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Isotope Fractionation by Photosynthetic Organisms

When inorganic carbon is converted into living matter, the light isotope of carbon tends to be preferentially fixed.

Is this isotope fractionation characteristic of all living matter? Are there any significant differences in isotope abundances among specific compounds and, if such differences exist, can they be related to known biochemical processes? In an effort to answer these questions, the isotopic composition of the carbon in amino acids isolated from cultures of a number of photosynthetic microorganisms grown in the laboratory has been investigated. The major effort has been devoted to the green alga, *Chlorella pyrenoidosa,* which can be grown on an inorganic medium with the sole source of carbon being the carbon dioxide fed to the system.

A typical experiment consists of (i) culturing the algae, (ii) hydrolyzing the protein formed, (iii) separating pure amino acids by ion-exchange chromatography, (iv) combusting a portion of the amino acids to carbon dioxide, (v) decarboxylating a portion of the amino acids with ninhydrin and purifying the liberated carbon dioxide, and (vi) performing an isotopic analysis on the carbon dioxide with the mass spectrometer.

Individual amino acids and specific carbons within a single substance were found to possess widely differing isotope ratios. Some values of C^{12}/C^{13} relative to input CO_2 are leucine $\delta = -24$ per mil; aspartic acid, $\delta = -6.6$; carboxyl carbons of asparatic acid, $\delta = +2$; arginine, $\delta = -19$; and guanidino carbon of arginine, $\delta = +6$.

Our data suggest that CO_2 is fixed in a number of ways by photosynthetic organisms, which gives rise to these differing isotopic fractionations.

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Morphological Variants of the Bacteriophage P_1

In studying the fine structures of various bacteriophages with E. Kellenberger, we discovered a case of morphological variation in the bacteriophage P_1. Such variations are of interest because they raise questions as to the way in which the synthesis and assembly of structural elements of bacteriophages are controlled in infected bacteria. P_1 is a large bacteriophage with a relatively massive contractile tail 2200 A long and 200 A in diameter. Stocks of P_1 prepared from single particles contain particles with polyhedral heads of two different diameters: 650 A and 900 A, respectively. The two kinds of particles have been separated by centrifugation in cesium chloride density gradients. The large-headed particles have higher densities than the small-headed particles. It is therefore likely that the large-headed particles contain a larger proportion of nucleic acid. The interest here is twofold: (i) what is the function (if any) of the extra nucleic acid, and (ii) how are the structural elements (capsomeres) arranged to make head membranes (capsids) of two different sizes?

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Reflexes from the Heart—with Special Reference to a New Cardioaccelerator Reflex

According to existing information, all reflexes from the heart serve to inhibit heart rate and are mediated via the afferent and efferent vagus. Increasing the pressures in the right atrium and left ventricle, as well as intracoronary injection of veratridine, cause reflex bradycardia. In anesthetized dogs, partial occlusion of the pulmonary artery causes increased pulmonary blood flow which is dependent on an intact sympathetic innervation of the heart. Neither the vagi nor the medullary centers participate in the increase in pulmonary blood flow. The most probable explanation is that the increase in pulmonary blood flow is due to reflex cardiac stimulation initiated by a rise in right ventricular systolic pressure and mediated entirely by the cardiac sympathetic nerves.

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Cheek Pouch of the Syrian Hamster and Tissue Transplantation Immunity

It is now generally accepted that grafts of skin and other tissues exchanged between unrelated members of the same species (*homografts*) are soon destroyed by their hosts as a consequence of an immunological reaction evoked by "foreign," genetically determined, isoantigens present in the grafts. Heterografts—those of which donor and recipient belong to *different* species—suffer an even more rapid rejection.

One well-known exception to this generalization is the acceptance of a variety of tissue homo- and heterografts implanted into the wall of the cheek pouches of Syrian hamsters (*Mesocricetus auratus*).

Experiments have been carried out to investigate the basis of this apparently privileged environment, using three inbred strains of hamster. Orthotopic skin homografts exchanged between members belonging to *different* strains are consistently rejected within 12 days. On the other hand, skin homografts transplanted to the cheek pouches survive and proliferate for a long time in "virgin" (that is, untreated) hosts. However, specific sensitization of the latter with orthotopic skin homografts before, or at any stage *after* implantation of homografts into their cheek pouches brings about destruction of the grafts. Thus, although incapable of sensitizing their hosts, homografts in the cheek pouch enjoy no exemption from a state of sensitization evoked independently.

It has also been found that if homografts of the "skin" constituting the wall of the cheek pouch are transplanted to recipient areas prepared in the integument, the majority live very much longer than homografts of normal body skin of much smaller size—indeed many survive indefinitely. Nevertheless, such grafts are fully susceptible to a state of sensitization elicited in their hosts by orthodox skin homografts or by donor leucocytes injected intravenously.

Further evidence has been obtained that the privileged status of the cheek pouch as a graft site and the uniqueness of cheek pouch "skin" homografts derive from properties of one of the ingredients—a layer of loosely packed areolar tissue.

This finding hints at the possible nature of mechanisms that may possibly prevent fetal mammals from sensitizing their mothers, and that may minimize the risk of occurrence of certain autoimmune diseases.

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Some Properties of Mitochondria from Cauliflower and Sweet Potatoes

The rate of oxygen utilization of many plant tissues is unaffected or even accelerated in the presence of cyanide or high partial pressure of carbon monoxide. Two hypotheses have been proposed in

the literature to explain the cyanide-insensitivity of such tissues. The first hypothesis suggests a pathway of electron transport to oxygen which is alternate to that provided by cytochrome oxidase. The second hypothesis is based on the assumption that there is an excess of oxidase compared with its substrate, cytochrome *c*. As yet there is insufficient evidence to allow a clear-cut decision between these two hypotheses.

In the experiments to be described a cyanide-sensitive tissue-cauliflower (*Brassica oleracea*) has been compared with a cyanide-insensitive one, sweet potato (*Ipomoea batatas*). In the presence of a suitable substrate the oxygen utilization of mitochondria prepared from both tissues is markedly influenced by adenosine diphosphate, the addition of which causes an increase of 5 to 10 times in the rate of oxygen utilization.

A careful study has been made of the respiratory capacities of these mitochondria. While no distinctive differences between the two types of mitochondria have been found, there are some marked characteristics that distinguish higher plant mitochondria from those derived from heartmuscle or liver. A study of the cytochrome components present in the mitochondria also shows no difference between the two types of plant mitochondria, but there are marked differences between the cytochromes present in plant and animal mitochondria.

The subtle difference between cyanide-sensitive and cyanide-insensitive plant tissues remains obscure in spite of the marked refinements that have been made in methods of mitochondrial preparation and in analytical methods.

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Virus Variants Obtained from Glycerinated Shope Papilloma

Shope papilloma virus prepared by alternate high and low speed centrifugation is usually thought to consist of fairly uniform particles. However, variations in size and density were observed in electron micrographs of our preparations, suggesting the presence of true structural variants. When such virus, prepared from glycerinated papilloma tissue, was suspended in cesium chloride solution having an initial specific gravity of 1.3070 and centrifuged at 35,000 rev/min for 24 hours, four distinct layers of opalescence appeared in the resulting density gradient. All four layers contained virus-like particles but only the lowest consistently produced papilloma on inoculation. This layer lost most of its activity when isolated and relayered, although initially active fractions were still active after storage for as much as 4 months in the cesium solution. The same four layers were obtained from different batches of papilloma of wild rabbits but not from glycerinated papilloma of domestic rabbits. The latter contain little or no virus. Suspensions of virus initially subjected to 100 freezings and thawings produced the same layers with corresponding infectivities. This stability, together with the consistency of isolation from different papillomas, suggests that the variants exist in the papilloma tissue of origin. The loss of infectivity of the lowest layer upon purification may possibly be due to separation from some necessary cofactor in the gradient.

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Use of Spider Monkeys (Ateles) in the Study of Gastric Secretion

Previous reports on the composition of gastric contents in monkeys have been confined almost entirely to *Macaca mulatta*. Material obtained by peroral intubation was found to have a *p*H above 3.5 in the fasting state. No acid response to histamine stimulation was obtained in some specimens. We have studied nine spider monkeys equipped with chronic gastric fistulae or innervated gastric pouches for periods up to 3 years. Fasting content from the fistula showed total acid concentrations of from 99–127 mEq/liter with the animal in its cage and less when restrained in a chair. The maximal total acid concentration after histamine stimulation was 130–141 mEq/liter, and after insulin hypoglycemia, 98–138 mEq/liter. Pepsin and electrolyte determinations were made also. An innervated gastric pouch was observed to secrete hydrogen ion at the rate of 0.4 mEq/hr during feeding. The cannulae of both fistulae and pouches were well tolerated and 24 hour collections with the animal in its cage or on a leash were obtained. The use of such animals in the study of nervous control of gastric secretion may have unique advantages including application to man.

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Conformations of Substituted Cyclopentanes. II. Ring D in the Steroids

While several authors have commented on the structure of *trans*-fused ring D in the steroids, no comprehensive structural study has been made. This report presents a conformational analysis which is consistent with recent experimental evidence relating to this five-membered ring system.

There are three important conformations to be considered consisting of two envelopes and one half-chair [Brutcher *et al., J. Am. Chem. Soc.* **81**, 4915 (1959)]. Envelope conformation I has C_{14} below the plane of C_{13}, C_{15}, C_{16}, C_{17}, while half-chair conformation II has C_{13} above and C_{14} below the C_{15}, C_{16}, C_{17} plane. An additional envelope conformation (III), hitherto neglected, has C_{13} above the plane of C_{14}, C_{15}, C_{16}, C_{17}. In particular, it is demonstrated through infrared techniques that 16-halo-17-keto steroids prefer envelope conformation I. This is in contrast to Shoppee's analysis of these haloketones. The conformations of steroids with other ring D substituents have been analyzed and will be discussed.

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Central Determination of Sensory Processes

Recent anatomical and physiological studies have implied that it is the diffuse reticular core of the central nervous system which exerts a vital tonic regulatory influence on the sensory systems, thereby modulating incoming sensory data in accordance with the central state of the organism or the behavioral significance of the stimulus.

We have obtained anatomical and electrophysiological evidence which reveals that there are direct neural pathways from the highest central level of sensory projection, the cerebral cortex, back to sensory relay stations of the somasthetic system. Observations of the electrical activity of individual second order nerve cells concerned with touch of body parts illustrate that these cells can be directly excited or blocked by neural activity generated in the same sensory cortical area to which these cells project. Spatial (somatotopic) relations are preserved throughout the system.

Such a system might operate so as to (i) refine the attributes of sensation, (ii) organize perception, (iii) form the early stage of mnemonic processes, (iv) set the receptive tone of the modality, or (v) initiate phenomena in the absence of peripheral stimuli.

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Selective Conversion in vitro of Phage-Specific Ribonucleic Acid to Deoxyribonucleotides

Uracil-2-C^{14} was incorporated into T6r$^+$-infected *Escherichia coli* strain B_{u-} to produce a ribonucleic acid (RNA) comprising 4 percent of the total RNA and mimicking the base composition of T6 deoxyribonucleic acid, rather than the bulk of *E. coli* RNA. The phage-specific RNA selectively disappears from extracts of infected cells. The fate of the labeled pyrimidine ribonucleotides was determined, using a system of analysis which could differentiate small amounts of labeled pyrimidine ribonucleotides, deoxyribonucleotides, and arabinonucleotides.

