National Academy Of Sciences Autumn Meeting

The National Academy of Sciences held its Autumn Meeting at Rice University, Houston, Texas, on October 19-21, 1970. Three symposia and two sessions of contributed papers comprised the scientific program. Abstracts of the contributed papers appear in this issue of the Proceedings.

Monday Evening Public Lecture—

DR. MICHAEL DeBAKEY, Baylor University College of Medicine: Cardiac Replacement: A Bioengineering Challenge

SCIENTIFIC SESSIONS

Monday Afternoon:

SYMPOSIUM ON THE GULF OF MEXICO

Chairman: HORACE R. BYERS

HORACE R. BYERS, Texas A&M University, College Station, Texas: The Gulf of Mexico, the Great North American Sink.

ANTON HALES, University of Texas, Dallas, Texas: Crust and Upper Mantle Structure in the Region of the Gulf of Mexico.

WILLIAM BRYANT, Texas A&M University, College Station, Texas: Geology as Revealed by Cores.

WORTH D. NOWLIN, JR., Texas A&M University, College Station, Texas: Circulation of the Waters of the Gulf.

ROBERT J. MENZIES, Florida State University, Tallahassee, Florida: Benthic Biology and Ecology of the Gulf.

JACK R. VAN LOPIK, Louisiana State University, Baton Rouge, Louisiana: The Coastal Zone—Problems and Prognosis.
Tuesday Morning:

SYMPOSIUM ON ENGINEERING IN MEDICINE AND BIOLOGY

Chairman: J. D. Hellums
Rice University
Houston, Texas

Richard J. Johns, Johns Hopkins School of Medicine, Baltimore, Maryland: Where is Biomedical Engineering Going?

Arne Troelstra, Rice University, Houston, Texas: The Eye as a Light Detector: Sensitivity, Dynamics, and Fluctuations.

Fred B. Vogt, University of Texas, Austin, Texas: Engineering and the Problems of Our Urban Society.

Homer R. Warner, Latter Day Saints Hospital, Salt Lake City, Utah: Computer Processing of Physiological Signals.

Constantine D. Armeniades, Rice University, Houston, Texas: Biomaterials Research in Artificial Circulatory Devices.

Wednesday Morning:

SYMPOSIUM ON PHYSICS IN SPACE

Chairman: William W. Rubey
Lunar Science Institute
Houston, Texas

Gary Latham, Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York: Results from the Apollo Passive Seismic Experiment.

F. C. Michel, Rice University, Houston, Texas: Interaction of the Solar Wind with the Moon and Planets.

Frank Low, Rice University, Houston, Texas: Recent Findings in Infrared Astronomy.

Herbert Friedman, Hulburt Center for Space Research, United States Naval Research Laboratory, Washington, D.C.: X-Ray and Gamma-Ray Astronomy.
CONTRIBUTED PAPERS

Tuesday Morning Session

Session Chairman: Richard B. Turner

Energy Transfer in Xylene-Biacetyl Systems: W. Albert Noyes, Jr. and D. A. Harter

Direct Fluorination of Organic and Inorganic Substances: J. L. Margrave, R. J. Lagow, and A. P. Conroy

The Heats of Hydrogenation of cis- and trans-2-Methylhexatriene: Resonance or Sigma Bond Energy?: Richard B. Turner and Milos Tichy

The Reaction of Trityl Radical with Thiophenol; Isotope Effects: Edward S. Lewis and M. Butler

The Ages of Lunar Maria: Luciano B. Ronca

The Role of Laboratory Studies in Space Science: Ronald F. Stebbings

Nepheloid Layer in the Gulf of Mexico: Maurice Ewing, Edward Thorndike, and Lawrence Sullivan

Crustal Section from Seismic Refraction Measurements near Victoria, Texas: J. Lamar Worzel, James Dorman, Robert Leyden, and Michael Hatziemmanuel

Wednesday Morning Session

Session Chairman: William E. Gordon

The Observability of Single Atoms in Biological Molecules: G. T. Trammell and J. R. Breedlove

On the Nature of Primordial Nuclei Acids: Joseph Nagyvary and Roberto G. Provenzale

Biosynthesis of dTTP in Escherichia coli. dCTP Deaminase, a New Enzyme Operative in the Pathway: Gerard A. O'Donovan and Jan Neuhard

Isozyme Activity During Early Development of Diploid and Androgenetic Haploid and Hybrid Frog Embryos: Stephen Subtelny and David A. Wright

Action of the NGF on Synthesis of Neurofilaments and Neurotubules in the Target Nerve Cells: Rita Levi-Montalcini and Pietro U. Angeletti

Louisiana Marshes and Estuaries: A Renewable Resource: Sherwood M. Gagliano

Should the Science-Based Food Industry be Expected to Advance?: Roger J. Williams

New Methodology to Reduce the Environment-Heredity Uncertainty about Dysgenics: William Shockley

Abstracts of Papers Presented at the Autumn Meeting
Houston, Texas, 19–21 October 1970

