the radioactivity of the ^{3}H peptidyl-puromycin, we could then calculate the number of peptide bonds formed and hence the number of active ribosomes.

The ^{3}H peptidyl-puromycin formed by ribosomes was contained in the void volume after chromatography on Sephadex G-10 with 0.05*M* tris HCl, *p*H 7.6, in 8*M* urea and was well separated from the radioactive puromycin. Of the total population, 25 percent of the ribosomes from normal animals were active; diabetes reduced the number to 9 percent. Insulin administered to diabetic animals caused a parallel increase in the ability of the ribosomes to catalyze protein synthesis (by 125 percent in 5 minutes) and of the number of active particles (by 150 percent). The difference in the capacity of normal and diabetic ribosomes to synthesize protein is the result of the latter having a smaller proportion of particles capable of forming peptide bonds. The use of radioactive puromycin to determine the number of active ribosomes in a population should prove a valuable tool in analyzing the mechanism of regulation of protein synthesis.

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Significance of Sialic Acid in the Temperature-Dependent Property of Cryoglobulins

The cryoglobulin from a patient with idiopathic cryoglobulinemia was found to have two components, 19*S* and 7*S*, in the molecular proportions of 1 to 3. Neither one was a cryoglobulin by itself, but together they constituted a classical cryoglobulin. By amino acid analysis they did not differ from normal 19*S* and 7*S* globulins, but a study of their carbohydrate components revealed total absence of sialic acid from the 7*S* component. Dextrose, glucosamine, and fucose were present in normal amounts. The 19*S* component also formed a cryoprecipitate with immunoglobulin G (IgG), from which all sialic acid had been removed. Normal IgM failed to effect similar results; on the other hand, the patient's 19*S* component did not form a cryoprecipitate with IgG which still contained its sialic acid. These findings suggest that the patient's 19*S* globulin represents a specific antibody against IgG which has been rendered antigenic by lack of sialic acid.

A second cryoglobulin from a patient with multiple myeloma was found to be composed of a 7*S* and a 3.5*S* component. The 3.5*S* component was a cryoglobulin, even when isolated from the 7*S* globulin. The 7*S* globulin had normal amino acid and carbohydrate constituents and was antigenically an IgG. The 3.5*S* component had normal amino acid components but completely lacked sialic acid. Dextrose, glucosamine, and fucose were present in small amounts.

The above findings suggest that the abnormal absence of sialic acid from a globulin renders it antigenic and may provoke the elaboration of specific antibodies. It also alters its solubility, making it dependent on a temperature of 37°C at physiological *p*H.

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