established an uncomfortable relation to science. He tends to think of it as all-powerful and unchallengeable, because ultimately exact and perfect. The really great scientists never fooled themselves on this matter of exactness. Newton would have been the first to welcome and praise the corrections Einstein brought to gravitational theory, for Newton himself, speaking of the check with which he calculated two aspects of the force of gravity—first, as necessary to hold the moon in its orbit and second, as necessary to make an apple fall to the ground—remarked simply, "I found them answer *pretty nearly.*"

The fact is that the average citizen tends to fear science, when he should, of course, learn about it, so that it can be an exciting intellectual companion and a useful servant. He tends to think that science is entirely mechanistic, and that its successes in the biological field depress the dignity of the inner man; whereas, as Robert Oppenheimer has said, he should ". . . have known that human life was far too broad, deep, subtle, and rich to be exhausted by anything the scientist would find out in his own field" (*4*).

Rather than pretending to be perfect and ultimate, any scientific theory represents only a stage of progress in successively better approximations. Concerning one of the most basic theories in physics, Oppenheimer said (*4*), ". . . it is a theory which is almost closed, almost self-sufficient, and almost perfect. Yet it has one odd feature: if you try to make it quite perfect, then it is nonsense." I would suggest that an absolutely critical distinction between science and religion may be that science never will and never can actually reach the final goal of perfection, whereas religion can do so and has done so.

The average citizen tends to think that science has destroyed the element of faith in religion; instead, he should realize that science is itself founded on faith. He tends to think that science is an ugly sort of foe of the gentler arts, whereas he should recognize that, as Bronowski has said (*3*, p. 250) "There is a likeness between the creative acts of the mind in art and in science. . . . The scientist or the artist takes two facts or experiences which are separate; he finds in them a likeness which had not been seen before; and he creates a unity by showing the likeness." This discovery of unity is at the center of science, and it is also at the center of art. Whenever Coleridge tried to define beauty he returned to a central deep thought. Beauty, he said, is "unity in variety."

We must all learn to understand this great modern intellectual force, to utilize it properly so that it may serve our lives and enrich our appreciation of the world around us, to respect the abilities of science at the same time that we realize its limitations, to know enough about science to be able intelligently to meet the responsibilities of modern citizenship. "I am strongly of the opinion," wrote Sir Edward V. Appleton, "that it is the scientist's mission not only to uncover nature but also to interpret his results to his fellow men. Scientific knowledge is itself neutral. It is the use that is made of it that is good or evil. Decisions concerning that use are not for the scientist alone. The layman must therefore make his own efforts at understanding. To assist him, the scientist must, in turn, be ready to leave his laboratory to act as a guide."

References and Notes

1. R. L. Heilbroner, "Public Relations; The Invisible Cell," *Harper's Magazine* 214, 23 (June 1957).
2. On the day on which I wrote this sentence I read, in an essay by E. B. White [*The New Yorker* 1957, 43 (27 July 1957)], "I see by the paper this morning that a steel drum containing radioactive sodium waste is floating at sea. . . . The news story says the Atomic Energy Commission has authorized the dumping of radioactive sodium waste in the ocean. I sometimes wonder about these cool assumptions of authority in areas of sea and sky. The sea doesn't belong to the Atomic Energy Commission; it belongs to me."
3. J. Bronowski, "Science and Human Values," *Universities Quart.* 10, No. 3, 247 (1956).
4. R. Oppenheimer, *Phys. Today* 10, No. 7, 12 (July 1957).

National Academy of Sciences

Abstracts of Papers Presented at the Autumn Meeting, 18–20 November 1957, Rockefeller Institute and New York Botanical Garden, New York

Pedigrees of Exconjugants in Escherichia coli K-12

Mating between morphologically distinguishable Hfr and F− bacteria (*Escherichia coli* K-12) has been observed in the light and electron microscopes. [J. Lederberg, *J. Bacteriol.* 71, 497 (1956); E. L. Wollman, F. Jacob, W. Hayes, *Cold Spring Harbor Symposia Quant. Biol.* 21, 141 (1956)]. In order to follow the subsequent details of recombination and segregation, individual couples of conjugating bacteria were isolated with a micromanipulator. After the mates had separated from each other, pedigrees of isolated exconjugants were obtained by isolating successive daughters and analyzing the genetic markers of clones derived from individual fifth to tenth generation bacteria. The Hfr exconjugants divided regularly and formed no recombinants. In contrast, a typical fertile F− exconjugant, in which recombination or segregation, or both, was occurring, divided irregularly to yield many dead bacteria, the F− parental type, F− types with altered morphologies, and a number of different viable recombinant types. The latter did not segregate to give pure clones until after the third, and sometimes not till after the tenth division. These results suggest (i) that the genetic material transferred from an Hfr to an F− bacterium persists in the F− bacterium for a number of divisions during which time it may recombine frequently with the F− genetic material; (ii) that many combinations may be nonviable; and (iii) that many viable recombinants may involve *morphological characters* not utilized in the genetic analysis.

The experiments reported here were carried out in the laboratory of Dr. A. Lwoff at the Institut Pasteur, Paris, while I held a Fulbright research scholarship and a fellowship from the John Simon Guggenheim Memorial Foundation.

THOMAS F. ANDERSON

Johnson Foundation, University of Pennsylvania

Structure of an Antibiotic Allenic Polyacetylene from the Basidiomycete Drosophila semivestita

Culture liquids of *Drosophila semivestita* contain several polyacetylenes. Among these are two which are closely related to or identical with drosophilins C and D, antibiotic polyacetylenes isolated previously from *Drosophila subatrata.*

For the polyacetylene corresponding to drosophilin C, formula I is proposed on the basis of its ultraviolet and infrared absorption spectra, its behavior with alkali, and analysis of its reduction product.

$$HC{\equiv}C{-}C{\equiv}C{-}CH{=}C{=}CH{-}CH{=}CHCH_2COOH \quad (I)$$

The alkali conversion product is be-

lieved to be II, on the basis of its ultraviolet absorption spectrum, which is that of an entriyne and is identical with that of an acid of this formula synthesized by Bohlmann. This acid would be the expected product of a conversion of I, closely analogous to the mycomycin → isomycomycin rearrangement.

$$H_3C—C\equiv C—C\equiv C—C\equiv C—CH=CHCH_2COOH \quad (II)$$

MARJORIE ANCHEL
New York Botanical Garden

Isolation of N-Acetylneuraminic Acid from Colominic Acid

The colocineogenic microorganism *Escherichia coli* K_{235} elaborates an acidic polysaccharide which can be isolated from the culture medium by suitable fractionation procedures. The purified material, termed colominic acid, contains nitrogen, acetyl, and carboxyl groups and is free of phosphorus, sulfur, and methoxyl. Tests for protein, hexosamine, pentoses, and hexuronic acids are negative. From the Ehrlich test it has been found that colominic acid gives such a high absorbance per unit of weight that it must be constituted solely of sialic or neuraminic acid-like substances. A crystalline material has been obtained from aqueous hydrolysates of the acid in high yield. A comparison of the elementary composition, infrared spectrum, optical rotation, and neutral equivalent of the unknown crystalline substance with those of N-acetylneuraminic acid has revealed that the two are identical. Colominic acid must therefore be regarded as an acidic homopolysaccharide of a unique type, composed of repeating units of N-acetylneuraminic acid. The exact arrangement of the monomer units in the macromolecule is as yet not known. Thus, for the first time, a substance containing sialic acid has been obtained from a source other than mammalian.