Angeletti, R. Hogue, R. A. Bradshaw, and R. G. Wade............. 5A
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Gagliano, Sherwood M............................................... 6A
Levi-Montalcini, Rita, and Pietro U. Angeletti......................... 7A
Lewis, Edward S., and M. Butler..................................... 7A
Margrave, J. L., R. J. Lagow, and A. P. Conroy...................... 8A
Nagyvary, Joseph, and Roberto G. Provenzale........................ 8A
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O’Donovan, Gerard A., and Jan Neuhard.............................. 10A
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Shockley, William..................................................... 10A
Stebbings, R. F.......................................................... 11A
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Structural Studies on Mouse Submaxillary Gland Nerve Growth Factor

Nerve growth factor (NGF) stimulates the growth of sympathetic and embryonic sensory nerve cells, and is necessary for the maintenance of their vitality in tissue culture. The physiological importance of this protein has been accentuated by the demonstration that antibodies specific for purified NGF cause the destruction of the sympathetic nervous system. Toward clarifying the molecular basis for its mechanism of action, we have begun to determine the primary structure of mouse submaxillary gland NGF. The subunit structure and amino acid composition of the protein have been established. Sedimentation equilibrium analysis of NGF in aqueous solution has confirmed previous reports of a molecular weight of 29,000 for the native molecule. Similar experiments in 6 M guanidine-HCl yielded a molecular weight of about 14,500 both for native NGF and for fully reduced and S-carboxymethylated NGF. These results suggest that native NGF is a dimer of two polypeptide chains. Support for these conclusions was obtained from direct structural analysis of the NGF molecule. Quantitative determination of the amino end group revealed the presence of two serine residues per molecule of native NGF. Analysis of the tryptic digest of S-[14C]carboxymethyl NGF resulted in the isolation of fifteen major peptides, or about half of the value predicted for a monomer of 29,000 molecular weight or a dimer of nonidentical subunits. Five of these peptides contained a total of six unique half-cystinyl residues, instead of the twelve expected. These results support the conclusion that native NGF of molecular weight 29,000 is composed of two identical, or very similar, subunits of 14,500 molecular weight. The two units, each of which contains three intramolecular disulfide bonds, are associated by noncovalent forces in the native molecule.

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Crustal Section from Seismic Refraction Measurements Near Victoria, Texas

Combined microearthquake and seismic refraction observations have been carried out on the Gulf coastal plain south of Victoria, Texas. A total of 108 seismograms with durations up to 24 hr each were written during June and July 1970. Included in these data are recordings of nine explosions detonated for two unreversed seismic refraction profiles. Though work is still in progress, it seems doubtful that any of the unidentified events recorded are local microearthquakes. Amplitudes of refracted arrivals across the strike were 20-40 times weaker than along the strike. Therefore, it was impractical to continue a refraction profile more than 40 km across the strike, although parallel to the coastline a profile was extended to 143 km, well into the range where first
arrivals are refracted through the mantle of the earth. The latter profile, which passes about 20 km southeast of Victoria, shows a thickness of about 30 km for the crust of the earth, including a 9-km sedimentary section. The crustal thickness thus appears to be about 3 km less, and the sedimentary thickness about the same, as on a parallel profile about 60 km inland measured by Cram (1961). A gradual seaward thinning of the continental crust may be indicated. This fact, together with the apparently uniform sedimentary thickness, if confirmed by further measurements, defines a type of continental margin distinctly different from others which have been more thoroughly investigated. We believe that techniques tested in this work offer a means of detailing the Gulf Coastal Margin.

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Nepheloid Layer in the Gulf of Mexico

Variation of light scattering with depth at 48 stations in the Gulf of Mexico is reported and used to establish that a nepheloid layer is a feature of the bottom waters of this region. Bottom currents with velocities of 4-8 cm/sec were measured at some of these stations. Photographs of the sea floor at all stations have been analyzed for evidence of a nepheloid layer and of currents. Scattering intensities are greatest in the northern and eastern parts of the Gulf, while the southwest area shows relatively clear water. From these data, inferences about the water circulation in the Gulf of Mexico are made in relation to its sill depth boundaries of 2,000 m in the Yucatan Channel and 800 m in the Florida Straits.

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Louisiana Marshes and Estuaries: A Renewable Resource

Coastal Louisiana wetlands are a product of Mississippi River Delta building that has occurred over a period of 3,000 to 5,000 years. The building process was a gradual one, for riverine and marine processes were very nearly balanced. In modern times man's use of the area (flood control, navigation improvement, exploitation of petroleum and other minerals, road building, etc.) has seriously altered the natural balance. As a result, overbank flooding has been virtually eliminated and river flow is confined to channels discharging into the outer shelf area. Most transported sediment is now deposited in the deep Gulf or along the continental shelf. Saltwater encroachment in the deltaic estuaries has been detrimental to fauna and flora. Even though considerable sediment deposition has resulted from the historic Atchafalaya River diversion and growth of subdeltas, comparative map studies indicate a net land loss rate of 16.5 square miles per year during the last 25-30 years. Land loss is only one symptom of general environmental deterioration.

A dynamic management plan is proposed for better utilization of combined discharge of fresh water and input of dissolved solids and transported sediments from the Mississippi River. Controlled flow into estuaries will reduce salinity encroachment and supply badly-needed nutrients. Large areas of new marshland and estuarine habitat can be built by controlled subdelta diversion. Studies of natural subdeltas indicate that these systems are amenable to en-
vironmental management; salinities and sediment deposition may be manipulated to enhance desired conditions.