In the presence of exogenous reduced triphosphopyridine nucleotide (TPNH) there was a rapid synthesis of pyrimidine deoxyribonucleotides from the ribonucleotides of the specific RNA. At least half of the label of the RNA which disappeared was converted to deoxynucleotide. About 20 percent of the isotope of the RNA appeared as 3′-ribonucleotide, and the system also generated a small amount of free labeled uracil. It was demonstrated that the absence of exogenous TPNH, but not of DPNH, inhibited the production of deoxyribonucleotides by over 80 percent. In the absence of TPNH in the incubation mixture, 5′-CMP accumulated in the acid-soluble fraction. The formation of dCMP was shown to be correlated with the disappearance of preformed, or newly generated, 5′-CMP.

In the presence of an excess of unlabeled UTP and CTP, the formation of labeled deoxyribonucleotides from labeled phage-specific RNA was markedly depressed, although the release of 5′-CMP was not depressed. On the other hand, deoxyribonucleotides could be formed rapidly from labeled acid-soluble nucleotides, and the presence of an excess of unlabeled phage-specific RNA did not depress this appearance of labeled deoxy compounds. It was concluded that phage-specific RNA released 5′-nucleotides to the acid-soluble fraction. In the presence of TPNH these 5′-ribonucleotides were rapidly converted to deoxyribonucleotides.

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Binding of Reduced Diphosphopyridine Nucleotide to Yeast Alcohol Dehydrogenase According to Chemical Relaxation by Temperature Jumps

The binding of DPNH (reduced diphosphopyridine nucleotide) to various dehydrogenases is associated with an increase in the fluorescence of DPNH [H. Theorell, *Adv. Enzymology* **20**, 31 (1958); *Acta Chem. Scand.* **14**, 933 (1960)]. The binding to YADH (yeast alcohol dehydrogenase) appeared to be most suitable for the investigation with the temperature jump method. The chemical relaxation of this system is followed fluorometrically with a pulse oscilloscope (Tektronix 545). Two different equilibrium shifts with their relaxation times τ_1 have been established so far; a small shift with $\tau_1 \sim 100$ msec and a larger shift with $\tau_2 \sim 100$ μsec. The first is attributed to the association step:

$$\text{YADH} + \text{DPNH} \rightleftarrows \text{YADH, DPNH}$$

The exact kinetic relationship has not as yet been established as some fast equilibria are expected to be coupled to the above reaction:

$$\text{YADH} + \text{H}^+ \rightleftarrows \text{YADH}^+ \quad (1)$$
$$\text{YAD}^- + \text{H}^+ \rightleftarrows \text{YADH}^2 \quad (2)$$

where reaction 1 shows equilibria involving hydrogen bonding, and reaction 2 shows equilibria involving refolding of enzyme (—parts).

One might also have to take into account reactions involving the transition of excitation energies, including also inter- and intramolecular resonance energy transfers and singlet-triplet conversions. The fast relaxation time τ_2 has so far been attributed to some *p*H effect. Detailed investigations on both τ_1 and τ_2 are in progress.

Temperature jumps are produced by the discharge of a high-voltage capacitor through the solution containing the reactants [*Z. Elektrochem.* **64**, 78 (1960)]. The fluorescence is excited at 360 mμ from a BH6 Hg-arc and measured at right angles to the exciting beam at 410 mμ. The current at the photomultiplier anode reaches 1 ma (amplification = 200). The signal-to-noise ratio in actual measurements of $\tau_2 \sim 100$ μsec is 100 with an equilibrium shift of 5 percent of the signal at *p*H 6.5. Artifacts due to the high voltage discharge through the enzyme solution are negligible above about 10 μsec after its triggering.

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Comparative Study of Genetic Effects Induced by X-Rays, Ultraviolets and 2-Aminopurine

In the *cys. C.* region of the chromosome of *Salmonella typhimurium*, three types of changes have been detected, namely, single site, short deletions, and long deletions. Data will be presented showing that relative frequencies of these three types differ among the mutants of spontaneous origin and mutants induced by x-rays, ultraviolet radiation, or 2-aminopurine. This research was carried out under the auspices of the U.S. Atomic Energy Commission.

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Correlation of Physical and Chemical Events in Sympathetic Ganglia

Pre- and postsynaptic slow potentials arise spontaneously in rat sympathetic ganglia after infection with pseudorabies virus. In early infections postsynaptic slow potentials are derivatives of the presynaptic slow potentials. In advanced infections postsynaptic slow potentials, and not their derivatives, are similar to presynaptic slow potentials; that is, both potentials are identical. In intermediate infections the first wave of a postsynaptic burst of activity is a derivative of the corresponding presynaptic wave; the remaining presynaptic and postsynaptic waves in corresponding bursts are identical. It is believed that the spontaneous origin and characteristic formation of these potentials are the result of a progressive inactivation by the virus of inhibiting and differentiating mechanisms located in the presynaptic nerve endings. It is suggested that within sympathetic ganglia there are mechanisms capable of forming and transmitting a wide range of responses from the multitude of nerve impulses reaching it from the central nervous system. At one extreme would be the formation and transmission of a response which is a perfective derivative of the presynaptic potential; the other extreme would be where no differentiation occurs. The role of acetylcholine is to increase the frequency and amplitude of the presynaptic potential. Because physostigmine can change an early stage of infection into a late stage within a matter of seconds, it is suggested that cholinesterase is a part of the differentiating mechanism.

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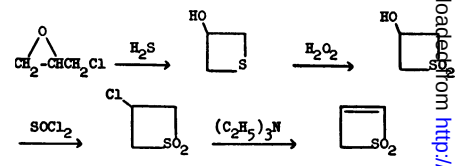
Synthesis and Reactions of Derivatives of Thietene and Thietane

The first synthesis of the previously unknown thietene sulfone has been accomplished by a four-step sequence.



Its structure has been confirmed by hydrogenation of the double bond (which gives the known thietane sulfone) and by its nuclear magnetic resonance spectrum. The NMR spectrum of thietene sulfone in deuterochloroform at 60 mc with tetramethylsilane as a standard shows sets of signals at 274 cy/sec for the methylene (CH_2) protons, at 408 cy/sec for the α-olefinic proton and 434 cy/sec for the β-olefinic proton. Therefore, the β-olefinic proton is least shielded from the applied magnetic field. This may be interpreted as indicating delocalization of the electrons in the system comprised of the carbon-carbon double bond and the sulfone group.

The infrared spectrum of thietene sulfone also is in agreement with the proposed structure and shows an unusually high C–H stretching frequency (3165 cm^{-1}) and an unusually low C=C stretching frequency (1543 cm^{-1}).

The carbon-carbon double bond is reactive towards various nucleophilic reagents. For example, dimethylamine and thietene sulfone give 3-dimethylaminothietane-1,1-dioxide. This is reduced by lithium aluminum hydride to 3-dimethylaminothietane. Pyrolysis of the quaternary ammonium hydroxide did not yield the unknown, four-membered, cyclic unsaturated sulfide, thietene.

Thietene sulfone gives a Diels-Alder adduct with anthracene which can be reduced with lithium aluminum hydride to the Diels-Alder adduct of thietene. Cracking this adduct gave anthracene and a nonvolatile oil, possibly a polymeric derivative of thietene.

The sulfone shows unusual behavior on reduction with lithium aluminum hydride. When the reduction was carried out at 45°C in a mixture of diethyl and di-*n*-butyl ethers, the only volatile product was

n-propyl mercaptan. No thietane was ever found. At 0 to 5°C the reduction was slow and the main product was still *n*-propyl mercaptan, but there were seven other components present (excluding solvent) as shown by gas chromatography. Sodium borohydride reduces the carbon-carbon double bond of thietene sulfone to give thietane sulfone.

When thietene sulfone is treated with aqueous sodium hydoxide, dimethyl sulfone is produced.

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Independent Biosynthesis of Hemin and Globin in Hemoglobin

Essential information has been lacking to permit a reliable interpretation of earlier findings on the relative labeling of the hemin and protein moieties of hemoglobin, with such agents as glycine-2-C^{14}. A major opinion has been that these disparate portions of the hemoglobin molecule are biosynthesized at the same rate and from the same amino acid pool. On the other hand, experimental data reported from our laboratory has supported the conclusion that there was a separation in the biosynthesis of the hemin and protein moieties of cytochrome *c*. The present work upon dog hemoglobin, labeled in vivo with glycine-2-C^{14}, supplies direct data upon the relative labeling per glycine-carbon in both hemin and globin. In most previous studies this necessary information has been derived by calculations, based upon questionable assumptions.

The amino acid spectrum of the globin of dog hemoglobin was determined quantitatively by the Moore and Stein technique. Relevant in the present connection there were 41.3 residues of glycine and 34.0 residues of serine in the protein molecule. The following labeling data were obtained: (i) The radioactivity in the globin was accounted for completely by that in glycine and serine. (ii) 58.9 percent of the globin radioactivity was present in the glycine. (iii) When the radioactivity values of the glycine and serine were adjusted for their relative molecular weights, the serine molecule had 90 percent of the activity of the glycine molecule, suggesting that these two amino acids had come to equilibrium quickly in the body pool or pools. (iv) The ratio of specific activities (counts per minute per milligram) of hemin to globin was 9.46, and that of counts per minute per mole of globin to hemin was 10.3. (v) The ratio of activities per glycine-carbon in globin to glycine-carbon in hemin was 1.18, a value probably significantly greater than unity. Since this ratio is not appreciably greater than unity, further work is in progress to fully establish its validity.

These findings do not lend support to the concept that in the biosynthesis of hemoglobin the hemin and protein moieties are made at the same rate from the same amino acid pool. They are consistent with the tentative viewpoint of an independence of hemin and globin biosynthesis in the construction of hemoglobin. They are also consistent with the possibility that the fabrication of the protein may be the rate limiting process in the synthesis of hemoglobin.

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Mechanisms of Gas Exchange and Oxygen Transport in Man

Rapid gas exchange between the pulmonary alveoli and the blood entering the pulmonary capillary bed has been recorded with a whole body plethysmograph to measure the volume of nitrous oxide absorbed per unit time after a single breath of 80 percent N_2O. The rate of gas absorption was proportional to the rate of blood flow, the gas concentration, and the solubility of the gas in blood. With this technique, pulmonary capillary blood flow was found to vary with the vascular pressure gradient during the cardiac cycle, flow lagging behind pressure by less than 0.1 second. The pressure-blood flow and pressure-blood volume characteristics were measured in isolated and perfused animal lungs. Abnormalities of these relationships were demonstrated in patients with pulmonary hypertension. The method yielded the same value for blood flow as that obtained by measuring the rate of oxygen transport from the lungs using the direct Fick method. The rate of pulmonary capillary blood flow also depends on the metabolic rate of the body. Therefore, a theoretical study was made of oxygen tension gradients within the tissues. The relationship between tissue oxygen tension and the rate of oxygen consumption, investigated by others, appears to indicate that oxygen tension at the site of utilization is much less than at the cell surface. A diffusion gradient between the cell surface and the site of utilization may exist if the radius of reaction is small, that is, if steady state reactions take place at small foci. Indirect evidence for this hypothesis has been adduced from the literature on chemical reaction rates at different oxygen tensions in whole cells, particulate fractions, and chemical extracts of cells. If it is true, such concentration gradients to and from small sites of reaction may explain the potential and osmotic gradients in cells.