GUY T. BARRY
Rockefeller Institute

On the Mechanism of Terpene Biogenesis

Earlier experiments had shown that the enzymatic conversion of 2-C^{14},5-di-T-mevalonic acid to squalene occurs with only a slight change in the ratio of the two isotopes, indicating that the δ-carbon atom of mevalonic acid remains reduced throughout the condensation process. Analogous results, now obtained with D-labeled mevalonic acid, strengthen the view that the isoprenoid intermediates are coupled by interaction of —CH_3— groups and not by a mechanism of the Claissen, aldol, or acyloin type. In a medium of D_2O, squalene synthesis from mevalonic acid proceeds with the uptake of 3 to 4 atoms of D from the solvent. This result is incompatible with any mechanism employing as condensing units 6-carbon compounds or C_5 structures containing isopropyl groups. The structural requirements for the "isoprenoid" intermediate dictated by these labeling experiments was discussed, and it was shown that a mechanism of squalene synthesis utilizing isoprene itself is in accord with all the evidence available.

KONRAD BLOCH, H. RILLING
Harvard University

Depolarized Muscle, a Missing Link

Tension in the intact frog muscle is insensitive to changes in *p*H (*p*H 5 to 9), and its Q_{10} is small (1.2 to 1.3). Tension in muscle "models" is strongly dependent on *p*H and temperature.

When membrane function is partly, temporarily, and reversibly suspended by depolarizing intact muscle with excess K, a preparation intermediate between intact muscle and its "models" is obtained which exhibits a similar temperature and *p*H dependence to that of muscle "models." This suggests that the genuine properties of the final contractile system (actomyosin+ATP +ions) in the intact muscle is somewhat obscured by the strong regulating influence of membrane function, and thus a comparison between them is not always meaningful.

If potassium-depolarized (nonpropagating) frog and turtle muscles are marked off into several segments along their length by a nontoxic fluorescent dye, the shortening of different portions of the muscle can be recorded accurately by a constant-speed camera. By use of such a method (Mashima and Csapo, 1957), earlier observations of our laboratory were confirmed and extended. Shortening is uniform along the length if the muscle is stimulated in a transverse alternating field but is greater in the middle portion in a longitudinal field. Longitudinal d-c activates the muscle throughout its length except at the extreme anodal end, and if the $[K]_0$ is sufficiently high only the middle portion of the muscle responds. Such high $[K]_0$ also greatly reduces the effect of the transverse d-c. Longitudinal d-c activates the cathodal end effectively if the rise in current strength is abrupt, while if it is slow the middle portion of the muscle shortens most. These observations show that effective internal currents are more directly linked to activation than is depolarization.

ARPAD CSAPO
Rockefeller Institute

In vitro Studies on Specificity of Action of Toxicants Which Function as Alkylating Agents

During studies on the kinetics of alkylation of amino acids and proteins by the fungicides 1-fluoro-2,4-dinitrobenzene (FDNB) and 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine, it was found that the former toxicant alkylated cysteine about 10^3 times more rapidly than substrates containing primary amino and carboxyl groups only. A C—S bond was first formed, but the reaction product rearranged rapidly to yield N-2,4-(dinitrophenyl)-cysteine. The two toxicants alkylated compounds containing sulfhydryl groups at about the same rates, but the *s*-triazine was about 20 times more reactive with substrates containing phenolic hydroxyl groups, and 30 to 50 times more reactive with *p*-aminobenzoic and nicotinic acids. Thus the *s*-triazine should be able to interfere with folic acid and pyridine nucleotide metabolism, while FDNB should be the more specific of the two compounds for the inactivation of sulfhydryl groups. The reactivities of these materials with bovine serum albumin were compared. The *s*-triazine alkylated it about 20 times more rapidly than FDNB, which is close to the ratio of reactivities observed for the phenolic hydroxyl group. However, the closeness of these ratios does not necessarily mean that the sites of action are at tyrosine residues, because the number of functional groups in the protein which participate may not be the same in both cases, and it has not been demonstrated whether energies of activation and steric factors will change in parallel ways when free amino acids are incorporated into protein molecules.

H. P. BURCHFIELD
Boyce Thompson Institute for Plant Research

Protein Intake and Resistance to Infection

In the underprivileged parts of the world, inadequate protein nutrition is extremely prevalent and a direct cause of much disease. It also has indirect deleterious effects by decreasing resistance to infection. As this particular aspect of the problem is of special importance among children, we have studied its manifestations in animals during the period of rapid growth.

Shortly after weaning, mice were allowed to feed *ad libitum* on diets containing all known nutritional factors, the protein and carbohydrate contents being the only variables. After they had been maintained for 2 weeks on the experimental diets, the animals were infected with doses of bacteria selected to produce either rapidly fatal or slowly evolving infections. The survival time served as the index of resistance.

All diets allowed normal growth rates of control (noninfected) animals. Yet, animals receiving 8 percent casein proved much more susceptible to all types of infection than did those receiving 20 percent casein. On the other hand, animals fed commercial pellets containing 22 percent protein were almost as susceptible as those receiving 8 percent casein. As pellets contain chiefly plant materials, it is apparent that the qualitative character of the protein, as well as the quantity fed, is of importance in determining resistance.

The resistance to infection of animals receiving 8 percent casein could be increased by supplementing their diets with amino acids. Here again, resistance varied independently of the growth curve. Thus, the resistance of young growing animals to bacterial infections is controlled by nutritional factors different from those involved in weight gain.

RENÉ J. DUBOS
RUSSELL W. SCHAEDLER
Rockefeller Institute

Role of α-Helical Configuration in Proteins and Protein Analogs

In previous work [Yang and Doty, *J. Am. Chem. Soc.* **79**, 761 (1957)], it was shown that solutions of synthetic polypeptides exhibited specific rotations and rotatory dispersions that were very dependent on the configuration of the polypeptide chains. By using a scale defined by these limiting behaviors, it was found that the rotatory properties of several proteins could be interpreted in terms of their helical content—that is, the fraction of residues in the α-helical configuration. The implementation of this procedure has now been improved, and self-consistent values of the helical contents of a number of proteins in a variety of solvents have been obtained. Furthermore, it has been possible to confirm these estimates of helical content by infrared spectral measurements in mixed solvent systems.

With the helical content of typical proteins reasonably well established, we have attempted to duplicate these features in synthetic polypeptides in physiological saline. This has been achieved with equimolar copolymers of L-lysine and L-glutamic acid which have random chain compositions but nearly uniform molecular weights and over-all chain compositions. Such polypeptides having molecular weights in the range from 25,000 to 100,000 are water soluble at all *p*H's. Their helix content in physiological saline varies from about 70 percent at *p*H 3 to 40 percent at *p*H 7 to 15 percent at *p*H 11. The removal of salt brings about complete denaturation, showing that the net effect of the charged groups is repulsive. These polypeptides resemble proteins in having a narrow isoelectric region (*p*H 6.8 to 7.1), in being denatured by urea, and in other ways.

P. Doty, K. Imahori
Harvard University

High-Accuracy Mechanical Integration by Shear in Viscous Liquids

Integration from the standpoint of instrumentation is provided by any device wherein the time rate of change of an output quantity is accurately proportional to the input quantity. Viscous shear in fluids is a very useful means of realizing integration when mechanical torque acts as the input and when the angular velocity of a rotor with respect to a stator is the output. This type of integration is widely used in situations requiring high accuracy for oscillatory inputs with output angles restricted to small magnitudes. This paper outlines the background of viscous shear integration and develops concepts for describing integrator operation. These concepts are applied to the practically important case of the single-degree-of-freedom integrating gyro unit as an illustration of viscous shear integrator operation. The physical dimensions and fluid properties associated with gyro-unit integrating components are taken as typical of the problems that may be solved by viscous shear integrators.