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Action of Nerve Growth Factor on Synthesis of Neurofilaments and Neurotubules in the Target Nerve Cells

The most outstanding feature of the effects of NGF is the massive and rapid production of neurofilaments and neurotubules in the target nerve cells. Embryonic sensory and sympathetic nerve cells cultured in the presence of NGF produce within a few hours a large amount of filamentous material which assembles in neurofilaments and neurotubules and converges toward the rapidly outgrowing axon. The same effect obtains in vivo both in newborn and adult animals. The dramatic hypertrophy induced by the NGF in sympathetic neurons is due to overproduction of neurofilaments and neurotubules which fill the cytoplasm and gather to form large bundles of nerve fibers. As a result, hyperinnervation of peripheral organs and tissues follows.

The growth stimulation of neurofilaments and neurotubules induced by NGF requires net protein synthesis and is not completely dependent on messenger RNA synthesis.

Colchicine and vinblastine at the respective concentrations of $10^{-7}$ and $10^{-8}$M completely prevent the outgrowth of nerve fibers in vitro. In adult mice, vinblastine (3 mg/kg) counteracts the volume increase elicited by NGF in sympathetic ganglia. Ultrastructural studies demonstrate that in the presence of the vinca alkaloid the NGF effect does not fully materialize. The synthesis of neurofilament material is still markedly stimulated, but its polymerization into organized structures is prevented.

These results suggest that NGF does not act on the assembly of filamentous proteins into neurofilaments and neurotubules but primarily on their synthesis. These and other observations to be reported favor the concept that these organelles are very dynamic structures within nerve cells, possibly endowed with broader functional significance than currently assumed.

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Reaction of Trityl Radical with Thiophenol; Isotope Effects

The reaction between “hexaphenylmethane” (I) and thiophenol yields mainly triphenylmethane and tritylphenyl sulfide at $25^\circ$C or higher, but at lower temperatures the isomerization of I to $p$-trityltriphenylmethane (II) predominates. Diphenyldisulfide is not found. A tritium label on the sulfhydryl group leads to tritium incorporations in both triphenylmethane and II.

The following mechanism is consistent with these observations:

$$\phi_3C\text H\rightarrow \phi_3CH + S\phi$$ (2)

$$\phi_3C\phi + HS\phi\rightarrow \phi_3CS\phi + S\phi$$ (3)

$$\phi S\phi + I\rightarrow \phi_3C\phi + SH$$ (4)

Assuming this mechanism, the tritium isotope effect can be calculated for reactions (2) and (5) from the activities of triphenylmethane and II and that of the thiophenol. The isotope effect on (2) is
large enough to suggest either major loss of bending vibrations in the transition state, or more likely a contribution of tunnelling. The isotope effect is larger than that in the attack of less stable radicals on thiophenol.

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Direct Fluorination of Organic and Inorganic Substances

A new technique has been developed—the La-Mar Process—for using elemental fluorine to prepare partially and perfluorinated organic and inorganic compounds. Thus, several polynuclear aromatic compounds (naphthalene, anthracene, tetracene, pentacene, coronene, etc.) have been converted to their perfluoroperhydro-derivatives. Aliphatic hydrocarbons (\(\text{C}_{12} \ldots \text{C}_{18} \ldots\), etc.) and related polymers (polyethylene, polypropylene, etc.) have been perfluorinated. Polystyrene, polycarbonates, natural rubber, adamantane, and many other materials have been fluorinated.

One can fluorinate either powders or liquids or surface-fluorinate shaped objects—thin films, beakers, bottles, tubes, etc.—and control the extent of fluorination by proper choice of parameters like temperature, flow rate, time of exposure, pressure of fluorine, etc.

Fluorination of compounds with functional groups—esters, acids, phenol and related compounds, etc.—is currently in progress. The fluorination of inorganic hydrides (\(\text{MBH}_4\), \(\text{MAI}_4\), \(\text{MH}_2\), \(\text{B}_{10}\text{H}_{14}\), carboranes, etc.) leads to perfluoro-analogs.

When graphite is fluorinated, one produces a solid product, \(\text{CF}_x\), which ranges in stoichiometry from \(\text{CF}_{6.7}\) (gray) to \(\text{CF}_{1.1}\) (white). This material is an outstanding solid lubricant, comparable with graphite or MoS\(_2\). It has excellent thermal stability, oxidation resistance and retains its lubricating character under heavy loads and in vacuum.

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On the Nature of Primordial Nucleic Acids

Current research on the origin of life has advanced one-sidedly on proteinoids, while there is no agreement yet on the prebiological formation of informational macromolecules. It is not even certain that the primitive polynucleotides consisted of the presently known nucleotides. Indeed, the organic chemist is unable to polymerize common nucleotides under presumed primitive-earth conditions. Research in our laboratory proceeds now under the consideration of three new concepts which are based on the assumption of "unnatural" polynucleotide analogs.