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Role of Adipsia in Lateral Hypothalamic Starvation

Feeding and drinking behavior is being studied in rats with lateral hypothalamic lesions. For some time after surgery these animals neither eat nor drink and will die in the presence of food and water. In the original descriptions of this phenomenon the cessation of feeding (aphagia) was emphasized and the lateral hypothalamus was seen as the site of a "feeding center."

However, if offered a liquid diet, many of these animals will recover feeding behavior, regulate calories, and maintain body weight after operation, although at this same time they will not accept tap water (adipsia) or dry food. They can then be weaned to a nonnutritive saccharine solution and as their weight declines they inevitably begin to eat dry food. If the saccharine is removed and only tap water is offered, they do not drink, and they stop eating dry food and begin to starve again. Intragastric hydration restores dry food intake. These animals, therefore, do not eat because they do not drink. In this stage of the recovery from lateral hypothalamic damage, adipsia, not aphagia, is the deficit produced by the lesions.

However, adipsia does not account for the entire syndrome immediately after operation. Depending upon lesion size, aphagia of variable duration may occur postoperatively despite adequate hydration.

In summary, except for this variable postoperative aphagia, the failure to eat and drink that is seen in rats with lateral hypothalamic lesions is caused by a prolonged adipsia.

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Phyllotactic Constants in Growth of the Shoot Apex of Xanthium

Consideration of the mathematical models of phyllotaxis suggests that the spiral arrangement of leaf primordia at the shoot apex of many plants can best be specified by quoting (i) the *divergence angle* between successive primordia and (ii) the *plastochron ratio*, that is, the ratio of distance of successive primordia from the center of the apex. The plastochron ratio can be interpreted as a growth rate. A statistical method for estimating these constants has been devised and applied to transverse sections of shoot apices of *Xanthium*. The resulting divergence angle of 133.25° and plastrochon ratio of about 0.5 differ significantly from theoretical values which have been quoted for a 2:3 phyllotactic pattern.

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Efficiency of Active Chloride Transport by the Gastric Mucosa

The net active transport of chloride by the gastic mucosa is the sum of the electric current of chloride ions and the hydrochloric acid secretion. Bullfrog gastric mucosa was mounted between two chambers and current was passed to maintain the natural membrane potential at zero so that the mucosa maintained a current through zero external resistance.

Oxygen consumption was measured polarographically and Cl^{36} was used to measure unidirectional fluxes. The ratio of the short-circuit current of chloride ions to oxygen molecules consumed in 50 experiments was from 0.9 to 5.4. Two values were certainly above 4.0 (5.1 and 5.4), the electrochemical equivalent of oxygen. When the sum of hydrochloric acid and the short-circuit chloride current was compared to the oxygen consumed, 13 of 22 values of the ratios were 4.0 (maximum of 7.3). Direct measurements of net actively transported chloride, while the transmembrane potential was held at zero, similarly gave ratios significantly above 4.0. Since some oxygen must be used by the tissue for other purposes, these results demonstrate clearly the inadequacy of a simple redox pump hypothesis, with oxygen as the sole electron acceptor, for the active transport of chloride across the gastric mucosa.

It is interesting that SO_4^{--} Ringer solution abolished the chloride current but that the oxygen consumption did not fall. In NO_3^- Ringer solution a current quite similar to that produced by chloride was maintained for several hours.

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Studies of Oxidative Phosphorylation Using Submito-chondrial "Digitonin" Particles

A series of studies has been undertaken to compare the properties of the endogenous pyridine nucleotides of rat-liver mitochondria and submitochondrial particles derived by digitonin treatment (RLMD) (Lehninger) and to relate these to the reactions associated with the phosphorylation of adenosine diphosphate occurring concomitant with the oxidation of substrates. These reactions involve the fluorometric measurement of very low concentrations of pyridine nucleotide which have been carried out simultaneously with the polarographic determination of the rate of oxygen utilization.

The following propositions were considered concerning RLMD and will be discussed: (i) The stoichiometry of pyridine-nucleotide concentration to cytochrome content and its interpretation in terms of units or assemblies of respiratory chains. (ii) The functional activity of this pyridine nucleotide and its relationship to betahydroxybutyric dehydrogenase and succinic dehydrogenase as well as other residual dehydrogenases retained during the isolation procedure of RLMD. (iii) The possible involvement of reduced pyridine nucleotides in oxidative phosphorylation and the changes in extent of steady-state reduction occurring during the phosphorylation reaction and, (iv) The number of sites of phosphorylation operative during betahydroxybutyrate or succinate oxidation, or both.

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Nature of the Biochemical Changes in Adenovirus Infected Cells

Adenovirus infection of HeLa cells results in a marked increase in deoxyribonucleic acid as well as protein. It was demonstrated that infected cells contain two species of DNA: the normal host cell component (termed water-soluble DNA), and a fraction which accumulates subsequent to infection (termed saline-soluble DNA because its nucleoprotein complex is soluble in 0.15*M* NaCl). Investigation of the synthesis of DNA in type 4 adenovirus infected cells utilizing radioisotopes demonstrated that the synthesis of saline-soluble DNA but not water-soluble DNA was increased. Detailed study of the saline-soluble DNA indicated that it was synthesized *de novo*; that it was derived from precursors in the cell and from the media; that its synthesis commenced 2 to 3 hours before detectable infectious virus, and that it was probably a viral precursor. Nucleotide and base analyses of DNA from uninfected and infected cells showed the saline-soluble DNA from infected cells to be unique to the infected cell in that it did not have the characteristic base-pairing as did normal host cell DNA. The guanine was particularly increased in content, and isotopic investigations indicated an increased synthesis of this purine.

Ribonucleic acid was increased approximately 30 percent by infection, but the RNA was not of unusual structure. Protein of infected cells was increased about 100 percent and, with Wilcox, was shown to consist of three immunologically distinct antigens separable from the viral particle.

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Some Properties of a Protein Component of the Cell in Constant Migration between Nucleus and Cytoplasm

Studies on nucleocytoplasmic interactions suggest that sizable biological molecules show only unidirectional movement. It is believed, for example, that ribonucleic acid migrates only from nucleus to cytoplasm and that energy sources presumably move only from cytoplasm to nucleus. Consequently, it was a unique observation to find, in *Amoeba proteus*, a protein(s) which is in constant, *nonrandom*, back-and-forth migration between nucleus and cytoplasm.

This can be demonstrated by grafting a nucleus with radioactively labeled protein into a cell with a nucleus. Within 1 hour, one finds that the cytoplasm is lightly labeled but the nucleus of the recipient cell is very markedly labeled (as is, of course, the grafted nucleus). This most likely represents a movement of the protein into the cytoplasm followed by a rapid return to the nucleus.

Additional experiments demonstrate that: (i) during this cycle of activity the material must be at least a polypeptide; and (ii) it contains methionine, lysine, and tryptophan (the only amino acids thus far tested). The presence of tryptophan argues against its being a histone.

Its primary localization in the nucleus suggests the protein may be related to the cell's genetic material. To test this, an investigation was made of its metabolic stability. While in some instances the protein was stable for at least 9 days and several cell divisions, in other cases it broke down after only 3 to 4 days and one cell division. Although not definitive, the evidence suggests that this protein does not have the stability one expects of genetic material.

The possible role of this protein in the physiology of the cell will be discussed.

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Genetic Alteration of Adenylosuccinase in Salmonella typhimurium

Mutations occurring in the *ade-B* locus of *Salmonella typhimurium* lead to the loss of activity of adenylosuccinase, a bifunctional desuccinylating enzyme required for two reactions in the biosynthesis of adenylic acid. Complete or partial restoration of enzymic activity can be obtained in such mutants by a variety of genetic changes. One such alteration involves a nonallelic suppressor mutation which allows phenotypic expression of the wild-type in presence of the original mutant gene. The suppressor mutant grows in the absence of adenine at one-third the growth rate of the original wild-type, accumulates the substrate of the first blocked reaction and contains adenylosuccinase activity at level of 5 to 15 percent of the wild-type activity. Comparisons of the restored activity in the suppressor with that of the wild-type show no significant differences with respect to K_m, heat stability, effect of inhibitors, and competition for substrates. However, striking differences are found in activities of extracts which have been dialized following precipitation of nucleic acids with streptomycin, protamine, or $MnCl_2$. Whereas the wild-type enzyme survives this treatment with no loss of activity, the suppressor enzyme is completely and irreversibly inactivated. Glutathione cannot restore activity but can partially prevent its loss if present during dialysis. The suppressor enzyme also differs from the wild-type enzyme in its resistance to inactivation at *p*H 8.0 and by antibodies prepared against wild-type enzyme. These differences may indicate that the *ade-B* mutation leads to an altered protein whose enzymic inactivity can be partially activated by a suppressor mutation.

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Morphogenesis and Cell Wall Structure in Growing Algal Cells

Elongating roots, stems, and other cylindrical plant organs extend through the elongation of individual cylindrical cells. The elongation process is especially

easy to study in certain algae where the cells occur either singly or in simple configuration. The expansion process may be considered as the result of the yielding of the semi-solid outermost part of the cell, the cell wall, to the osmotic pressure developed in the large vacuole in the cell interior. By this model of growth, the highly polar (cylindrical) expansion of the cell must be a function of inequalities in the ability of the wall to yield in various directions. The wall is strong in the transverse direction; it is weak in the longitudinal direction. Strength is associated with the alignment of a great many crystalline cellulose microfibrils in the transverse direction inside the wall. During the elongation process new transverse microfibrils are added to the inner surface of the wall. Experiments with *Nitella* involving (i) the artificial cessation of expansion in part of an elongating cylinder, (ii) the virtual elimination of the transverse component of expansion, and (iii) the virtual elimination of the longitudinal component, indicate that alignment takes place along lines at right angles to the direction of the maximum surface expansion. When expansion is artificially stopped, the alignment becomes very poor. When either the transverse or the longitudinal component is restored, alignment is perpendicular to this component. Thus the alignment aspect of wall synthesis bears a perpendicular relation to the direction of maximum wall expansion at the time of synthesis.

PAUL H. GREEN
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Studies of Antibody Formation by Transfer of Lymph Node Cells

Studies of the site of synthesis of antibodies have indicated that they are produced chiefly in organs of the lymphatic system. More recently, it has been found that cells can be obtained from lymph nodes of animals injected with antigens and transferred to other (recipient) animals with the appearance in the serum of the recipients of antibodies to the antigen injected into the donor animals. In the studies to be reported here it has been found that lymph node cells can be obtained from uninjected donor animals, incubated in vitro with certain antigens and transferred to recipient animals, with the subsequent appearance of the corresponding antibody. In the in vitro incubation of lymph node cells with antigen it has been found that within 5 minutes of incubation enough of the antigen has been taken up by the cells to lead to maximal formation of antibody by these cells; and, with certain assumptions, it has been possible to estimate that at most a few hundred molecules of this antigen are taken up by each cell during this incubation.