The essential problem is that of causing shearing forces in a thin fluid layer between a cylindrical rotor and a cylindrical stator to produce a torque on the rotor with its magnitude closely proportional to the relative angular velocity between the two cylinders. For practical applications, this proportionality should be at least as accurate as 1 part in 100,000. Physical properties of a fluid which influence the accuracy obtainable with this configuration are discussed. It was found essential to use a Newtonian fluid in an isothermal state. A detailed mathematical analysis of the fluid motion determines limits for physical dimensions, frequency, and amplitude of rotor motion.

C. S. Draper, M. Finston
Massachusetts Institute of Technology

Conductance of Tetrabutylammonium Tetraphenylboride

The tetraphenylboride ion has essentially the same symmetry, size, and structure as the tetrabutylammonium ion; together, these ions provide a 1/1 salt which is well approximated physically by the idealized model in which the ions are represented by charged spheres of equal size. The conductance has been measured in propylene carbonate (dielectric constant $D = 65.1$), nitrobenzene ($D = 34.82$), acetonitrile ($D = 36.0$), and in mixtures of acetonitrile or nitrobenzene with carbon tetrachloride. Down to a dielectric constant of about 20, the conductance curves lie above the Onsager tangent (anabatic phoreograms). Such curves have previously been considered characteristic of "strong electrolytes" such as potassium chloride in water. The present results show that anabatic curves can appear in solvents of moderately low dielectric constant, provided that the ions are large enough. The positive deviations from the Onsager limiting tangent can be accounted for by the conductance theory, which allows for the effects of nonzero ion size. [R. M. Fuoss and L. Onsager, *Proc. Natl. Acad. Sci.* (*U.S.*) **41**, 274, 1010 (1955); *J. Phys. Chem.* **61**, 668 (1957)]. Viscosity measurements lead to a hydrodynamic radius equal to 5.4 A, which agrees with the electrostatic radius found in propylene carbonate and with estimates made on molecular models. In the other solvents, the electrostatic radius is larger.

Raymond M. Fuoss,
Joan B. Berkowitz,
Ernest Hirsch, Sergio Petrucci
Yale University

Colicine K

Colicines are agents of unknown nature which are elaborated by certain strains of *Escherichia coli* and which have the ability to kill specifically certain other strains of enteric bacilli. These substances are presumed to be related to bacteriophage yet differ in that they cannot be transmitted by serial passage. Colicines have never been obtained as pure chemical entities, and hence it has not been possible to compare their properties with those of bacterial viruses.

When grown under appropriate conditions, a strain of *E. coli* known as K_{235} elaborates colicine K into the medium. From the culture it has been possible to obtain a substance having potent colicine K activity. This material is a lipocarbohydrate-protein complex and is believed to be identical with the O antigen of the microorganism from which it is derived. In experimental animals it elicits agglutinins for the parent cell, precipitins for the substance itself, and antibodies which neutralize the colicine K activity of the antigenic complex. The complex may be degraded by chemical means into its lipocarbohydrate and protein components. The protein fraction contains all of the colicine K activity of the original substance. A comparison of the immunological properties of this protein with those of phage T_6, the virus to which colicine K is presumably related, reveals no such relationship.

If colicine K activity is indeed an inherent property of the O antigen of *E. coli* K_{235}, it should be possible to obtain a noncolicineogenic variant and to compare the properties of its O antigen with those of the colicineogenic parent. Such a variant has been obtained, and a study has been instituted to elucidate this salient point.

Walther F. Goebel, Guy T. Barry,
Tsunehisa Amano
Rockefeller Institute

Bactericidal Substances in White Blood Cells

The cellular theory of resistance to infection, formulated by Metchnikoff over 50 years ago, stresses the role of polymorphonuclear leukocytes in eliminating microorganisms from animal tissues. These cells commonly migrate to the site of bacterial invasion and help to control infection by engulfing and destroying the microbes. Recently interest has focused on biochemical factors which endow leukocytic cytoplasm with unique capacity to kill bacteria.

Lysozyme was the first bactericidal substance recognized to be present in polymorphonuclear leukocytes. Crystalline lysozyme is a basic low-molecular-weight protein which degrades enzymatically certain aminopolysaccharides. The few Gram-positive microorganisms whose cell wall is composed of this carbohydrate are promptly killed by lysozyme.

Another white cell component, called phagocytin, may be responsible for intracellular destruction of bacteria not affected by lysozyme. Phagocytin has not been isolated in pure form; however, low concentrations (less than 1 μg/ml) of preparations available at present cause rapid death of various Gram-negative enteric bacilli. Phagocytin appears to be a protein which alters the cell wall of susceptible microorganisms, but its exact mechanism of action is not yet known.

Finally, but by no means of least importance, local accumulations of organic acids in leukocytic cytoplasm may harm bac-

teria. Present evidence suggests that the intracellular *p*H about ingested particles is near 4.5. This degree of acidity, acting either alone or in conjunction with phagocytin or lysozyme, is lethal or inhibitory to many microorganisms.

JAMES G. HIRSCH
Rockefeller Institute

Antinuclear "Antibodies" in Lupus Erythematosus

Lupus erythematosus is a disease of human beings which is characterized by the presence of antibodies to various body cells and tissues. Serum from certain patients with this disease can induce specific morphological changes in the nuclei of polymorphonuclear leukocytes *in vitro*. The responsible serum factor is a gamma globulin which has a specific affinity for isolated cell nuclei and nuclear nucleoprotein. Localization of gamma globulin on cell nuclei during *in vitro* induction of the cell changes can be demonstrated by the fluorescent antibody technique.

These sera also contain factors capable of fixing complement with whole-cell nuclei, isolated nuclear deoxyribonucleic acid, and isolated histone. One or more of these factors may be present in any individual serum. Nuclei and deoxyribonucleic acid from widely different organs and species will participate in these reactions. The factors which fix complement with nuclei and with deoxyribonucleic acid are separable by absorption.

Preliminary experiments with rabbits indicate that antibodies to nuclear constituents can develop, but they appear to lack the broad species reactivity characteristic of the serum factors in lupus erythematosus.

The evidence obtained suggests that the lupus erythematosus serum factors are antibodies to nuclear constituents, including deoxyribonucleic acid. However, proof that they are true antibodies, as well as elucidation of their role in this disease, awaits further investigation.

H. R. HOLMAN, W. C. ROBBINS, H. DEICHER, H. G. KUNKEL
Rockefeller Institute

Relationship of Spotted-wilt Virus in Northern and Southern Hemispheres

Identification of spotted-wilt virus in the Northern Hemisphere with the Australian virus first described under that name has never been attempted by importation of virus strains for comparative study, nor have specific antisera been available. Instead, seeds of plants carrying known genes for resistance have been exchanged. Such a technique can be applied to a variety of other phytopathogenic viruses, but its availability has not been generally recognized. The number of applicable genes is now substantial. Without exception they have proved virus specific or strain specific. This technique, in contrast to transportation of the viruses themselves, is beneficial to all national interests. In the case of spotted-wilt virus, a single-gene resistance that proved effective in the eastern United States proved applicable experimentally to some strains in Australia. It was effective also in the Hawaiian Islands, although less effective than a single-gene resistance developed there earlier. Both resistances were ineffectual under field conditions in Western Australia, but Finlay found that the F_1 hybrid between the two resistant stocks was resistant in the field. He showed that each parental line had contributed two additional resistance genes, one of which was common to both parents. Recognized heterogeneity of Australian strains of spotted-wilt virus may reflect efficient transmission by *Frankliniella schultzei*, a thrips confined to the Southern Hemisphere. Less efficient vectors in the Northern Hemisphere may not transmit all strains.

FRANCIS O. HOLMES
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Countercurrent Distribution Studies with Proteins

Recently attempts have been made to find solvent systems suitable for fractionating proteins by countercurrent distribution (C.C.D.). The greatest difficulty is not that of finding two-phase systems which will partition the proteins but rather that of preventing denaturation during the distribution. An investigation of the enhancement of stability against denaturation by addition of certain substances to the system is being made.