1. The thermodynamic argument. The chemical evolution of polynucleotides had its start with prototypes of high thermodynamic stability and high entropy. The priority of the currently natural polynucleotides is ruled out since the same conditions which lead to their formation also cause their degradation. Polynucleotides containing arabinose instead of ribose or deoxyribose fulfill our criteria best. According to the observations made in the laboratory of Schramm and ours, polyarabinonucleotides are uniquely stable. This property could have ensured the continuous growth of the chain length. Poly aU is completely devoid of any secondary structure and is incapable of expressing the information that is carried in its rigidly locked uracil moieties. For this reason, poly aU represents a sort of sleeping beauty of evolution which could have been revived by a configuration change to poly rU. Several ways of arabino \(\rightarrow\) ribo conversion are feasible.
2. Evolutionary role of thiophosphate. While the contemporary thinking is restricted to phosphates, thiophosphate and its polymers should have been very common in the sulfur-rich, oxygen-free, reducing atmosphere of the primitive earth. As a consequence, nucleotide analogs with sulfur substitution in both the heterocyclic and carbohydrate moieties are expected to have been a dominant type. We have obtained such a thiopolymer from thymidine and polythiophosphate.

3. The mechanism of polymerization. The mechanism via phosphate activation is widely held and is, indeed, supported by the principle of continuity. However, it is inherently inefficient in aqueous solution. We feel that a displacement reaction involving an activated primary carbon and a phosphate could be a more suitable mechanism of primitive phosphorylation, since it is possible to bring about the reaction in water and at higher temperature. The possible involvement of thiophosphate, the most powerful nucleophile hitherto known, would extend the temperature range down to 0°C. We have carried out such polymerizations and studied the prototypical poly 5'-thiodeoxyribonucleotides. Optical rotatory dispersion and circular dichroism data show that these polyribonucleotides possess secondary structures. This new approach raises the question as to the nature of prebiotic carbon activation.

Finally, our interpretation of recent results obtained in our and other laboratories suggest that pyrimidine polynucleotides could have evolved prior to the purine polynucleotides.

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Energy Transfer in Xylene–Biacetyl Systems

One method of measuring triplet state formation in photochemical systems is to preferentially excite emission from the triplet state of biacetyl by energy transfer from the triplet state of the donor molecule. For certain molecules, particularly simple aromatic molecules, the lowest excited singlet state lies high enough to cause excitation of biacetyl to its second excited singlet state, and biacetyl molecules excited to this state form the first excited singlet state with negligible yield. If this were not so one would necessarily have to take account of the essentially quantitative cross-over of biacetyl molecules in the first excited singlet state to the lowest-lying triplet state, thus leading to phosphorescent emission. This would, of course, invalidate calculation of triplet-state yields of aromatic molecules.

This method was first applied by Ishikawa (to benzene). The method has also been used for several other simple aromatic molecules and at least in some cases there is good agreement with triplet-state yields based on the isomerization of cis-2-butene as proposed by Cundall. However there began to appear cases in which the sum of fluorescent yield and triplet-state yield exceeded unity by more than the experimental error and this has led to a detailed reexamination of the biacetyl method. The method was reasonably successful for benzene because the biacetyl pressure was low, about 0.1 torr. At this pressure, excited benzene molecules in the \(^1A_\text{so}\) state transferred negligible excitation to the biacetyl because of their short lifetimes (about 75 nsec, as shown by E. K. C. Lee). It can now be shown that at higher pressures biacetyl molecules are excited to both the first and second excited singlet states, and the former cross over to the lowest triplet state and phosphoresce. The kinetics of the system have been worked out for the xylenes and will be discussed.

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Biosynthesis of dTTP in *Escherichia coli*. dCTP Deaminase, a New Enzyme Operative in the Pathway

A strain of *Escherichia coli* HD1038, isolated as a pyrimidine over-producer, was found to contain a dCTP pool 10 times as large and a dTTP pool half as large as in the parent strain. Mutant and parent strains grow with the same generation time in minimal medium. Addition of any pyrimidine deoxyribonucleoside to the growth medium resulted in a marked increase in the dCTP pool in the mutant strain and a reduction of the dCTP pool to normal levels. The addition of ribonucleosides or purine deoxyribonucleosides to the medium did not alter the anomalous pool sizes significantly.

Although thymine-stressed mutants display characteristics quite similar to the mutant described here (i.e. high dCTP, low dTTP) they differ markedly in other respects. Unlike thymine-stressed strains, HD1038 has a low DUMP pool and its pool sizes return to normal on deoxyribosine addition.

A new pathway for the synthesis of DUMP was described recently for *Salmonella typhimurium* and a novel enzyme, dCTP deaminase, was isolated. In this paper we describe *E. coli* mutant deficient in this enzyme. The isolation of such a mutant allows dTTP biosynthesis to be examined more closely than was hitherto possible. The biosynthesis in the mutant and in the parent strain of *E. coli* is discussed.