This experimental situation, involving the transfer of cells among rabbits within a genetically heterogenous stock, has introduced immunologic reactions to antigens of the transferred cells themselves. The prior injection of donors' leukocytes to prospective recipients was found to stimulate an immunologic reaction against the donors' individual cell-antigens which led to the suppression of antibody formation by donors' lymph node cells subsequently transferred to these recipients. In sera of rabbits injected with leukocytes of other rabbits, it has been possible to demonstrate the presence of antibodies to rabbit cell antigens, since these sera can cause suppression of the synthesis of antibody by transferred lymph node cells.

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Role of Phosphorylase Activation in the Cardiovascular Action of Drugs

In the perfused rat heart it has been found that the increase in force of contraction produced by sympathomimetic amines is closely associated with an increase in the activity of phosphorylase *a*. Methyl xanthines, such as theophylline, in doses which produce cardiac stimulation also cause activation of phosphorylase.

In the intact rat, the level of activity of cardiac phosphorylase *a* varied widely depending on the prior treatment of the animal. Decapitation caused a marked increase, and anesthesia with pentobarbital or ether a decrease, in enzyme activity. Pretreatment of the animals with hexamethonium or bretylium, which by different mechanisms depress the sympathetic nervous system, caused a decrease in phosphorylase *a* activity in the heart. Administration of reserpine which has been shown to deplete the heart of catecholamines was also found to decrease the activity of phosphorylase *a*.

Finally, it was observed that in hearts perfused with Locke solution there was a rapid fall in phosphorylase *a* activity.

The experimental findings described above will be discussed in relation to the work of Sutherland, Rall, and co-workers on the action of drugs on the intracellular synthesis of cyclic 3′, 5′-adenylic acid.

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Recent Work at the National Radio Astronomy Observatory

The first observing instrument at the National Radio Astronomy Observatory, an 85-foot diameter radio telescope, has now been in operation about 18 months. The general research program of this instrument will be briefly outlined. Much of the work, by staff and visiting astronomers, is directed to the general problem of galactic structure and evolution, through observations of regions and objects of particular interest in our galaxy and of the discrete sources of radio emission that lie beyond the galaxy. The current status of two continuing programs in this area will be discussed. These two programs are concerned with the determination of precise positions of radio sources and the study of spectra of sources.

The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with the National Science Foundation.

D. S. HEESCHEN
National Radio Astronomy Observatory

Cross-reactions between Salmonella and Pneumococcus

Type-specificity in *Salmonella* has been shown by Westphal, Staub, and co-workers to be due largely to terminal nonreducing groups of 3, 6-dideoxy-sugars in the so-called O-lipopolysaccharide antigens in the cell walls of these microorganisms. In *Pneumococcus,* type-specificity derives from the unique structure and chemical composition of the extramural capsular polysaccharide of each type, and dideoxy-sugars have not yet been found. The three sugars, D-galactose, D-glucose, and L-rhamnose occur frequently in the type-specific and group-specific sequences of *Salmonella* and in the capsular substances of *Pneumococcus*, so that cross-reactions of precipitation or agglutination between a member of one of these two large groups of pathogens with a strain of the other group would not be surprising if one or more of these sugars should occur multiply and similarly linked in the determinant antigens of both. Several instances of such cross-reactivity are recorded in the present study, in addition to the few already noted, and are discussed in terms of the incomplete present chemical knowledge of the polysaccharides involved.

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Persistent Viral Infections

The majority of viral infections of man or animal remain silent. Furthermore, many viruses may persist in the host after recovery from illness. Several factors conceivably control these states of latency, but their pertinence and importance cannot readily be assessed in intact hosts. Cell cultures offer a simplified system for study of latency, and many examples of persistent viral infections in cell populations have been recorded [H. S. Ginsberg, *Progr. med. Viol.* **1**, 36 (1958)]. Some of these require antiviral sera, inhibitors, or changes in cultural conditions. In others, interference and interferon production limit viral reproduction, as shown by my associates and myself [Henle, *et al., J. Exptl. Med.* **108**, 537 (1958); Bergs, *et al., ibid.*, 561; Deinhardt, *et al., ibid.*, 573; Henle, *et al., ibid.* **110**, 525 (1959)].

On inoculation of MCN cultures with Newcastle disease virus (NDV) maximally 30 percent of the cells became infected but the incidence rapidly decreased and was maintained at about 1 percent for years. Yet, infected lines showed reduced

growth rates, increased aerobic glycolysis, and resistance to superinfection with other viruses. Cures by anti-NDV serum were rare but cloning under antibody yielded virus-free populations indistinguishable from cloned parent cells.

Infected cells contained only one infectious unit at a time. On transfer of a few to uninfected cultures the infection spread slowly with doubling of virus every 6 to 8 hours until about 1 percent of the cells became infected. At this stage, all remaining cells resisted superinfection. A component other than virus was produced which induced resistance and resembled interferon [A. Isaacs and B. W. Lacey, Eds., *Virus Growth and Variation* (Cambridge Univ. Press, London, 1959) pp. 102-121].

Evidently MCN cells vary in competence, which is not genetically controlled. Interferon, produced by NDV infection, reduces the number of competent cells. Since interferon protection is transitory, interferon and virus compete for cells regaining competence. If the virus wins, more interferon is produced, in turn; when protection wears off, more virus can be replicated. Thus, interferon and virus are kept in balance and both virus and cells persist in culture.

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Loss of Phenotypic Traits by Differentiated Cells in vitro

Do the cultured progeny of differentiated tissue cells inherit the unique somatic traits of their parental cells much as bacteria inherit genes determining constitutive or inducible enzymes or as paramecia inherit genes determining serotypes?

An unambiguous approach to this problem requires that; (i) the initial population of differentiated cells be homogeneous, and (ii) all the differentiated cells divide during the experiment. These conditions are satisfied by liberating chondrocytes from the matrix of embryonic cartilage (by means of trypsin), rearing them in culture for varying periods of time, and then testing the progeny of the chondrocytes for their capacity to differentiate into cartilage cells.

Freshly liberated chondrocytes immediately organ-cultured (that is, before they divide mitotically) proceed to form new matrix. Over 98 percent of these cells synthesize chondroitin sulfate and incorporate sulfur-35 into ester bound sulfate. After being cultured as a monolayer for 8 days (generation time 30 hours), the progeny of chondrocytes when organ-cultured or grafted to the coeloem (i) fail to differentiate into chondrocytes, (ii) fail to synthesize chondroitin sulfate, and (iii) fail to incorporate sulfur-35 into ester bound sulfate.

These results suggest that (i) the differentiated state of a cultured tissue cell may not survive rapid multiple mitotic divisions and that (ii) the differentiated state of a cultured tissue cell is not exclusively (primarily?) a function of the genetic constitution of the cell's nucleus.

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New Experiments on Interactions of Electrons with Quantized Lattice Vibrations (Phonons)

Interactions of electrons with phonons have been investigated on samples which allow the process to be studied in the classical configuration of source, propagation medium, and receiver [see also K. Hubner and W. Shockley, *Phys. Rev. Letters* **4**, 10, 504 (1960)]. This is accomplished with very thin plates of single crystal silicon, into which phosphorus has been diffused in order to render the regions close to the surface electron conductive. (Such procedures are common practice in the semiconductor industry.) The resulting structure is like a three-layer sandwich with the two outer layers containing free electrons capable of carrying current and the middle layer forming an electrically isolating barrier between them. An electric field is applied along one of the outer layers, and the resulting flow of electrons disturbs the equilibrium distribution of the phonons due to electron-lattice interactions. This disturbance propagates through the sample and in turn acts upon the electrons in the other outer layer, resulting in a measurable induced electric field. The approximate range of the effect is an average mean path of the phonons, although some effect is detected out to 1 mm at 77°K, about 10 mean free paths. Systematic measurements of the induced field and its dependence on thickness and chemical purity of the middle layer yield interesting information on propagation of atomic vibrations in silicon. In addition, this effect might have practical applications, including possibly a direct current transformer.

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Orientation of Cell Growth by Polarized Radiation

Four years ago it was reported that zygotes of the intertidal "rock weed," *Fucus*, germinate parallel to the vibration plane after exposure to plane polarized visible light [L. Jaffe, *Science* **123**, 1081 (1956)]. Further study led to the conclusion that this "polarotropic" response is a variant of the general phenomenon of phototropism and is mediated by a periclinal orientation of dichroic photoreceptor molecules; that is, the axes of maximum molecular absorption lie parallel to the nearby surface of the zygote [L. Jaffe, *Exptl. Cell Research* **15**, 282 (1958)]. Similar polarotropic responses were also discovered in the spores of a moss, a fern, and the imperfect fungus, *Botrytis* [E. Bünning and H. Etzold, *Ber. deut. botan. Ges.* **71**, 304 (1958)].

In this paper, we report proof that the polarotropic response of *Botrytis* spores is also a variant of phototropism mediated by an orientation of dichroic photoreceptor molecules with respect to the nearby surface. However, in this case it is shown that the axes of maximum absorption of these molecules, unlike those in *Fucus*, are anticlinal; that is, they are perpendicular to the nearby surface. We also report a somewhat less rigorous analysis of the polarotropic responses of the spores of the fern, *Osmunda*, indicating periclinal photoreceptors similar to those in *Fucus*. Again, we report a preliminary study of the polarotropic responses of the spores of the moss, *Funaria*. This indicates a situation which is anomalous not only in a high responsiveness to red light, but also in the presence of at least two different tropic photoreceptor molecules, one of which is *un*oriented while the other is anticlinal. Finally, we report a qualitatively new aspect of the tropic responses of *Botrytis* spores to light, the phenomenon of *centering*.

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Role of Disulfide Pairing in the Biosynthesis of Antibody

The demonstration that the integrity of the disulfide bonds of rabbit antibody is essential for the maintenance of its immunologic specificity has directed our attention to the possible significance of disulfide formation during the biosynthesis of antibody. The relatively high cystine content of γ-globulin in general and of the immunologically active fragments from papain-treated rabbit antibody in particular means that the number of ways of pairing of half-cystines may be very large. A statistical calculation based on the assumption of variable pairing shows, for example, that there are 1.3×10^6 patterns of pairing for the formation of only four disulfides from 16 SH groups. The concept of variable pairing thus provides adequate scope for the broad spectrum of immunologic specificity. It is proposed that the nature of the antigenic group governs the particular pattern of pairing of the corresponding antibody and that this pattern is compatible with and serves to stabilize that conformation of the combining region which is complementary to the structure of the antigenic determinant. In addition to its possible immunological relevance, the notion of variable pairing provides a molecular basis for the explanation of the physical heterogeneity of γ-globulins.