Systems of ethanol, water, and a relatively high content of ammonium sulfate offer a sufficiently stable environment for C.C.D. of ribonuclease and lysozyme up to 4000 transfers without any loss of their enzymatic activities. On the other hand, in a system of ethanol, *n*-propanol, water, and ammonium sulfate, the serum albumins undergo a slow, continuous transformation. The incorporation of a small amount of sodium caprylate into the system stabilizes the albumins enough to permit C.C.D. to 1500 transfers. Under these conditions, human serum albumin is found to contain three components of partition ratios—0.22, 0.49, and 0.60—while crystalline bovine serum albumin shows three components of partition ratios—1.0, 1.4, and 3.0. The band spread suggests each component to be a mixture of still more closely related substances. Mixtures of human and bovine serum albumins are separable by this technique, with all six bands appearing.

TE PIAO KING, LYMAN C. CRAIG
Rockefeller Institute

Genetic Control of Enzymatic Fine Structure as Revealed by Substrate Affinities

The deoxyribonucleate particles controlling sulfonamide resistance in pneumococci have been shown to contain three linked marker regions. These three markers may remain linked, be separated, or reassembled by recombination during transformation so that the unit types, designated *a*, *b*, and *d*, and their four combinations can all be produced. The genetic fine structure of this system can also be recognized by its phenotypic manifestations since each of these seven genotypes gives a stable bacterial strain showing its own characteristic degree of resistance toward sulfanilamide. The effect of sulfanilamide is reversed by *p*-aminobenzoate for each of these and the sensitive strain.

Study of the effects of the markers *a*, *b*, and *d* indicates that they are so highly interdependent that it is concluded that all three determine properties of a single enzyme utilizing *p*-aminobenzoate. Activity of this enzyme and its sensitivity to sulfanilamide can be followed by measuring the synthesis of folic acid. The resistance of the enzyme system proves to be a reliable measure of the resistance of the strain which bears it.

The small changes induced in the enzyme by the unit markers *a*, *b*, and *d* have been further analyzed through their effects upon affinities for such related drugs as sulfaguanidine, *p*-nitrobenzoate, and *p*-aminosalicylate. Variation of acidity and spatial relations in the drugs has revealed sharp qualitative differences between the different phenotypes. The fine structure of the deoxyribonucleate appears to determine corresponding fine structure in the enzyme protein, which may be explored by means of the affinities for *p*-aminobenzoate and systematically chosen inhibitory analogs.

ROLLIN D. HOTCHKISS
AUDREY H. EVANS
Rockefeller Institute

Chemical Aeronomy

The chemical structure of the earth's atmosphere is primarily a result of the action of solar radiation on the atmospheric gases. The study of the chemical reactions between the various atmospheric constituents has been designated "chemical aeronomy," and the region of the atmosphere where these reactions are most prevalent is called the "chemosphere." Nitric oxide is present in the upper region of the chemosphere. Its concentration is determined by the relative rates of the reactions between atomic nitrogen and molecular oxygen and between atomic nitrogen and nitric oxide. Many of the aeronomic chemical reactions produce the luminous emission of the night airglow. The atomic lines and molecular bands of the airglow indicate what chemical reactions are taking place and what some of the physical conditions are at the level of emission. The Herzberg and atmospheric bands of oxygen which are prominent airglow emissions have been produced and studied in laboratory afterglows. Some still unidentified airglow emissions have also been obtained in laboratory sources. The technique of artificially producing an airglow by rocket seeding experiments makes possible the detection of some of the atmospheric constituents and suggests, as well, a method of measuring the temperature of the upper atmosphere.

JOSEPH KAPLAN
C. A. BARTH
University of California, Los Angeles

On the Question of Reabsorption in Sweat Glands

Whether reabsorption takes place in sweat glands has been regarded as controversial. Direct evidence is lacking, although reabsorption of water has been postulated to account for concentration differences between slowly and profusely secreted sweat, and absorption of sodium to account for differences between plasma and sweat.

Two simple experiments appear to provide the requisite direct evidence of reabsorption and reveal something of the dynamics of sweat formation and reabsorption. Sweat emergence at the surface of the cat's footpad in response to stimulation of the secretomotor nerve has been observed visually, and its latency in various circumstances of stimulation has been measured.

In one experiment stimulations, standardized in frequency and duration, were applied to the motor nerve, the rest interval between stimulations being varied. After prolonged rest 60-sec stimulation may be required for beginning emergence, after brief rest, but 2.5 sec. Over most of the range, latency varies linearly with duration of rest interval. This experiment indicates that: reabsorption does occur; sweat formation is a rapid process; latency for sweat formation (as distinct from emergence) is about 2.5 sec; reabsorption is a slow process; and reabsorption takes place near the base of the gland.

In the other experiment the first stimulation is standardized and the brief rest period is fixed. Frequency of the second stimulation is varied. Emergence latency in response to the second stimulation varies, within limits, inversely as its frequency. At low frequencies there may be no visible emergence. This experiment indicates that a balance can be struck between formation and reabsorption.

DAVID P. C. LLOYD
Rockefeller Institute

Acquired Immunity from Infection by Strains of Aster-Yellows Virus in Aster Leafhopper

During recent years it has been shown that certain plant viruses, including that of aster yellows, multiply in their insect vectors as well as in plants. Indeed, the specificity and long retention of these viruses by their vectors seem to depend on this ability. Among the different strains of aster-yellows virus occurring in nature there is one known as ordinary aster-yellows virus and another known as the California strain of aster-yellows virus. Both are transmitted by the aster leafhopper, *Macrosteles fascifrons*.

When a colony of this insect is exposed to ordinary aster yellows for about 2 weeks, a period sufficient to render it viruliferous for ordinary aster-yellows virus, and then exposed to California aster-yellows virus for a like period, which period is sufficient to render a virus-free colony viruliferous for California aster-yellows virus, the colony transmits only ordinary aster-yellows virus. On the other hand, if a colony is allowed to feed on a plant with California aster yellows and subsequently on a plant with ordinary aster yellows for appropriate periods, it transmits California aster-yellows virus only. Use of individual insects instead of colonies gives essentially the same results. In other words, multiplication of one strain of aster-yellows virus in the vector insect protects it against transmitting virus of a closely related strain. Thus, it has been shown for the first time that two closely related strains of a plant virus protect against each other in an insect vector.

L. O. KUNKEL
Rockefeller Institute

Movement of Ions and Compounds Into and Out of Fungus Spores

When spores of representative species of fungi labeled with S^{35}, P^{32}, Zn^{65} are suspended in several changes of distilled water, from 15 to 30 percent of the labeled compounds is rapidly released into the ambient solution. The loss of these cell contents does not reduce germination. The compounds remaining do not leach out even on long further suspension in water. The results show that a considerable portion of the cell contents is not protected to any great degree from the external environment but that substances essential for germination are more firmly held. However, washed spores, under certain conditions, readily lose additional cell contents or take up ions and compounds. When subjected to sublethal doses of some fungicides, such as silver and some organic toxicants, washed spores can lose a further relatively large proportion of their contents without loss of germination capacity. Washed spores also take up lethal doses of fungicides very rapidly, in a few minutes or less. Accumulation of ions and compounds by fungus cells probably involves a number of processes, of which reaction with cell constituents is important. These constituents are readily available in large quantities to the fungicides applied. This affords an explanation for the large doses which are quickly taken up and firmly bound and which are required to produce toxicity.