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The Ages of Lunar Maria

The previously presented geomorphologic index (Geol. Soc. Amer. Bull., 81, pp. 337–352) is a quantitative description of the geomorphology of lunar surfaces. In the case of the maria but not of the highlands, the geomorphic index is proportional to the age of the mare surfaces, thus permitting the determination of their relative ages. The geomorphic indices of the maria show that each mare surface is not the result of a single effusion, but of several that occurred over a span of time. From a stratigraphic point of view, there is a lateral interfingering of the effusions of different maria. Certain maria, such as Tranquillitatis, have older surfaces than other maria, meaning that effusions started earlier in some maria than in others, or that older effusions were buried by younger ones. Young effusions are lacking on the surface of certain maria, such as Humorum, indicating that the end of the effusive activity was not contemporaneous for all the maria.

A preliminary translation of the geomorphic index into age in years is attempted by using the Apollo 11 and 12 radiogenic ages. By using the range of values obtained by four models, the most recent effusion can have any age between zero and $2.6 \times 10^9$ years. Older effusions, however, can be dated with a smaller range. The oldest appear to have an age of more than $4 \times 10^9$ years. The principal conclusion of the study is that the mare effusions were not a relatively short-lived phenomenon but occurred throughout a considerable portion of the lunar geological history.

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New Methodology to Reduce the Environment-Heredity Uncertainty About Dysgenics

of racial composition, estimated from "tracer genes," on IQ for half-siblings in environments controlled to the degree of being in the same illegitimate nonwhite slum family. A new proposal combines improved accuracy in determining racial composition of populations (T. E. Reed, Science 22 Aug 70 finds 22 \pm 1% Caucasian genes in Oakland, California, negroes) with population IQ differences (R. Heber describes environmentally matched Milwaukee school populations averaging 30 IQ points difference depending on mother's IQ being above or below 80). The hypothesis that the difference arises from racial mixes of 33 and 11% for the two populations respectively results in the testable prediction of 84 versus 30 instances of Reed's Caucasian gene for the two groups of 300 children each. A Negro university offers a comparable research possibility for which environmental influences of Caucasian genes might be negative rather than positive. Racial compositions could be determined for test populations selected on the basis of higher and lower performance on both nationally standardized tests and alternatively on any locally accepted measures of intellectual achievement. Resulting facts would be relevant to important national social objectives. Estimates of the variance of racial composition support the feasibility of this proposal.

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Role of Laboratory Studies in Space Science

During the past two decades space research has been greatly expanded and accelerated through the use of rockets, satellites, and ground-based telescopes. These have collectively provided the bulk of the primary data regarding the nature and composition of the earth's environment. The interpretation and understanding of these data depends on a knowledge of the collision properties of the various charged and neutral species present. These data have been provided primarily through laboratory investigations by means of a variety of experimental techniques. Of these some seek to accurately reproduce in the laboratory certain atmospheric phenomena while others isolate discrete atmospheric species, which are then studied as they react with each other. This paper will briefly but critically review the major laboratory techniques currently in use and identify major problem areas.

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Observability of Single Atoms in Biological Molecules

To form an image of a single molecule in which the images of the individual atoms are resolved, the wavelength of the illuminating radiation must be similar to or smaller than the interatomic spacing (\( \lambda < 1 \text{Å} \)), and each atom imaged must scatter (or absorb) at least one quantum of radiation. Although the effects of elastically scattered quanta on molecular structure may be ignored, the inelastic collisions will lead (with a certain probability) to molecular dissociation or rearrangement, and the "image" formed will be a composite picture of the molecule over the history of its irradiation and may bear little relation to its original structure. On the basis of estimates of the molecular damage caused in the observation process we conclude that "molecular microscopy" of biological molecules in which the individual atoms are resolved is impossible with an electron or x-ray microscope. Similar conclusions are reached with respect to microscopes using all other possible illuminating radiations, with the possible exception of neutrons.

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Isozyme Activity During Early Development of Diploid and Androgenetic Haploid and Hybrid Frog Embryos

Cross fertilization of geographic races of the frog, *Rana pipiens*, produces viable diploid hybrids. Starch gel electrophoresis of extracts from the different races of frogs and their hybrid progeny reveal different forms of certain isozymes. Distinct isozyme bands identifiable as maternal in origin are present from the very beginning of embryonic development, while the paternal and hybrid forms first appear as discrete bands in the extracts of hybrids at various post-neurula stages of development. Thus, paternal and "hybrid" molecules of phosphogluconate dehydrogenase (EC 1.1.1.-43) are first detected at the tail bud stage; the B subunit of lactate dehydrogenase appears slightly later, at the muscular response stage, while gene expression for soluble isocitrate dehydrogenase and mitochondrial malate dehydrogenase becomes evident still later, when heart beat is detected in the embryo.

Androgenetic haploid hybrids can be produced by removal of the nucleus from the egg of one race after artificial insemination with sperm from another race. Such haploids display different degrees of abnormalities during embryogenesis: some arrest at about the neurula stage, while others develop abnormally through hatching. We determined the paternal (nuclear) and the maternal (cytoplasmic) contributions to the various isozyme forms in these androgenetic haploid hybrids. The maternal and paternal forms of dehydrogenases are evident at the same time in embryonic development as the diploid controls, even though the haploids are grossly abnormal and retarded in morphogenesis. However, the "hybrid" isozymes, composed of maternal and paternal subunits, are not obtained with extracts from androgenetic haploid hybrids.