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Problem of Specificity of Nuclear Differentiation in Rana pipiens

Previous work has shown that the majority of test embryos derived from late gastrula endoderm nuclear transfers exhibit a pattern of deficiencies consistent with the origin of the donor nuclei. In others the developmental deficiencies do not appear to conform to any specific pattern. To test whether these nuclear changes are *specific* for endoderm two additional types of analyses were carried out. In the first, test blastulae and gastrulae were joined in parabiosis with gastrula hosts obtained from normally fertilized eggs. In the second, at the time the parabiotic combinations were performed, chromosome preparations of the experimental embryos were made.

Since the deficient differentiation of the nuclear transplant embryos was not improved by parabiotic union with normal hosts, the nuclear condition responsible for these deficiencies appears to be intrinsic. The chromosomal analysis showed that embryos which *do not* conform to the endoderm pattern of deficiencies are aneuploid and therefore do not provide a valid test of the properties of the nuclei prior to transplantation. The valid cases for the significance of nuclear changes that accompany embryonic development are those in which the chromosome complement remains euploid following nuclear transfer, and these consistently exhibit the "endoderm" pattern of deficiencies. These results strengthen the case for specificity of nuclear differentiation but do not yet provide the final proof.

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A Hypothesis of the Pre- and Postsynaptic Sites of Action of Acetylcholine in Neurohumoral Transmission

As a neurohumoral transmitter, acetylcholine (ACh) is liberated by the axonal terminals of cholinergic fibers and activates the postsynaptic neuron. In certain non-neuronal tissues (cilia, smooth and cardiac muscle), ACh acts as a "local hormone," producing its effects on the same cells from which it is liberated. Several findings from our own and other laboratories are best explained by the assumption that ACh acts in both these capacities at cholinergic and certain noncholinergic synaptic sites.

Cholinergic neurons contain high concentrations of acetylcholinesterase throughout their entire lengths; in adrenergic neurons the enzyme is scarcely detectable; concentrations are intermediate in others (for example, primary afferent neurons), the transmitters of which are unknown. Volle has found recently that intra-arterially injected ACh and carbamylcholine in the cat superior cervical ganglion probably activate presynaptic terminals, causing them to liberate ACh at the site of transmission. By DiCastro's procedure, Matsumura has established functional reinnervation of the cat superior cervical ganglion by the afferent vagal neurons and has obtained pharmacological and histochemical evidence that they liberate ACh in limited amounts. Our finding of acetylcholinesterase in the terminals of the cholinergically controlled hypothalamiconeurohypophyseal fibers, and the recent report from De Robertis' laboratory [*Endocrinol.* **66**, 741 (1960)] that these terminals contain two distinct populations of vesicles indicates that ACh is released, and in turn releases the hormones from the same terminals. The report of Burn and Rand [*Brit. J. Pharmacol.* **15**, 56 (1960)] of cholinergic involvement in the release of catecholamines by adrenergic fibers can be explained similarly.

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An Image Intensifier System for the Study of Rare Decay Modes of Elementary Particles

Filamentary chamber-image intensifier systems have been developed which yield pictures of charged particle tracks limited in spatial resolution only by the unit filament size, and which exhibit a time resolution of about 1 μsec. This device, used in conjunction with auxiliary particle counters, is well suited to the study of rare decay modes of elementary particles such as, for example, π- and K-mesons. The method employs a filamentary chamber divided into two or more regions. In one of the regions the incident mesons stop and subsequently decay. The other regions are traversed by the decay products. One face of the chamber is viewed by the image intensifier system and the opposite face is viewed by photomultiplier tubes, one for each of the separate chamber regions. It is required to trigger the image intensifier system that a counter telescope, including one of the chamber photomultipliers, indicates that a meson has stopped in the proper region, and also that appropriate delayed coincidences obtain between that stopping event and pulses from the other chamber photomultipliers which indicate the passage of a decay particle. Under these conditions the event is photographed and, in addition, the time sequence of the several counter outputs is available for recording. The system is capable of utilizing a large incident meson current and accepts decay product particles over a large solid angle. The counter selection procedure limits the number of photographs necessary to observe a given decay mode and facilitates the extraction of useful data from the photographs that are taken.

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Detection Thresholds: a Problem Reconsidered

W. P. Tanner and his collaborators have argued against thresholds playing a role in the detection of stimuli in a background of noise that makes them barely perceptible. Plots of the conditional probability of saying a stimulus is present when it is versus the probability of the same response when it is not are nonlinear as predicted by their threshold-free detection theory. By contrast, their version of a threshold model predicts it to be a line segment through (1,1), which is clearly rejected by the data. Two peculiarities of this model are pointed out. An alternative is suggested that overcomes them and that predicts the function to consist of a segment from (0,0) to (p,p') and another from (p,p') to (1,1). When compared with published data, this is as adequate as the prediction from the threshold-free model.

Consider now an experiment in which either noise alone, noise plus a tone of one frequency, or noise plus a tone of a different frequency is presented on a trial; the subject is required both to detect and to identify on each trial. If there is no detection threshold, the undetected responses should therefore exhibit differential identification; whereas, if there is a threshold, this would not be anticipated. Data from Elizabeth Shipley's thesis are presented which show no differential identification among the undetected responses, suggesting, but not proving, that detection thresholds exist.

The significance for psychophysical theory of this, as yet unresolved, question of thresholds is discussed.

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On Bird Species Diversity

The number of breeding bird species varies from habitat to habitat and from latitude to latitude. To understand the causes of this change, both bird censuses and a series of habitat diversity measures were taken in a wide variety of habitats and latitudes. Partial regression shows that (i) addition of new horizontal layers of vegetation increases bird species diversity, (ii) the three horizontal layers (0 to 2, 2 to 25, and over 25 feet) are about equally effective in controlling the number of bird species, and (iii) plant species diversity has no effect on bird species diversity except insofar as it affects foliage height diversity mentioned in (i).

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Preparation and Properties of Some New Derivatives of Silane and Disilane

Since silicon falls immediately below carbon in the periodic table, it is of interest to compare the properties of simple

silicon compounds with those of their carbon analogs. Substances containing $-SiH_3$ or $-SiH_2SiH_3$ groups may therefore be considered the silicon analogs of methyl and ethyl compounds respectively. Species containing these groups have been prepared and characterized in order to determine the effect of d_π-p_π bonding on their physical and chemical properties.

The synthesis of Si_2H_6 and of its partly substituted derivatives such as $CH_3SiH_2SiH_2CH_3$ has been carried out by means of a Wurtz-type reaction. Derivatives of Si_2H_6 such as SiH_3SiH_2I, $(SiH_3SiH_2)_2O$, $(SiH_3SiH_2)_2S$, $(SiH_3SiH_2)_3N$, and $(SiH_3SiH_2)_2NCH_3$ have been prepared, in addition to the completely methylated species $Si(CH_3)_3Si(CH_3)_2OH$, $[Si(CH_3)_3Si(CH_3)_2]_2O$, and $(CH_3)_3Si\text{-}O\text{-}Si(CH_3)_2\text{-}O\text{-}Si(CH_3)_2\text{-}Si(CH_3)_3$.

The compounds $SiH_3\text{-}O\text{-}CH_3$ and $SiH_3\text{-}S\text{-}CH_3$ have been synthesized from $[SiH_3N(CH_3)_3]I$ and CH_3OH or CH_3SH respectively. The base strength of $SiH_3\text{-}O\text{-}CH_3$ relative to $(SiH_3)_2O$ and $(CH_3)_2O$ has been investigated by means of its reaction with BF_3 and B_2H_6.

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The Growth Fraction: A New Concept Applied to Tumors

The possibility exists that, like the normal tissues from whence they arose, various tumors are mixtures of proliferating and nonproliferating cells. The Growth Fraction (G.F.) is suggested as an index of such mixtures and is defined as the ratio of proliferating cells (C_p) to total cells (C_t).

Mitotic figures represent a sample containing only proliferating cells. However, the bulk of proliferating cells are in interphase and as such are not distinguishable from nonproliferating cells. With very few exceptions, a second reliable indication of mitotic activity is the incorporation of thymidine into the nuclei of cells. After a brief exposure to tritiated thymidine and the subsequent passage of enough time to permit the cells to randomly distribute themselves in the mitotic cycle, the labeled cells can be identified on an autoradiograph. Within certain limits the following relationships should then apply (labels are marked with an asterisk):

$$\text{mitoses}^*/\text{mitoses} = C_p^*/C_p$$
$$\text{cells}^*/\text{cells} = C_p^*/C_t$$
$$\frac{\text{cells}^*/\text{cells}}{\text{mitoses}^*/\text{mitoses}} = \frac{C_p}{C_t} = \text{G.F.}$$

This formulation of Growth Fraction is potentially vulnerable to a number of technical and theoretical aberrations A sizable traffic of cells between the two populations would confuse the issue, and the assumption that the tumor can be represented by only two cell populations may be an oversimplification. Nevertheless the method as applied to spontaneous breast tumors growing in vivo in the C3H mouse indicates that the derived Growth Fraction is reasonably stable in any one tumor between the second and ninth days after injection of tritiated thymidine. In addition, a series of 14 tumors measured on the 5th day give a mean Growth Fraction of 0.61 (standard deviation of the mean, 0.029). Further studies attempting to confirm these results by an independent method are in progress.

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Protein Uptake by Pinocytosis in Amoebae

The uptake of macromolecular substances has been studied chiefly in free-living amoebae, but many observations on other cell types by electron microscopy suggest that the process is of wider significance. In amoebae, proteins which induce pinocytosis have been shown to be bound first to the cell surface before being taken up in vesicles. The mechanism of this initial binding reaction was studied by comparing the *p*H dependence of binding for two closely related proteins, ferritin and methylated ferritin. Ferritin behaves as a typical ampholyte, isoelectric at *p*H 4.4; methylated ferritin, in which carboxyl groups are blocked by esterification, is isoelectric at *p*H 6 to 7. Observations on living amoebae, confirmed by electron microscopy, demonstrated that binding depends upon net charge, the proteins being bound in the cationic form. The receptor substance on the cell surface, which other evidence suggested might be a mucopolysaccharide, was studied. Mass cultures of amoebae yielded after tryptic digestion a metachromatic, acidic polysaccharide.

Ferritin and methylated ferritin were also used for a study by electron microscopy of the changes which occur within the pinocytosis vesicle after uptake. The results show that there is no gross breakdown in the structure or function of the vesicle membrane: neither ferritin nor methylated ferritin particles escape through the membrane or pass into the microvesicles which are formed in great numbers from the primary vesicle. The evidence from this and other studies indicates that selective exchange mechanisms operate in both directions, between the vesicle and the surrounding cytoplasm. This conclusion leads to a clearer view of the relationship between pinocytosis and active transport at the molecular level.