LAWRENCE P. MILLER,
S. E. A. MCCALLAN
Boyce Thompson Institute for Plant Research

Mechanism of Action of the Fungicide Dichlone (2,3-dichloro-1,4-napthoquinone)

Dichlone at toxic concentrations accelerates endogenous oxygen consumption by conidia of *Neurospora sitophila*, typical of other compounds which uncouple phosphorylation from oxidation. Endogenous phosphorus accumulates in the cell as inorganic phosphate in proportion to loss of viability of the conidia. The inorganic phosphate is derived from organic phosphates, including 2 percent perchloric acid soluble phosphates, phospholipids, and ribonucleic acid. Dichlone thus prevents maintenance of a steady state with regard to these compounds in poisoned conidia. Oxidation of exogenously supplied glucose, acetate, or lactate by conidia was inhibited by concentrations of dichlone that inhibited germination of conidia. Inhibition of acetate metabolism was reflected in the amounts of Krebs-cycle acids in conidia. Normally, incubation of conidia with acetate results in large increases in the amounts of citric, succinic, and malic acids, which are derived from acetate. However, when conidia were poisoned with dichlone, these acids tended to disappear. Thus the enzymes of the Krebs cycle, the cytochrome system, and cytochrome oxidase were unaffected, but the pathway from acetate to citrate was blocked. This pathway involves coenzyme A which reacts *in vitro* with dichlone, and it is suggested that the block occurs because of inactivation of coenzyme A. Since coenzyme A functions in many synthetic processes, including lipid and protein synthesis, it is concluded that dichlone acts nonspecifically to prevent conidia from carrying on the essential intermediary interconversions and phosphorylation processes necessary to maintain a dynamic state in the protoplasm.

ROBERT G. OWENS
Boyce Thompson Institute for Plant Research

Restoration of Sodium-Deficient Frog Nerve Fibers by Onium Ions

The following onium ions are able to restore the ability to conduct impulses to sodium-deficient frog A fibers: formanidinium, guanidinium, aminoguanidinium, hydrazinium, hydroxylammonium, and ammonium. In the form of free bases, hydrazine, hydroxylamine, and ammonia do not restore sodium-deficient nerve fibers.

More than 70 derivatives of the restoring ions have been tested on sodium-deficient nerve. Any change in structure—even as slight a change as the substitution of a hydrogen atom by a methyl group—results in a loss by the ion of the ability to replace sodium. On the other hand, there are changes—such as the introduction of an *n*-amyl group in the guanidinium ion—that convert the ion into an anesthetic agent.

It is concluded that the restoring ions do not simply exchange for sodium ions because of similar physical properties. Inside the nerve fibers the restoring ions take part in those electrochemical reactions that underlie the production of the nerve impulse.

R. LORENTE DE NÓ,
L. M. H. LARRAMENDI, F. VIDAL,
J. GARCIA-BILBAO
Rockefeller Institute

Comparative Study of Fine Structure of Some Invertebrate Photoreceptors

Receptor cells of the eyes of animals from four classes of anthropods have been studied. For Arachnoidea, *Limulus polyphemus* and a spider (family not identi-

fied) were examined; for Crustacea, *Callinectes*; for Chilopoda, *Scutigera*; and for Insecta, *Tenebrio molitor*. The rhabdoms of the receptor cells of these eyes are honeycomb-like structures composed of closely packed microvilli of a surface of the cell. The microvilli composing the rhabdoms range from 50 to 150 mμ in diameter.

The double retina of the mollusk, *Pecten irradians,* is under investigation. The distal layer of this retina is composed of cells which are presumed to be sense cells. On their septal surface, immediately underlying the lens, are concentric or coiled laminated structures, the three-dimensional morphology of which is not yet known. These laminae are continuous, with ciliary stalks and basal bodies which in transverse section show the nine peripheral double filaments characteristic of cilia. Thus these sense cells, like the rods and cones of the vertebrate retina, have appendages which are highly specialized ciliary derivatives.

WILLIAM H. MILLER
Rockefeller Institute

The Structure of Atidine, a Diterpene Alkaloid

The two genera *Aconitum* and *Delphinium* elaborate several series of closely related alkaloids. The occurrence in related plant species of mixtures of chemically related alkaloids is of considerable genetic interest and has stimulated much research recently. These alkaloids fall into two broad groups. The first comprises the very poisonous, highly oxygenated ester bases called the aconitines. The second or atisine group includes a series of simpler and less toxic bases. Recently a few members of the latter class have been shown to incorporate the perhydrophenanthrene skeleton and to be modified diterpenes.

In an effort to gain more insight into the structure of the highly toxic aconitines, a systematic study of the simpler alkaloids of *Aconitum heterophyllum* has been undertaken. Of these, only the structure of atisine has been heretofore elucidated. We wish to report on structural studies carried out on a new alkaloid, atidine, isolated from *Aconitum heterophyllum*. Chemical and spectral studies suggested a pentacyclic, tertiary base of the dihydroatisine type, and containing a ketone in a six-membered ring. This has been proved by the conversion of atidine to dihydroatisine. Atidine has also been related chemically to the *Delphinium* alkaloid, ajaconine. This work demonstrates that both atidine and dihydroajaconine possess the same stereochemistry as dihydroatisine.

S. WILLIAM PELLETIER
Rockefeller Institute

Studies on 2-Thiouracil and Plant Virus Synthesis

The capacity of thiouracil to inhibit the multiplication of certain plant viruses has been previously established. The incorporation of this compound into tobacco mosaic virus particles has been demonstrated, and such particles are presumed to be incapable of reduplication. The number of such particles produced in thiouracil-treated infected plants did not appear sufficient to account for the observed reduction in virus multiplication, and it seemed likely that other factors entered into an impaired virus synthesis. In order to gain an understanding of the physiological basis for thiouracil inhibitions of plant virus multiplication, healthy tobacco plants and plants infected with cucumber mosaic virus were grown in nutrient solutions with and without thiouracil and were examined for differences in certain important biochemical constituents. A number of changes were observed, but of special interest in the thiouracil-treated plants was a reduction in nucleic acid and protein sythesis without an apparent decrease in the production of free amino acids and amides. These findings were further substantiated by time-course and C^{14} O_2 fixation studies. The C^{14} studies also showed qualitative differences in labeling of the bulk proteins of plants subjected to thiouracil treatment and actual fixation of more total carbon during the exposure to C^{14} O_2 than nontreated plants. Experiments with thiouracil-S^{35} indicate that it is incorporated into ribonucleic acid, and that it is also metabolized into a number of as yet unidentified compounds. It is suggested that thiouracil inhibits virus multiplication because of its incorporation into normal and virus nucleic acids, resulting in their qualitative change and in their quantitative reduction, and because of its inhibition of virus protein synthesis resulting from an aberrant nucleic acid metabolism.

CLARK A. PORTER, L. H. WEINSTEIN
Boyce Thompson Institute for Plant Research

Fields of Inhibitory Influence of Single Receptor Units in the Lateral Eye of Limulus

In the lateral eye of *Limulus,* each receptor unit (ommatidium) discharges impulses at a frequency determined principally by the intensity of light incident upon it. However, each receptor unit exerts an inhibitory action on its neighbors and is in turn inhibited by them. Thus, when activity is recorded simultaneously from two receptor units near one another, mutual inhibition of each one by the other is observed: the degree of inhibition of each (measured by the decrease in the frequency of its discharge of nerve impulses) is greater the higher the frequency of discharge of the other. For each receptor unit there is a threshold frequency below which it exerts no inhibition on a particular neighboring receptor. Above this threshold the strength of the inhibitory action increases with a constant coefficient. Experiments in which the activity was recorded from receptor units at various distances from one another show that, in general, the more widely separated two units are, the higher the thresholds and the smaller the inhibitory coefficients of their mutual interaction. Thus each receptor unit exerts inhibitory influences upon other receptors in the retina surrounding it, the extent of its field of action depending on its frequency of discharge. One physiological significance of such a mechanism is that it enhances the differences in the neural responses resulting from intensity differences in the pattern of illumination on the retina, and thus enhances information about borders of objects and other similar discontinuities in the animal's visual environment.