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Heats of Hydrogenation of cis- and trans-2-Methylhexatriene: Resonance or Sigma Bond Energy?

The heats of hydrogenation (acetic acid solution, 25°C) of *cis* - and trans-2-methylhexatriene have been measured in order to test the suggestion of M. J. S. Dewar and N. H. Schmeising that conjugative stabilization in butadiene results not from resonance interaction, but from a strengthening of the sp²–sp² sigma bond relative to sp³–sp³ sigma bonds in model systems. Cis- and trans-2-methylhexatriene were selected since the *cis* compound, but not the *trans*, should be nonplanar, a view derived from extensive previous work in the carotenoid and vitamin A fields.

The heat of isomerization (*cis* → *trans*) obtained for the methylhexatriene pair is −4.7 kcal/mol. The corresponding value for the unsubstituted hexatrienes (planar) is −1.1 kcal/mol (unpublished results of R. B. Turner and W. von E. Doering). If the conformational postulates are correct, planarity seems to be an important factor in conjugative stabilization. The results are thus consistent with the resonance hypothesis.

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Should the Science-Based Food Industry Be Expected to Advance?

In all other science-based industries (transportation, textiles, communication, electronics, etc.) we take periodic if not revolutionary advance for granted. Should not the food industry be providing us continuously with food of better and better quality?

That this is not being done in the crucial milling and baking industry is shown by the following experiment, 128 weanling rats of four strains were placed on two bread diets. Half of them received commercial "enriched" bread, the others received the same bread supplemented, in accordance with modern nutritional knowledge, with small amounts of minerals, vitamins, and one amino acid (lysine). The two breads were undistinguishable to the baker. The supplemented bread would cost perhaps 5% more.

After about 2 weeks the least responsive strain of rats was growing almost five times as rapidly on the supplemented bread; the most responsive strain grew eight times as fast. After 12 weeks two-thirds of the animals on the "enriched" bread had died of malnutrition while practically all the other animals were alive and growing.

Because of the consistency observed with four strains of rats, it is readily inferred that many other mammals, including man, would yield similar results.

"Enrichment" started in the baking and milling industry nearly thirty years ago. In the intervening years there has been no nutritional improvement or advance whatever. The quality of the nutrition furnished children and adults who consume "enriched" products as well as many processed foods is far below what scientific advance should demand.

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National Academy Of Sciences Autumn Meeting

The National Academy of Sciences held its Autumn Meeting at Rice University, Houston, Texas, on October 19–21, 1970. Three symposia and two sessions of contributed papers comprised the scientific program. Abstracts of the contributed papers appear in this issue of the Proceedings.

Monday Evening Public Lecture—

DR. MICHAEL DeBAKEY, Baylor University College of Medicine: Cardiac Replacement: A Bioengineering Challenge

SCIENTIFIC SESSIONS

Monday Afternoon:

SYMPOSIUM ON THE GULF OF MEXICO

Chairman: HORACE R. BYERS

HORACE R. BYERS, Texas A&M University, College Station, Texas: The Gulf of Mexico, the Great North American Sink.

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Tuesday Morning:

**SYMPOSIUM ON ENGINEERING IN MEDICINE AND BIOLOGY**

*Chairman:* J. D. HELLUMS  
Rice University  
Houston, Texas

**Richard J. Johns,** Johns Hopkins School of Medicine, Baltimore, Maryland:  *Where is Biomedical Engineering Going?*

**Arne Troelstra,** Rice University, Houston, Texas: *The Eye as a Light Detector: Sensitivity, Dynamics, and Fluctuations.*

**Fred B. Vogt,** University of Texas, Austin, Texas: *Engineering and the Problems of Our Urban Society.*

**Homer R. Warner,** Latter Day Saints Hospital, Salt Lake City, Utah: *Computer Processing of Physiological Signals.*

**Constantine D. Armeniades,** Rice University, Houston, Texas: *Biomaterials Research in Artificial Circulatory Devices.*

Wednesday Morning:

**SYMPOSIUM ON PHYSICS IN SPACE**

*Chairman:* William W. Rubey  
Lunar Science Institute  
Houston, Texas

**Gary Latham,** Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York: *Results from the Apollo Passive Seismic Experiment.*

**F. C. Michel,** Rice University, Houston, Texas: *Interaction of the Solar Wind with the Moon and Planets.*

**Frank Low,** Rice University, Houston, Texas: *Recent Findings in Infrared Astronomy.*

**Herbert Friedman,** Hulburt Center for Space Research, United States Naval Research Laboratory, Washington, D.C.: *X-Ray and Gamma-Ray Astronomy.*
CONTRIBUTED PAPERS

Tuesday Morning Session

Session Chairman: Richard B. Turner

Energy Transfer in Xylene-Biacetyl Systems: W. Albert Noyes, Jr. and D. A. Harter

Direct Fluorination of Organic and Inorganic Substances: J. L. Margrave, R. J. Lagow, and A. P. Conroy

The Heats of Hydrogenation of cis- and trans-2-Methylhexatriene: Resonance or Sigma Bond Energy?: Richard B. Turner and Milós Tichý