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Hypothalamic Regulation of Luteinizing Hormone Secretion

Ovarian ascorbic acid depletion in rats pretreated with gonadotrophins was used as the assay for luteinizing hormone (LH). Mere insertion of an electrode into the median eminence was found to evoke hypophysial LH release, whereas passage of the electrode into the posterior hypothalamus had no effect. Lesions in the median eminence and in the suprachiasmatic region (the former inducing persistent diestrus and the latter constant vaginal estrus) prevented the rise in plasma LH which followed ovariectomy and were associated with subnormal values for hypophysial LH content. The rise in pituitary LH content which follows ovariectomy was prevented by the median eminence lesions but still occurred after suprachiasmatic lesions. Acid extracts of median eminence tissue from rat and rabbit evoked ascorbic acid depletion in the assay rats. Experiments with hypophysectomized assay rats showed that at least part of this activity of the extracts was caused by LH secretion. The activity could not be accounted for by the content of known pharmacological agents in the extract, such as vasopressin, oxytocin, histamine, serotonin, epinephrine, or substance P. The nature of the active material is unknown. Extracts from cerebral cortex were devoid of activity. It is suggested that a humoral agent, designated LH-releasing factor, secreted by the median eminence, regulates the secretion of LH by the adenohypophysis.

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Role of Corpus Callosum in Transfer of Visual Discriminations in the Cat

In the normal cat, visual discriminations learned with one eye are immediately transferred to the other eye by certain midline commissures passing between the two halves of the brain. The cat's visual apparatus includes a number of these midline commissures, such as the optic chiasm, the corpus callosum, and the posterior commissure. Sperry and his associates have shown that if both the optic chiasm and the corpus callosum are mid-sagittally sectioned in the cat, pattern discriminations fail to transfer from one eye to the other and the cat must relearn the pattern discrimination with the initially untrained eye. Complete transfer occurs between the eyes, however, if only one of these commissures is sectioned.

In testing brightness discrimination transfer, we have demonstrated that, in cats with the optic chiasm and the corpus callosum sectioned, simple suprathreshold brightness discriminations will transfer completely from one eye to the other as in normal cats. However, near-threshold brightness discriminations fail to transfer from one eye to the other as pattern discriminations also fail to transfer.

So far as tested, visual functions dependent upon the visual cortex (pattern and threshold brightness discriminations) require the corpus callosum for transfer from eye to eye when the optic chiasm has been sectioned; visual functions not dependent upon the visual cortex (suprathreshold brightness discriminations) do not require the corpus callosum for transfer.

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Initiation of Enzyme Formation by Birth

An earlier study of glycogen metabolism in mammalian liver unexpectedly revealed that glucose-6-phosphatase is absent in the fetus. Further examination of the liver showed that this enzyme appears first at term, increasing rapidly to adult levels immediately after birth. It seemed possible that all enzymes unique to liver and subserving special liver functions would have this same pattern of development in the mammal. Subsequent work in our laboratory and elsewhere has supported this idea.

We are now attempting to determine the factors initiating the formation of the unique liver enzymes after birth. Tryptophan pyrrolase was chosen as the subject of study. First, compounds known to increase enzyme activity in the adult liver such as substrate and adrenal cortical hormones were tested in the fetus. None of these compounds were able to stimulate enzyme formation in fetal liver and one would suppose they do not limit enzyme formation during fetal life. Secondly, the effect of birth and maturity on enzyme formation was studied by varying the gestation period. Tryptophan pyrrolase was studied in the rabbit, a species in which the gestation time can be shortened or lengthened by several days without interfering with growth or morphological development. Premature delivery resulted in an immediate and rapid increase in enzyme activity. Prolongation of the gestation time prevented enzyme formation until after delivery. Therefore, it would seem that some factor in the uterine environment represses formation of the unique liver enzymes.

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A Minute Chromosome in Human Chronic Granulocytic Leukemia

In seven cases thus far investigated (five males, two females), a minute chromosome has been observed replacing one of the four smallest autosomes in the chromosome complement of cells of chronic granulocytic leukemia cultured from peripheral blood. No abnormality was observed in the cells of four cases of *acute* granulocytic leukemia in adults or of six cases of acute leukemia in children. There have been several recent reports of chromosome abnormalities in a number of cases of human leukemia [including two of the seven cases reported here: Nowell and Hungerford, *J. Natl. Cancer Inst.* **25**, 85 (1960)], but no series has appeared in which there was a consistent change typical of a particular type of leukemia.

Cells of the five new cases were obtained from peripheral blood (and bone marrow in one instance), grown in culture for 24–72 hours, and processed for cytological examination by a recently developed air-drying technique (Moorhead, *et al., Exptl. Cell Research*, in press). The patients varied from asymptomatic untreated cases to extensively treated cases of several years' duration in terminal myeloblastic crisis. All seven individuals showed a similar minute chromosome, and none showed any other frequent or regular chromosome change. In most of the cases, cells with normal chromosomes were also observed. Thus, the minute is not a part of the normal chromosome constitution of such individuals.

The findings suggest a causal relationship between the chromosome abnormality observed and chronic granulocytic leukemia.

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DAVID A. HUNGERFORD
Institute for Cancer Research

Cytoplasmic Ribonucleic Acid Which Is Nucleolar and Nuclear Dependent and Its Relation to Amino Acid Incorporation

A comparative study has been made of the kinetics of nucleoside incorporation into ribonucleic acid of the nucleolus, extranucleolar portions of the nucleus, and cytoplasm of normal HeLa cells and cells in which the nucleolus is inactivated by means of localized ultraviolet microirradiation. Based on these studies, a model for RNA synthesis in actively growing cells is proposed in which (i) approximately two-thirds of the cytoplasmic RNA is synthesized in the nucleolus and the other one-third in the nucleus, and (ii) the nucleolar and nuclear RNA's are independent in their synthesis and movement to the cytoplasm.

A parallel study has been made of amino acid incorporation into normal and nucleolus-inactivated cells, and, in contrast to the above, no evidence of a significant nucleolar or nuclear dependence is found when the incorporation is followed for periods which are short compared to a cell generation time. These results support the belief that nucleoside and amino acid incorporation need not necessarily be simultaneous.

These experiments were carried out together with Prof. Maurice Errera while I was an American Cancer Society fellow in the laboratory of Prof. J. Brachet, Brussels.

ROBERT P. PERRY
Institute for Cancer Research

Chemically Induced Life-Shortening and Its Probable Genetic Basis

Exposure of animals, including man, to ionizing radiation results in acute effects and in such delayed effects as cancer appearing long afterwards, and life-shortening in general. The acute effects seem based in chromosomal damage. More recently, experiments with *Drosophila* have shown that life-shortening has a similar basis (Oster 1959, Ostertag and Muller, 1959). For such studies, appropriate stocks have been synthesized. Exposure of preimaginal stages that are soon followed, normally, by a period of intensive cell proliferation, growth, and differentiation leads to a relatively early onset of damage. Thus, death resulting shortly before or after eclosion, following treatment, probably represents a life-shortening comparable in principle to that observed in higher forms.

Since many carcinogenic chemicals possess other radiomimetic properties (for example, produce chromosomal breaks), it is essential to know whether they can also shorten the life-span otherwise than by causing cancer.

Stocks were made up containing chromosomes (ring-chromosomes) which allowed for normal functioning of the cells but which were more easily lost following breakage than normally structured chromosomes (rod-chromosomes). Untreated controls containing either type of chromosome had good survival rates (794/800) for the period studied, from larva to adult, while feeding nitrogen mustard to larvae resulted in lower survival (595/900) amongst the individuals containing the chromosomes which were more susceptible to induced damage than amongst those containing normal chromosomes (735/900). These results indicate that chemical carcinogens may cause premature "ageing" via the formation of chromosomal breaks and point to an especially insidious effect following contact with such chemicals.

This work was supported by National Institutes of Health grant CY-4615 C1.

IRWIN I. OSTER
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Mechanical Properties of Blood Vessels and the Regulation of the Cardiovascular System

The mechanical properties of blood vessels determine their biological functions, that is, the dissipation and distribution of the mechanical energy produced by the heart and, therefore, the distribution of blood and blood flow.

These properties have historically been referred to by the entirely descriptive term "tone." We have recently been able, for the first time, to define analytically and evaluate quantitatively the parameters of tone, therefore making it possible to quantitatively analyze the biological effects upon the blood vessels of such things as the nervous and endocrine systems, aging, and disease. These mechanical properties are defined by the relationships between the force tending to cause vessel wall motion (stress) and the resulting motion (strain). Instantaneous radial stress (blood pressure) and strain (vessel diameter) from multiple sites in the vascular system have been recorded on magnetic tape and the data have been analyzed using digital or analog computers, or both. The properties of arteries which describe their "tone" can now be stated as a discrete equation whose terms can be evaluated. Moreover, since the so-called "pressure receptors" lying within blood vessel walls are really strain receptors, we have studied the interrelationship of blood pressure, vessel wall strain,

vessel wall mechanical properties, and the activity of nerve receptors lying in the vessel walls (for example, carotid sinus). It has been found that the mechanical properties of the receptor-containing vessels undergo reflex changes which suggest that the concept of blood pressure regulation as a physiological principal must be re-examined.

L. H. PETERSON
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Localization in Electron Microscopy of the Contractile Proteins of Striated Muscle by Antibody Staining

The contractile proteins, myosin and actin, as well as the digestion products of myosin, the meromyosins, have been localized in the striated muscle fibril by the fluorescent antibody technique at the level of the light microscope. It is desirable, however, to obtain localization at the level of the electron microscope. This would permit description of the distribution of individual protein species within the fine structure of the muscle filament. This might be extended to a study of the distribution of proteins in the relaxed and contracted states which should add to our understanding of the contractile apparatus. To this end three methods, of general applicability in electron microscopy, have been devised. (i) To increase electron scattering, mercury, in addition to fluorescein, was introduced into the antibody molecule. This permitted direct comparison between light and electron microscopy. (ii) Unmodified antibody was visualized in osmium-fixed material as a change in morphology due to the adherence of the antibody to the antigenic structures. (iii) Tissue stained with the unmodified antibody was extracted with solvents in which the combined antigen and antibody was selectively insoluble.

The results obtained from these three methods for electron microscopy are consistent with one another and with the results obtained by light microscopy. Higher resolution must be achieved, however, for more precise localization of individual proteins in muscle.

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Theory of Solid He^3

A theoretical analysis is given of the properties of solid He^3 on the basis of: (i) a gas-phase potential modified at small interatomic distances; (ii) a variational type wave function constructed from a properly antisymmetrized product of individual atom orbitals localized on the various lattice points; (iii) a Dirac vector model to describe the symmetry energy with an exchange integral deduced from (i) and (ii); (iv) a spin-wave and a Kramers-Opechowski approximation at "low" and "high" temperatures respectively for a calculation of the free energy of the nuclear spins; and (v) a Debye phonon model for the description of the vibrationally excited states of the solid. On this basis, calculated values at low pressures and temperatures ($p \approx 30$ atm; $T \leqq 1°K$) are presented for: (i) the cohesive energy; (ii) the root mean square deviation of the atom from its lattice site; (iii) the nuclear magnetic susceptibility which corresponds to an antiferromagnetic behavior with Curie temperature T_C; (iv) the variation (decrease) of T_C with increasing pressure corresponding to a possible nuclear antiferromagnetic to nuclear ferromagnetic transition at high pressures; (v) the specific heat which exhibits an anomaly at $T \approx T_C$ associated with the alignment of the nuclear spins; (vi) the thermal expansion coefficient which becomes negative below about 0.5°K; (vii) the melting curve which is characterized by a minimum, and, in addition, if liquid He^3 never becomes a superfluid, by a maximum; (viii) certain properties of solidified isotopic mixtures of He^3 and He^4. Comparison of the theory is made with available data.