FLOYD RATLIFF, H. K. HARTLINE
Rockefeller Institute

Permanent Damage Done to Rats by Prolonged Feeding of Several Common Therapeutic Drugs and Hormones

It was found that prolonged feeding (1 to 4 months) of any one of a variety of drugs and hormones in common therapeutic use in medicine—a sulfa drug, sulfamerazine; the antithyroid drugs, propylthiouracil and thiouracil; the barbiturate, barbital; an antipyretic, pyramidon; the female hormones, estradiol or progesterone—may produce damage from which the rat never recovers.

The damage does not interfere with life; it cannot be seen, and the rat appears to be normal. Its presence can be detected only by taking daily records of spontaneous running activity, food intake, and water intake over long periods. Then it shows up in a marked disturbance to homeostasis, as great and very regular cycles in running activity and food intake —cycles such as have never been seen in a normal rat. The length of the cycles may range from 14 to 58 days. Each cycle consists of two sharply defined phases, one of activity and the other of almost total inactivity; food intake may be more than double in the inactive phase.

The appearance of these abnormal cycles indicates that damage has most likely been done to the brain—the hypothalamus—either through direct action or indirectly through the exhaustion of cells that results from the animal's efforts to reestablish homeostasis which was disturbed by the drugs and hormones.

Evidence was presented showing that the prolonged use of these substances may produce severe mental and physical changes in man, and that it may be responsible for some of the periodic diseases and psychoses in man.

CURT P. RICHTER
Johns Hopkins Hospital

Intraspecific Categories of Manihot esculenta

Manihot esculenta (Euphorbiaceae), a complex of tropical food plants, has a large number of unclassified subspecific units. In this study the variability of the species has been intensively examined in two areas where the crop is important—Jamaica and Costa Rica. The 75 cultivars found here are probably representative types of a much larger area, inasmuch as man has introduced many variants in his efforts to improve the crop.

After examination of plants in the field and of specially prepared herbarium specimens, it is possible to make a few rather definitive groups and from these to judge something of the total range of variation which can be expected in other regions of the growth of cassava.

The framework of taxonomic categories proposed for a classification of the subspecific entities of *Manihot esculenta* is: (i) convariants, the equivalent of subspecies in wild plant taxonomy; (ii) subconvariants, comparable with "series"; (iii) cultivars, the cultivated counterpart of the taxonomist's "variety."

In *Manihot esculenta* two convariants exist, and below this category many subconvariants are found. In each of the convariants the characteristics which differentiate the subordinate groups are almost identical and parallel categories are established. In this classification the importance of individual cultivars is subordinate to those units designated as sub-convariants. Some inferences about the origin of the cultivated forms may be drawn after this classification is proposed.

DAVID J. ROGERS
New York Botanical Garden

The Magneto-Ionic Expander Isotope Separator and the Boltzmann Equation in Absence of Particle Collisions

The application of the magneto-ionic expander isotope separator (M-I.E.I.S.) to uranium was considered probably highly successful in the report last spring [*Science* **125**, 751 (1957)].

Three Americans [G. F. Chew, M. L. Goldberger, F. E. Low, *Proc. Roy. Soc.* (*London*) **A236**, 112 (1956)], have considered the relationship of the Boltzmann equation with single mass ions in the absence of particle collisions (Eq. 1 of Chew, Goldberger, and Low)

$$\frac{\partial f}{\partial t} + (\mathbf{v} \cdot \text{grad}) f + (e/M)(\mathbf{E} + \mathbf{v} \times \mathbf{B}) \cdot \text{grad}_{\mathbf{v}} f = 0 \quad (1)$$

and the derived one-fluid hydromagnetic equation (Eqs. 27 and 28 of Chew, Goldberger, and Low)

$$\rho_0 \frac{d\mathbf{u}_0}{dt} = -\text{div}\overleftrightarrow{P}_0 + \mathbf{j}_1 \times \mathbf{B} \quad (27)\ \&\ (28)$$

For the case of a heavy discharge and a strong enough magnetic field, the one equation leads to the other for the discharge in the M-I.E.I.S. [J. Slepian, *J. Franklin Inst.* **263**, No. 2 (1957); *Nuclear Sci. and Eng.*, Nov. (1957)] for the case of a single mass ion. $\mathbf{j}_1$ is the net induced current density [G. F. Chew, M. L. Goldberger, F. E. Low, *Proc. Roy. Soc.* (*London*) **A236**, 115 (1956)].

In the M-I.E.I.S. we have at each terminal slat,

$$\int \mathbf{j}_1 \cdot d\mathbf{S} = 0$$

For the two expander side electrodes $\mathbf{j}_1$ is parallel to the surface. $\mathbf{j}_1$ is therefore nearly parallel to the surface at all electrodes. Hence the M-I.E.I.S. output is at the same total energy as the input.

We have (Eq. 19 of Chew, Goldberger, and Low)

$$\overleftrightarrow{P}_0 = M\int dv (\mathbf{v} - \mathbf{u}_0)(\mathbf{u} - \mathbf{u}_0) f_0 \quad (19)$$

closely related to the mean energy of random motion; it becomes small at the output slats; the energy of mean motion of the ions becomes nearly the whole energy.

Letting the ions be of two masses, M and $M + \partial M$, the ions become separated and enriched by an amount per unit length given by

$$\frac{\text{Total energy at slats}}{\text{random energy at slats}} \cdot \frac{\partial M}{2M}$$

JOSEPH SLEPIAN
Pittsburgh, Pennsylvania

Enzymatic Reactions at a Liquid-Solid Interphase

Previous studies have shown that protein multilayers deposited on a solid slide were acted upon by proteolytic enzymes even if the layers were protected by a thin plastic blanket upon which a drop of enzyme solution was deposited. Three possible explanations could be offered for such a phenomenon: (i) The enzyme molecules went through the blanket by an ordinary diffusion process, under the influence of a gradient of concentration. (ii) The enzyme molecules were forced through the blanket on account of specific or nonspecific forces to come into contact with the protein layers. (iii) The enzymatic action took place across the blanket, before intimate contact.

A considerable body of experimental evidence now strongly indicates that the first explanation is incorrect, since the minimum thickness of a blanket to prevent an enzymatic action from taking place was a function of the number and the mode of deposition of the protein layers and also depended in a periodic fashion on the number of layers of fatty acid serving as anchorage for the protein layers. The length of the chain of the fatty acid molecules was also found to be an important factor.

The most recent experiments show that the second explanation is the correct one and that specific long-range forces are involved. The material located under the blanket must be of protein nature sensitive to trypsin action, for instance, to permit trypsin molecules in solution on top of the blanket to go through it.

ALEXANDER ROTHEN
Rockefeller Institute

Structure of Pathogen Populations and Problems of Natural Resistance to Infection

In an analysis of natural resistance to infection in mouse salmonellosis, we have been led to the operational necessity of specifying that the test pathogen population be genetically heterogeneous with respect to virulence. To simplify the experimental analysis, the simplest kind of heterogeneous population has been arranged: a combination in specified dose and ratio of two clonal populations of a highly virulent and an avirulent genotype of *Salmonella typhimurium*. By means of an indifferent genetic marker, these two strains are capable of being separately identified by a differential medium upon recovery from the infected host.

When survivorship of mice in this simplified heterogeneous *Salmonella* infection has been arranged by a potent, naturally occurring nutritional factor, it has been found that in such surviving mice, 1 month after infection, a small latent *Salmonella* population is recoverable from the spleen. This population is composed exclusively of descendant cells from the *virulent* members of the original infecting population, as identified by the genetic marker and by virulence tests, in mice, of subcultures. Mouse survivorship has thus been achieved, not by genotypic selection of avirulent pathogens by the host, but by another process. This silent entertainment of potentially fully virulent *Salmonella* was considered from the standpoint of adaptive changes in the now-experienced mouse, and from the view of *Salmonellae* phenotypically adapted to a state of avirulence. No decision is possible as yet between these alternative views, but some experiments presented lend heuristic argument in favor of the *Salmonella* phenotypic adaptation hypothesis.