The Reaction of Trityl Radical with Thiophenol; Isotope Effects: Edward S. Lewis and M. Butler

The Ages of Lunar Maria: Luciano B. Ronca

The Role of Laboratory Studies in Space Science: Ronald F. Stebbings

Nepheloid Layer in the Gulf of Mexico: Maurice Ewing, Edward Thorndike, and Lawrence Sullivan

Crustal Section from Seismic Refraction Measurements near Victoria, Texas: J. Lamar Worzel, James Dorman, Robert Leyden, and Michael Hatziemmanuel

Wednesday Morning Session

Session Chairman: William E. Gordon

The Observability of Single Atoms in Biological Molecules: G. T. Trammell and J. R. Breedlove

On the Nature of Primordial Nuclei Acids: Joseph Nagyvary and Roberto G. Provenzale

Biosynthesis of dTTP in Escherichia coli. dCTP Deaminase, a New Enzyme Operative in the Pathway: Gerard A. O'Donovan and Jan Neuhard

Isozyme Activity During Early Development of Diploid and Androgenetic Haploid and Hybrid Frog Embryos: Stephen Subtelny and David A. Wright

Action of the NGF on Synthesis of Neurofilaments and Neurotubules in the Target Nerve Cells: Rita Levi-Montalcini and Pietro U. Angeletti

Louisiana Marshes and Estuaries: A Renewable Resource: Sherwood M. Gagliano

Should the Science-Based Food Industry be Expected to Advance?: Roger J. Williams

New Methodology to Reduce the Environment-Heredity Uncertainty about Dysgenics: William Shockley

Abstracts of Papers Presented at the Autumn Meeting
Houston, Texas, 19–21 October 1970

Angeletti, R. Hogue, R. A. Bradshaw, and R. G. Wade

Dorman, James, J. Lamar Worzel, Robert Leyden, and Michael Hatziemmanuel

Ewing, Maurice, Edward Thorndike, and Lawrence Sullivan

Gagliano, Sherwood M.

Levi-Montalcini, Rita, and Pietro U. Angeletti

Lewis, Edward S., and M. Butler

Margrave, J. L., R. J. Lagow, and A. P. Conroy

Nagyvary, Joseph, and Roberto G. Provenzale

Noyes, W. Albert, Jr., and D. A. Harter

O’Donovan, Gerard A., and Jan Neuhard

Ronca, L. B.

Shockley, William

Stebbings, R. F.

Subtelny, Stephen, and David A. Wright

Trammell, G. T., and J. R. Breedlove

Turner, Richard B., and Milos Tichy

Williams, Roger J.
Abstracts of Papers Presented at the Autumn Meeting
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Structural Studies on Mouse Submaxillary Gland Nerve Growth Factor

Nerve growth factor (NGF) stimulates the growth of sympathetic and embryonic sensory nerve cells, and is necessary for the maintenance of their vitality in tissue culture. The physiological importance of this protein has been accentuated by the demonstration that antibodies specific for purified NGF cause the destruction of the sympathetic nervous system. Toward clarifying the molecular basis for its mechanism of action, we have begun to determine the primary structure of mouse submaxillary gland NGF. The subunit structure and amino acid composition of the protein have been established. Sedimentation equilibrium analysis of NGF in aqueous solution has confirmed previous reports of a molecular weight of 29,000 for the native molecule. Similar experiments in 6 M guanidine-HCl yielded a molecular weight of about 14,500 both for native NGF and for fully reduced and S-carboxymethylated NGF. These results suggest that native NGF is a dimer of two polypeptide chains. Support for these conclusions was obtained from direct structural analysis of the NGF molecule. Quantitative determination of the amino end group revealed the presence of two serine residues per molecule of native NGF. Analysis of the tryptic digest of S-[¹⁴C]carboxymethyl NGF resulted in the isolation of fifteen major peptides, or about half of the value predicted for a monomer of 29,000 molecular weight or a dimer of nonidentical subunits. Five of these peptides contained a total of six unique half-cystinyl residues, instead of the twelve expected. These results support the conclusion that native NGF of molecular weight 29,000 is composed of two identical, or very similar, subunits of 14,500 molecular weight. The two units, each of which contains three intramolecular disulfide bonds, are associated by noncovalent forces in the native molecule.

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Crustal Section from Seismic Refraction Measurements Near Victoria, Texas

Combined microearthquake and seismic refraction observations have been carried out on the Gulf coastal plain south of Victoria, Texas. A total of 108 seismograms with durations up to 24 hr each were written during June and July 1970. Included in these data are recordings of nine explosions detonated for two unreversed seismic refraction profiles. Though work is still in progress, it seems doubtful that any of the unidentified events recorded are local microearthquakes. Amplitudes of refracted arrivals across the strike were 20–40 times weaker than along the strike. Therefore, it was impractical to continue a refraction profile more than 40 km across the strike, although parallel to the coastline a profile was extended to 143 km, well into the range where first
arrivals are refracted through the mantle of the earth. The latter profile, which passes about 20 km southeast of Victoria, shows a thickness of about 30 km for the crust of the earth, including a 9-km sedimentary section. The crustal thickness thus appears to be about 3 km less, and the sedimentary thickness about the same, as on a parallel profile about 60 km inland measured by Cram (1961). A gradual seaward thinning of the continental crust may be indicated. This fact, together with the apparently uniform sedimentary thickness, if confirmed by further measurements, defines a type of continental margin distinctly different from others which have been more thoroughly investigated. We believe that techniques tested in this work offer a means of detailing the Gulf Coastal Margin.