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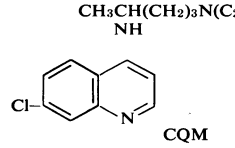
Mechanism of Biological Alkylations

In spite of the interesting and important variety of biological effects of polyfunctional alkylating agents, the amazing spectrum of compounds possessing similar biological and clinical activities, and the many investigations of the mechanism of action of these compounds, many of the most important details of their mode of action remain obscure.

As one part of our studies of this problem, we have prepared chloroquine mustard (CQM) labeled with tritium in the quinoline nucleus.



CQM

This material has been found to be rapidly and irreversibly bound by protein, DNA, and RNA, either in the intact animal, in tissue homogenates, or in solutions of these biological substrates. The tissue distribution in the mouse is very similar to that of HN-2, $CH_3N(C_2H_4Cl)_2$, except for brain (higher in CQM) and kidney (higher in $C^{14}H_3$ HN-2). The former is reflected in the higher central nervous system toxicity of CQM; the latter, in the demethylating function of the liver.

In reaction with DNA ($10^{-2}M$ in phosphorus), less than 2 percent of $10^{-4}M$ CQM was extractable by chloroform, even after 20 minutes at 90°C. In reaction with RNA ($3 \times 10^{-2}M$), 18.5 percent was extractable without hydrolysis at 90°C. For bovine serum albumin ($10^{-4}M$) only 1.5 percent was extractable before hydrolysis at 90°C, 15 percent after. In contrast, reaction of CQM in water in the absence of biological substrate gave 32 percent extractable before heating to 90°C, an additional 54 percent after.

With larger equivalents of CQM, over 2 moles of the alkylating agent were bound per nucleotide unit of DNA. Most of the bound CQM reacted without liberation of acid and was not freed by hot alkaline hydrolysis (less than 5 percent chloroform-extractable). This would indicate that most of the binding has occurred through the tertiary ring nitrogen atoms of DNA, rather than through hydroxyl, amino, or phosphate oxygen groups.

CHARLES C. PRICE, ROBERT J. RUTMAN,
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Neurological Basis of Behavior in the Cat

Recent findings indicate the brain is served by two sensory systems. The first system includes the several specific pathways which selectively subserve vision, audition, skin sense and joint-muscle sense, each of which shows an exquisite degree of anatomical and physiological localization. The second system, called nonspecific, is much less localized in function and provides areas of interaction of many sensory modalities from many parts of the body. These lie in the reticular formation of the brainstem and are believed to be the sites of integration of neural processes which form the basis of adaptive behavior. That this hypothesis must be seriously questioned is seen from the following experiments. Lesions were placed in the upper midbrain so as to interrupt most of the specific sensory pathways, leaving intact most of the reticular formation. Such animals become automata, characterized by stereotyped, restless behavior, in which there is little or no vestige of their preoperative personalities. They are mute and without facial expression. There is great deficit of attention and their response to visual, auditory, tactile, and painful stimuli is largely generalized activation. They show little or no rage, fear or pleasure responses, somatic or autonomic. Social and sexual relations with other animals are virtually lacking. Thus, when the forebrain is deprived of sensory information via the specific pathways, the remaining portions of the nervous system including the reticular formation are unable to maintain the repertoire of attentive, affective, and adaptive behavior.

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Appearance of Discretely Sedimenting Components in Deoxyribonucleic Acid above pH 9

When calf thymus, salmon sperm, and bacteriophage deoxyribonucleic acids are studied in the ultracentrifuge at *p*H values above 9 at low concentrations (below 0.01 percent), the continuous distribution of sedimentation coefficients seen at lower *p*H values changes dramatically. Several

extremely sharp and discretely sedimenting components are seen by both ultraviolet and schlieren optics. The multiple components behave in a normal manner; the log x versus t plots are straight. The transition from a continuum distribution to a number of discrete components occurs progressively over a pH range of about ½ a pH unit, starting at pH 8.8. The effect is completely reversible and no denaturation of the DNA occurs. In spite of the dramatic change in the distribution of sedimentation coefficients, the change in viscosity is not great. The resolution of discrete components requires some convective disturbance; a superimposed density gradient (for example, sucrose) prevents the formation of the discrete components although considerable sharpening of the pattern occurs. At low ionic strengths, (0.007), the discrete components are present until the speed of the ultracentrifuge is lowered to 15,000 rev/min. At higher ionic strengths, (0.1), the speed must be reduced to 40,000 rev/min before reproducible results are obtained, probably due to the formation of a salt density gradient at higher speeds. The number of discrete components is a sensitive function of the DNA concentration from 0.001 to 0.01 percent. The effects of deoxyribonuclease and chymotrypsin on the discrete components have been studied. These results will be discussed with reference to the significance of the polydispersity of DNA as seen in the ultracentrifuge.

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Discriminative Classical Conditioning under Curare Can Later Control Discriminative Avoidance Responses in the Normal State

Dogs were trained to avoid shock in response to a signal. The avoidance response was the pressing of a panel, the signal (S^0) was a light going out, the shock was of 4 ma intensity (applied to the hind toe pads), and the time interval between signal onset and shock onset was 10 sec. If the correct response occurred during the time interval between signal onset and the usual shock presentation time, no shock was given and the signal was terminated. Intertrial intervals were varied systematically, with a mean of 1.5 min.

After the dogs were reliably pressing the panel in response to the signal, with response latencies of 3 sec or shorter, they were totally curarized. While the dogs were completely immobilized under curare, a Pavlovian discriminative conditioning session was carried out. A new signal (S^+) was consistently paired with shock, using a delayed procedure and a time interval of 10 sec between S^+ onset and shock onset. The shock duration was 5 sec. On some trials a contrasting signal (S^-) was presented for 15 sec, but it was not paired with shock. A sequence of 99 discriminative conditioning trials was presented, ending with an S^+ trial. The S^+ and S^- trials were partly randomized in a special sequence. After this conditioning session, the dogs were given 48 hours to recover from the various physiological effects of curarization. Next they were returned to the training situation in the normal, undrugged state, and the three previously used stimuli (S^0, S^+ and S^-) were presented. The latency of panel-pressing responses was recorded during these tests of the efficacy of the three stimuli.

The dogs responded in a way consistent with their discriminative Pavlovian conditioning experience. There were frequent panel presses in response to S^0 and S^+, very few in response to S^-. When a dog pressed a panel in the presence of S^-, the latency of the response was often long.

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Mechanisms of Bile Acid Formation

The cleavage and oxidation of the cholesterol side-chain which takes place in liver mitochondria requires, in addition to ATP, AMP, DPN, Mg^{++}, and boiled supernatant factor, supplementation with gluthathione and citrate or malate. A requirement for TPNH can also be demonstrated when the mitochondria are pretreated by extraction with potassium chloride solution. The boiled supernatant factor is obtained in most active form only if the microsomes are removed before boiling. Experiments with C_{25}- as well as C_{26}- and C_{27}- labelled cholesterol indicate that all three terminal carbon atoms can be oxidized to carbon dioxide by this system. Small yields of labelled acetone can be obtained from such systems; carbons 26 or 27 of cholesterol are converted to methyl carbons of acetone while carbon 25 becomes the carbonyl group of acetone.

The identification of acetone as a cleavage product is somewhat surprising in view of the findings by Bergstrom and his group, as well as by our own laboratory, that trihydroxycoprostanoic acid is efficiently converted to cholic acid. Experiments in our laboratory with trihydroxycoprostane have shown that the terminal carbon atoms of this sterol are more readily converted to carbon dioxide than is cholesterol in the rat liver system. Furthermore, additional experiments have demonstrated that trihydroxycoprostane is readily converted by rat liver homogenates to trihydroxycoprostanic acid, a naturally occurring reptilian bile acid, and that this acid is subsequently converted to cholic acid and carbon dioxide. If the latter pathway is eventually found to be a major metabolic route to cholic acid, then the production of acetone must be considered a minor pathway representing a relatively nonspecific action of an oxidase upon cholesterol and trihydroxycoprostane.

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Estimating the Forces of the Human Heart: Their Diminution as Age Advances and before Disease Develops

Studies on the cardiac forces made during recent years in this laboratory have taken three directions. (i) In mathematical studies, Noordergraaf, working both in Philadelphia and in Utrecht, Holland, using data on blood pressure and elasticity of vessels throughout the body, has calculated the movement of the body's center of gravity at each instant during the cardiac cycle. The forces resulting from this movement correspond closely in magnitude and time with those recorded from the body by the "force" ballistocardiograph. (ii) In experiments (with Schnabel and Mayock) systole was simulated in fresh cadavers in such a manner that the initial "cardiac" forces could be computed. Good agreement was found between the magnitude of these forces and the forces of reaction recorded by the "force" ballistocardiograph. (iii) In empirical studies (with Hildreth and Wood) 200 persons, originally healthy, have been followed for 17 years or longer after their first ballistocardiograms. Cardiac forces diminish as age advances even though health is maintained; from the regression of this normal relationship a "physiological age" for the heart of any subject can be computed from his ballistocardiogram. In our series, the group who later developed coronary heart disease exhibited at the initial test cardiac forces significantly smaller for their age than normal; before clinical manifestations appeared, their hearts behaved like those of much older persons.

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Direct Measurement of Deoxyribonucleic Acid Content of Genetic Loci in Drosophila

The giant chromosomes of the Diptera provide a unique material for direct measurement of the deoxyribonucleic acid (DNA) content of discrete chromosomal regions (the bands) by the use of microspectrophotometric methods. It is known that individual genetic loci are represented in the giant chromosomes by characteristic cross bands, highly differentiated from each other in their structure. They range from single lines through pairs of lines ("doublets") to quite complex patterns. A study of the distribution of DNA content in the various types of band in *Drosophila melanogaster* carried out earlier in this laboratory showed a variation ranging over 2 to 3 logarithmic units, the distribution of quantities for single and double bands overlapping. A calculation of the number of nucleotide pairs contained in a minimal unit resolvable by the ultraviolet microscope, with appropriate assumptions with regard to polyteny, leads to the order of magnitude of 50,000 nucleotide pairs, a result consonant with current measurements of DNA molecular weight. This would be the maximal DNA content for a single locus.

The critical assumption involved in the calculation is that the number of replications leading to polyteny is the same for all loci. This has been tested as follows: Feeding of larvae for the first 24 hours after hatching on a medium containing tritiated thymidine is followed by transferral to a standard medium. Microspectrophotometric measurements of DNA in the fully grown chromosomes (total DNA synthesized) are followed by autoradiographic measurements of thymidine incorporation in the same chromosome region (DNA synthesized during the early replications). If replications during development are equivalent at the different loci, the ratio of grain count to total DNA should be constant throughout a chromosome. Preliminary data indicate disproportionately high (twofold) early synthesis for certain regions, which form puffs during the much later prepupal period. The result may eventually be of considerable importance for discussions of nuclear differentiation. In the single bands used for the calculation given above such a twofold disproportionate synthesis could have reduced the calculated value to 25,000 nucleotide pairs.