HOWARD A. SCHNEIDER
Rockefeller Institute

Ribonucleic Acid Synthesis and Influenza Virus Multiplication

New and highly active inhibitors of ribonucleic acid (RNA) biosynthesis have been developed and employed in studies on the biochemical kinetics of influenza B virus multiplication. 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) at a concentration of 38 μ*M* caused 50 percent inhibition of incorporation of adenosine-8-C^{14} into RNA of the chorioallantoic membrane from 10 to 11 day embryonated chicken eggs *in vitro*. Incorporation of C^{14}-L-alanine into the protein fraction and oxygen uptake of the membrane were unaffected. Yield of virus was reduced by 75 percent. Adenosine was capable of blocking the inhibitory effect of DRB on virus multiplication only when low concentrations of DRB were employed to give 50 to 75 percent reduction in yield. Guanosine was ineffective as a blocker. A congener of DRB, 5-(or 6-)bromo-4,6-(or 5,7-)dichloro-1-β-D-ribofuranosylbenzimidazole, is the most active inhibitor of virus multiplication reported; at a concentration of 1.8 μ*M* or 0.72 μg/ml, it caused 75 percent reduction in yield. The benzimidazole derivatives were synthesized by Karl Folkers and Clifford H. Shunk of Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey.

In single-cycle experiments with influenza B virus, no new virus particles appeared in control membranes for 3 hours following infection. DRB reduced the yield of virus only if given within this 3-hour interval; it had no effect when given after the logarithmic increase in virus had begun. It may be inferred that synthesis of virus RNA is completed within the so-called latent period and thus precedes completion of new virus particles. There is similar indirect evidence that synthesis of virus protein continues

for a short time beyond the latent period. The period of logarithmic increase appears to represent primarily the assembly phase.

IGOR TAMM
Rockefeller Institute

Provocation of Masked Hog Cholera Virus in Lungworm-Infested Swine by Ascaris Larvae

Consideration of the epidemiology of hog cholera suggests that its causative virus must be perpetuated in nature in a nonporcine reservoir intermediate host. Experiments conducted with the swine lungworm indicate that this nematode can serve as a reservoir host for the hog cholera virus but also that it harbors the virus in a masked or occult noninfective form. Swine fed lungworm larvae containing the masked hog cholera virus ordinarily do not come down with hog cholera. However, the appearance of good health shown by such animals is misleading because all that is required to bring them down with a fatal attack of hog cholera is the application of some relatively innocuous provocative stress. In the experiments reported, migrating *Ascaris* larvae supplied the stimulus that provoked masked hog cholera virus to infectivity.

RICHARD E. SHOPE
Rockefeller Institute

Amino Acid Turnover in Brain Compared with Turnover in Other Tissues

A litter of mice was produced whose tissues were labeled by feeding C^{14}-glycine and serine to their dam for 18 days (6 days before and 12 days after the young were born). One young mouse was killed the day C^{14} feeding was stopped, the remainder at intervals up to 136 days thereafter.

Five sets of tissues from these animals were analyzed for radioactivity: (i) brain, (ii) heart and lungs, (iii) liver, (iv) kidney and spleen, and (v) skeletal muscle.

The liver, kidney, and spleen in the 136-day animal showed no radioactivity, whereas the brain and the tissues containing muscle were still at high levels.

The implications of these findings were briefly discussed.

JOSEPH W. STILL
George Washington University School of Medicine

Enhanced Folic and Folinic Acid Contents of Erythrocytes Infected with Malaria Parasites

There is good evidence that folic acid plays some important role in the metabolism of malaria parasites. Thus certain antimalarial drugs appear to act by interfering with the utilization of folic or folinic acids. The extracellular survival *in vitro* of a species of bird malaria (*Plasmodium lophurae*) has been found to be prolonged by the presence of folinic acid in the culture medium. Assays of the folic and folinic acid contents of the erythrocytes of normal ducks and of ducks infected with *P. lophurae* have now shown that infected red cells contain much more of both of these growth factors than uninfected ones. Only about one-fifth to one-third of this increase is contained in the parasites themselves, the other four-fifths to two-thirds being contained within the infected host cell material. The observations are consistent with the hypothesis that invasion of an erythrocyte by a parasite alters the folic acid metabolism of the host cell in a manner of benefit to the parasite.

WILLIAM TRAGER
Rockefeller Institute

Some Statistical Observations on Cooperative Study of Human Pulmonary Pathology

Stanley P. Reimann of the Lankenau Hospital in Philadelphia arranged, for the Scientific Advisory Board of the Tobacco Industry Research Committee, a study by a number of pathologists in different centers to examine the conditions at autopsy in the bronchial mucosae of unselected cases; he has reported thereon. The object here is to give some of the statistical details with special reference to differences between different centers. The pathologists classified their slides according to the worst condition found thereon, from normal through hyperplasia and metaplasia to carcinoma. The percentages of slides in the different classes differed widely from place to place. To see whether the pathologists were differing, a selected sample of 40 slides was presented to all of them, and marked differences were found. Further studies are in progress to enable the pathologists to allow for their systematic differences in making comparisons between their findings.

EDWIN B. WILSON, MARY H. BURKE
Office of Naval Research and
Tobacco Industry Research Committee

Probable Evolutionary Relationship of Serotonin and Indoleacetic Acid

This paper presents evidence for the idea that serotonin (5-hydroxytryptamine) is the hormone in animals corresponding to indoleacetic acid in plants. There is first the relationship in chemical structure, since serotonin is the base (modified by hydroxylation) corresponding to indoleacetic acid. Secondly, there is mounting evidence of a resemblance in basic modes of action of the two substances. Indoleacetic acid changes the permeability of plant cells, causing them to take up water and thus to elongate. Serotonin is known to cause muscles to contract. It is now shown that this action is associated with a change in the permeability of the muscle cell to calcium ions. When calcium ions are sequestered with Versene, serotonin no longer is able to cause isolated rat uterine muscle to contract. In the absence of Versene, calcium ions can replace serotonin in causing contraction, provided that sufficient calcium is used. Serotonin is 70,000 times as active as $CaCl_2$ on rat uterus. Finally, it is well known that indoleacetic acid can be replaced by compounds such as 2,4-dichlorophenoxyacetic acid as a growth regulator for plants, and that both auxinlike and antiauxin effects of 2,4-D can be shown. The corresponding amine—that is, 2,4-dichlorophenoxyethylamine—shows serotoninlike and antiserotonin actions on various animal tissues. A close correlation exists between the types of structural alteration which change the auxin activity of 2,4-D and those which affect the serotoninlike actions of 2,4-dichlorophenoxyethylamine. Some of these substituted phenoxyethylamines are very active as pro- and antiserotonins.

D. W. WOOLLEY
Rockefeller Institute

Axenic Serial Culture in Cell-Free Medium of Entamoeba invadens, a Pathogenic Amoeba of Snakes

A strain of *Entamoeba invadens,* ordinarily grown in cultures of mixed bacterial species, was established bacteria-free by Miller (1951, 1953) with the aid of fresh liver tissue. With extracts of raw liver sterilized by filtration, Miller reported some but no continued growth.

The present study started with a Miller culture received (from Dr. E. Meerovitch, Institute of Parasitology, Macdonald College, Quebec) in serum-saline containing a piece of hamster liver. Axenic growth in a cell-free medium was subsequently initiated, and serial cultures were maintained for 16 months thereafter.

The basic medium was autoclaved liver broth, used with or without peptone, and supplemented with a crude extract from raw liver prepared as a chilled, acid infusion and sterilized by Seitz filtration (Stoll, 1953). When, in addition, mucin is supplied to this medium of combined heat-stable and heat-labile liver infusions, there is striking enhancement in yield and survival of the entamoebae. No antibiotics are employed. The cultures are vaseline sealed.

One interest that attaches to the successful continuous cultivation of *E. invadens* axenically in the absence of cells is that it may be a step toward a similar result with such a species of the genus as *E. histolytica,* the pathogenic amoeba of man. Another is that the avidity for mucin displayed by *E. invadens* in axenic culture, as found by Ratcliffe (1934) in mixed bacterial cultures with the parasite, may be a clue to the natural state of affairs in the host-parasite relation of this genus.

NORMAN R. STOLL
Rockefeller Institute

Hypothesis for Mechanism of Action of Chymotrypsin

Investigations of the active site of chymotrypsin have shown that a serine molecule is acylated during the reaction, and they have elucidated a partial peptide sequence in the neighborhood of this serine residue. Kinetic investigations have

implicated histidine as part of the catalytically active center. Many studies have delineated the chemical and stereochemical specificities of the enzyme toward substrates and inhibitors.

The foregoing data, obtained by numerous investigators, form the basis for the following detailed mechanism for the action of chymotrypsin. It is here assumed that the protein in the neighborhood of the active site is coiled in a right-handed alpha helix, and that the amino acid sequence is His-*X*-Gly-Asp-Ser-Gly-Glu-Ala-Val, where *X* is some unassigned amino acid. (This sequence, except for the histidine and *X*, is the one previously established.) The alpha helix brings the serine, histidine, and aspartate ion groups into juxtaposition. In the Michaelis complex, the phenyl group of a typical substrate lies between methyl and isopropyl groups; in the reaction complex, serine and aspartate ions are hydrogen-bonded to the substrate as it reacts with the imidazole group of histidine; the model predicts an easy transfer of an acyl group between serine and histidine. The reaction complex explains both the chemical and the stereochemical specificities of the enzyme and several peculiarities found in studies of kinetics and inhibition. The mechanism is illustrated by photographs of scale models, which show the fit of enzyme and substrate —of "lock and key"—for chymotrypsin.

F. H. WESTHEIMER
Harvard University

Cardiac Mechanisms that Limit Operation of Ventricular Suction

Recent experimental evidence indicates that the relaxing ventricles can develop suction under certain artificial experimental conditions [Brecher, *Circulation Research* (Jan. 1958)]. Before such evidence can be used to revive the discarded concept that ventricular filling of the normally beating heart is aided by, or due to, aspiration, it is necessary to consider the way in which the cardiac pump operates.

During early moments of ventricular relaxation, elastic stresses created during contraction are released. Thus the effective left ventricular pressure falls, say, from 100 to 10 mm-Hg in approximately 0.08 sec. If blood could enter the ventricular chamber during this phase of diastole, such a rapid drop in pressure would unquestionably constitute a potent aspirating force. However, both the inlet and outlet valves remain closed until this major fall of pressure has taken place. It is not until a succeeding interval of about 0.1 sec that blood rushes from the atria into the ventricles. An aspirating force could, therefore, aid the positive left atrial pressure in transferring blood only during this short interval.

Crucial evidence is still required that the small remnant of elastic recoil still operative at the end of relaxation can create sufficient suction to be of significance in filling the normally-beating heart. Dynamically it must be shown that the concordant declines of atrial and ventricular pressures are due to a more rapid rate of ventricular relaxation than of filling from the atria.

CARL J. WIGGERS
Frank E. Bunts Educational Institute

Lysogenic Conversion in Salmonella typhimurium

Salmonella typhimurium strain LT2 becomes serologically different following its lysogenization by phage P22. The difference resides in a well-known diagnostic antigen of the *Salmonella* group: somatic antigen O1.The lysogenized cells are agglutinable by anti-O1 serum, while the nonlysogenized cells are not. "Curing" not only causes loss of the bacteriophage but also the antigen O1. All attempts to demonstrate a masked antigen O1 in the nonlysogenic cells have failed. There is no obvious serological relationship between the O1 antigen and any antigens in the bacteriophage as demonstrated by unsuccessful attempts at cross absorption of sera. The new antigen is detectable about 7 minutes following infection. It is not only produced by the temperate phage but also by virulent mutants of this phage. It is therefore not a consequence of lysogenization. The chemical nature of this antigen is unknown.

A certain relative of phage P22 does not cause the production of antigen O1 but rather some as yet unclassified antigen. This phage is also partially serologically different from P22. The genes controlling these two properties have been mapped by crossing the two phages. It would seem that these properties are controlled by unit factors, for the segregants exhibit only parental properties and not intermediate ones. As yet it has proved impossible to separate by recombination the serological specificity of the phage and its conversion property, indicating that the same gene may be involved. The meaning of this and related phenomena was discussed.

NORTON D. ZINDER
Rockefeller Institute

Identification of Blood Characteristics Common to Alcoholic Males

A series of 53 alcoholic men and 41 control individuals entered into this study. The control individuals had demonstrated the ability to consume alcoholic liquors in moderation. Basal blood chemistry, basal blood morphology, and urine chemistry were studied, as well as other items, including the reaction of the individuals to prolonged glucose tolerance tests.

Of a group of items chosen because preliminary studies indicated that they might show significant differences, 11 were found to be significantly different at high confidence levels. These were: (i) total leukocyte count, (ii) lymphocyte count, (iii) eosinophil count, (iv) serum sodium, (v) serum potassium, (vi) serum calcium, (vii) blood glucose, (viii) urinary creatinine, (ix) urinary hippuric acid, (x) urinary sodium, and (xi) urinary chloride.

While the evidence is far from complete, there is a strong presumption that a number of these items are under genetic control; if this is the case, we have laid the groundwork for tests which may be applied to the identification of alcoholism-prone individuals during youth.

ROGER J. WILLIAMS, RICHARD B. PELTON, HERTTA-MAIJA HAKKINEN, LORENE L. ROGERS
Clayton Foundation for Research, Biochemical Institute, and *Department of Chemistry, University of Texas, Austin, Texas*

Ultraviolet Color Translating Microscope—New Tool for Studying Anatomy of Living Systems

This paper reports initial results with the ultraviolet color-translating microscope, a new instrument which uses a combination of sequential color television and ultraviolet microspectrophotometric techniques. A brief discussion of the operation is given [V. K. Zworykin and F. L. Hatke, *Science* **126**, 805 (1957)]. In the instrument the specimen is illuminated sequentially by three selected wavelengths in the ultraviolet or visible and the image reproduced on a color television receiver. The minimum bandwidth in the present instrument is 5 mμ, which permits the differentiation of small absorption shifts in the specimen. The ultraviolet dosage to the specimen is kept to a minimum by ultrafractionating the light into approximately 1-msec bursts, 1/60 sec apart. There is evidence to indicate that such intermittent illumination reduces radiation damage to the specimen. Previous methods of examining living tissue required photography or a continuous illumination using an ultraviolet-sensitive Vidicon. The reduced ultraviolet dosage using a special image Orthicon has permitted the continuous observation of such specimens as tissue cultures, muscle fibers, and connective tissues for periods which would previously have caused rapid necrosis and death.

In addition to color photographs of living unfixed specimens, motion pictures showing initial successful application to the study of amoeboid cells, Kupfer cells, capillary circulation, mast cells, and other elements in connective tissues in the mesentery, all in the living state, are shown. Some suggestions for the development of improved optics were made.

V. K. ZWORYKIN
Radio Corporation of America
F. HATKE, C. BERKLEY
Rockefeller Institute