This work was aided by grant C-1613 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service, and by an institutional grant from the American Cancer Society.

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Institute for Cancer Research

Cytological Evidence for the Nature of Action of Hooded Gene in Barley

The dominant gene hooded in barley replaces the awns of the lemmas with a complex structure consisting of two lemma-like bodies superimposed end to end, the lower one oriented inversely. It usually produces a palea and floral organs, which may be rudimentary or well developed, depending upon gene expression. The apical "lemma" may be much reduced, or narrowed to an awnlike structure, but when well developed it also produces a palea and rudimentary floral organs. The two "lemmas" of the hood are connected by a rachiola-like structure, which represents an "island" of axis tissue, completely separated from the remainder of the floral axis by tissue of the original lemma, an appendage. In ontogeny, the first evidence of hood production is a swelling on the adaxial side of the young lemma primordium. In cross section, this swelling shows an epidermal layer with much smaller, more actively dividing cells than the corresponding layer in the awned genotype. The subepidermal cells are larger, but they differ from corresponding cells of awned in their planes of division. Immediately above the procambial strand of this swelling is a layer of cells bearing starch inclusions, which is lacking in awned. Thus histological structure and cell behavior resemble that of the young floral axis. Evidence from estimates of nuclear and cell size in developing organs of various ages suggests that the phenomena described are associated with differential rates of synthesis of nuclear and cytoplasmic material.

G. LEDYARD STEBBINS
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Temperature Dependence of Thermal Inactivation Rates at Very High Temperatures

The theory of absolute reaction rates predicts the temperature dependence of thermal inactivation rate constants for biological materials. However, theory and experiment have been compared only over a limited temperature range because of the experimental difficulties of heating and cooling materials rapidly. We have developed an instrument which allows liquid suspensions to be heated or cooled in times comparable to 1 msec and permits exposure to thermal square waves of amplitudes between 30° and 100°C, and durations between 10 msec and 100 sec.

In this instrument the pistons of three large syringes holding, respectively, the test suspension at a noninjurious temperature, hot buffer, and cold buffer, are coupled together and driven mechanically. The test fluid and the hot buffer are driven into a mixing chamber, thereby establishing an equilibrium temperature. The output from the mixing chamber is driven at a predetermined speed through a flow tube of variable length that is held at the equilibrium temperature. This output is mixed in a second chamber with the cold buffer, thereby suddenly cooling the test material.

We have studied the thermal inactivation of haploid yeast by manual techniques in the temperature range from 48° to 58°C, and by our instrument, from 56° to 74°C. The experimental temperature dependence agrees well with theory over the entire range studied. This instrument is used to study a variety of phenomena, in which reactions are started and subsequently stopped before assay.

THOMAS H. WOOD
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Intercellular Transfer of Hemolysin

Intercellular transfer of rabbit antisheep hemolysin has been used as a relative inverse measure of avidity of the hemolysin-cell union. The percent transfer was determined in 50 percent units by adding unsensitized Cr^{51}-labeled red cells to sensitized unlabeled red cells and ascertaining the percent Cr^{51}-cell lysis after adding complement. In previous work [W. H. Taliaferro, L. G. Taliaferro, Pizzi, *J. Infectious Diseases* **105**, 197 (1959)], we studied the gamma-1 Forssman hemolysin of large molecular weight which occurs in normal rabbit serums and in immune serums after one antigen injection or reinjection [Stelos and Taliaferro, *Anal. Chem.* **31**, 845 (1959)]. This gamma-1 hemolysin in both normal and immune serums was composed of a nonavid component which transferred rapidly within 10 minutes and an avid component which transferred much more slowly. Immune serums collected one week or more after the injection of antigen were progressively more avid. In the present work, we used immune serums from rabbits repeatedly immunized until they contained not only the gamma-1 Forssman hemolysin but also the gamma-2 Forssman hemolysin of smaller molecular size. We found that the latter transferred more rapidly than the former. Thus, as the gamma-1 hemolysin decreased and the gamma-2 hemolysin increased during immunization, the percent intercellular transfer increased, that is, avidity decreased. The avidity of the gamma-2 fraction was intermediate between normal gamma-1 and immune gamma-1 hemolysin.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

WILLIAM H. TALIAFERRO
LUCY GRAVES TALIAFERRO
Argonne National Laboratory

Investigation of Disease Resistance and the Mathematical Problem of Patterns

The investigation of innate resistance to diseases both of infectious and noninfectious nature (including such maladies as gout, arthritis, coronary disease, cancer, mental disease, alcoholism, and so forth) holds great promise but has been hampered by two main obstacles. First, sufficient detailed data of the right sort are not available and are difficult to collect. These data should include measurements of those inborn anatomical, physiological, and biochemical characteristics of individuals which are pertinent to the disease under consideration. The delineation between innate and adaptive characteristics has received little attention and is not always easy; the selection of those characteristics which are pertinent to a particular disease may likewise be difficult.

Further progress has been made in our attempts to collect data related to susceptibility to alcoholism, but an additional obstacle has been encountered in the existence of confusing interethnic differences which need to be taken into account but are difficult to interpret.

A second most basic obstacle to the investigation of innate susceptibilities is the lack of suitable mathematical techniques whereby the *patterns* of different individuals can be recognized, compared, and contrasted in a quantitative manner. Only recently have mathematicians become interested in problems which are related significantly to this basic obstacle.

The lack of these fundamental techniques has been and continues to be a serious deterrent to the laborious collection of the vast amount of data which will be essential before disease susceptibilities and resistances can be adequately investigated. The handling of such data will necessarily entail the use of high-speed computers.

ROGER J. WILLIAMS
University of Texas

Learning and Problem-Solution in the Marmoset

Comparative studies of the learning and problem-solution ability of the marmoset have been carried out in the light of existing data on the rat and the rhesus monkey. The marmoset is the most primitive of the simian primates. While its brain has typical primate conformation, it is not as developed as that of the rhesus monkey, its cerebral cortex, for example, being virtually smooth. Given the marmoset's intermediate phylogenetic status, and particularly its intermediate position in neurological development, the experimental question is to determine its relative behavioral capacities. To this end, marmosets were trained to displace the correct one of two or more stimulus-objects to find food. In simple discrimination learning, requiring consistent response to a specific stimulus, the rat, marmoset, and rhesus monkey all perform at about the same level. Where the tests require discrimination on the basis of symbolic process (delayed reaction) or general principle (learning set and oddity problems), the rat fails almost completely and the rhesus monkey succeeds to a high degree. The marmoset performs at very close to the level of the rhesus monkey. Analysis of errors in these tests shows that the rat is very much stimulus-bound in its behavior whereas the marmoset, like the rhesus monkey, can go beyond simple, stimulus-response habits and solve problems on the basis of symbols and general principles.

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Association Affairs

Programs Planned for the AAAS New York Meeting

Section and society programs in the medical sciences, dentistry, and pharmacy and in the history and philosophy of science to be presented at the New York meeting are given here. Programs in mathematics, physics, chemistry, astronomy, geology and geography, the biological sciences, anthropology, psychology, and the social and economic sciences have been previously announced [*Science* **132**, 1259 (28 Oct. 1960); **132**, 1318 (4 Nov. 1960); **132**, 1403 (11 Nov. 1960)].

Medical Sciences

Section N. Five-session symposium, cosponsored by the American Physiological Society and the Society of General Physiologists: "Biophysics of Physiological and Pharmacological Actions," arranged by Abraham M. Shanes, National Institutes of Health; 26–28 Dec.

Part I: "Elementary Systems," with T. Shedlovsky, Rockefeller Institute, presiding; 26 Dec. After an introduction by Shanes, papers will be presented on mechanisms of active cation transport (Joseph F. Hoffman, National Heart Institute); ion transport across the erythrocyte membrane (R. L. Post, Vanderbilt University School of Medicine); electrogenesis in frog skin (T. Hoshiko, Western Reserve University School of Medicine); the interaction of drugs with model systems (Norman L. Gershfeld, National Institutes of Health).

Part II: "Nerve," with K. S. Cole, National Institutes of Health, presiding; 26 Dec. Papers will be presented on nerve structure (J. D. Robertson, McLean Hospital, Boston); ionic movements in nerve fibers at rest and during recovery (W. P. Hurlbut, Rockefeller Institute); ionic permeability changes underlying nerve excitation (F. A. Dodge, Rockefeller Institute); spike generation and sodium ions (K. Koketsu, University of Illinois College of Medicine); possible mechanisms underlying the production of after-potentials in nerve fibers (J. M. Ritchie, Albert Einstein School of Medicine); energetics of activity (B. C. Abbott, University of California, Los Angeles); metabolism in relation to function in nerve cells as illustrated by an excised sympathetic ganglion (M. G. Larrabee, Johns Hopkins University).

Part III: "Muscle I: Membrane Properties," with Alexander Sandow, Institute for Muscle Diseases, presiding; 27 Dec. Papers will be presented on the influence of ions on the membrane potential of muscle fibers (P. Horowicz, Washington University School of Medicine); ion fluxes (R. Swan, Cornell University Medical College); potassium movement in muscle membrane — anomalous rectification (W. Freygang, National Institutes of Health); electrical activity of skeletal muscle (Gertrude Falk, University of Washington); calcium movements in striated muscle during contraction, and contractures with or without membrane depolarization (C. Paul Bianchi, Institute for Muscle Disease); the role of extracellular calcium ions in excitation-contraction coupling in skeletal muscle (George B. Frank, University of Manitoba, Winnipeg, Canada); correlation of calcium uptake and contractility in frog rectus abdominis muscle (Abraham M. Shanes); "relaxation response" of tonic muscle fibers (William G. Van der Kloot, New York University School of Medicine).

Part IV: "Muscle II: Contractile Properties," with Wallace O. Fenn, University of Rochester Medical School, presiding; 27 Dec. The first paper will be the vice-presidential address of Section N, "The Mode of Action of Drugs," by Carl F. Schmidt, University of Pennsylvania. After the address, the 16th Theobald Smith Award, given by Eli Lilly and Company, will be presented. Papers will then be presented on structure and function of twitch and slow striated muscle fibers (Lee D. Peachey, Columbia University); general energetics of contraction (Alexander Sandow); the immediate energy source for contraction of muscle (R. E. Davies and D. F. Cain, University of Pennsylvania); the nature of the contractile mechanism in muscle (R. J. Podolsky, Naval Medical Research Institute); general physiology and pharmacology of junctional transmission (Harry Grundfest, Columbia University).

Part V: "Muscle III," with C. Ladd Prosser, University of Illinois, presiding; 28 Dec. Papers will be presented on some current theories and problems in cardiac electrophysiology (B. Hoffman, New York Downstate Medical Center); possible electrochemical factors in heart electrophysiology (J. W. Woodbury, University of Washington School of Medicine); electrolyte metabolism in myocardial tissue (W. C. Holland, University of Mississippi Medical Center); electrochemistry of smooth muscle and its relationship to contraction (L. Hurwitz, Vanderbilt University School of Medicine); potassium and the mechanical responsiveness of artery strips (L. Barr, University of Michigan School of Medicine); electrolytes and contraction in cardiac muscle (Saul Winegrad, National Institutes of Health).

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