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Nepheloid Layer in the Gulf of Mexico

Variation of light scattering with depth at 48 stations in the Gulf of Mexico is reported and used to establish that a nepheloid layer is a feature of the bottom waters of this region. Bottom currents with velocities of 4-8 cm/sec were measured at some of these stations. Photographs of the sea floor at all stations have been analyzed for evidence of a nepheloid layer and of currents. Scattering intensities are greatest in the northern and eastern parts of the Gulf, while the southwest area shows relatively clear water. From these data, inferences about the water circulation in the Gulf of Mexico are made in relation to its sill depth boundaries of 2,000 m in the Yucatan Channel and 800 m in the Florida Straits.

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Louisiana Marshes and Estuaries: A Renewable Resource

Coastal Louisiana wetlands are a product of Mississippi River Delta building that has occurred over a period of 3,000 to 5,000 years. The building process was a gradual one, for riverine and marine processes were very nearly balanced. In modern times man's use of the area (flood control, navigation improvement, exploitation of petroleum and other minerals, road building, etc.) has seriously altered the natural balance. As a result, overbank flooding has been virtually eliminated and river flow is confined to channels discharging into the outer shelf area. Most transported sediment is now deposited in the deep Gulf or along the continental shelf. Saltwater encroachment in the deltaic estuaries has been detrimental to fauna and flora. Even though considerable sediment deposition has resulted from the historic Atchafalaya River diversion and growth of subdeltas, comparative map studies indicate a net land loss rate of 16.5 square miles per year during the last 25-30 years. Land loss is only one symptom of general environmental deterioration.

A dynamic management plan is proposed for better utilization of combined discharge of fresh water and input of dissolved solids and transported sediments from the Mississippi River. Controlled flow into estuaries will reduce salinity encroachment and supply badly-needed nutrients. Large areas of new marshland and estuarine habitat can be built by controlled subdelta diversion. Studies of natural subdeltas indicate that these systems are amenable to en-
vieronmental management; salinities and sediment deposition may be manipulated to enhance desired conditions.

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Action of Nerve Growth Factor on Synthesis of Neurofilaments and Neurotubules in the Target Nerve Cells

The most outstanding feature of the effects of NGF is the massive and rapid production of neurofilaments and neurotubules in the target nerve cells. Embryonic sensory and sympathetic nerve cells cultured in the presence of NGF produce within a few hours a large amount of filamentous material which assembles in neurofilaments and neurotubules and converges toward the rapidly outgrowing axon. The same effect obtains in vivo both in newborn and adult animals. The dramatic hypertrophy induced by the NGF in sympathetic neurons is due to overproduction of neurofilaments and neurotubules which fill the cytoplasm and gather to form large bundles of nerve fibers. As a result, hyperinnervation of peripheral organs and tissues follows.

The growth stimulation of neurofilaments and neurotubules induced by NGF requires net protein synthesis and is not completely dependent on messenger RNA synthesis.

Colchicine and vinblastine at the respective concentrations of $10^{-7}$ and $10^{-8}$ M completely prevent the outgrowth of nerve fibers in vitro. In adult mice, vinblastine (3 mg/kg) counteracts the volume increase elicited by NGF in sympathetic ganglia. Ultrastructural studies demonstrate that in the presence of the vinca alkaloid the NGF effect does not fully materialize. The synthesis of neurofilament material is still markedly stimulated, but its polymerization into organized structures is prevented.

These results suggest that NGF does not act on the assembly of filamentous proteins into neurofilaments and neurotubules but primarily on their synthesis. These and other observations to be reported favor the concept that these organelles are very dynamic structures within nerve cells, possibly endowed with broader functional significance than currently assumed.

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Reaction of Trityl Radical with Thiophenol; Isotope Effects

The reaction between "hexaphenylmethane" (I) and thiophenol yields mainly triphenylmethane and tritylphenyl sulfide at $25^\circ$C or higher, but at lower temperatures the isomerization of I to p-trityltriphenylmethane (II) predominates. Diphenyl disulfide is not found. A tritium label on the sulphydryl group leads to tritium incorporations in both triphenylmethane and II.

The following mechanism is consistent with these observations:

\[
\phi_3C + \text{HS} \rightarrow \phi_3CH + \text{S} (2)
\]

\[
\phi_3C + \text{S} \rightarrow \phi_3CS (3)
\]

\[
(1)
\]

Assuming this mechanism, the tritium isotope effect can be calculated for reactions (2) and (5) from the activities of triphenylmethane and II and that of the thiophenol. The isotope effect on (2) is
large enough to suggest either major loss of bending vibrations in the transition state, or more likely a contribution of tunnelling. The isotope effect is larger than that in the attack of less stable radicals on thiophenol.

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Direct Fluorination of Organic and Inorganic Substances

A new technique has been developed—the La-Mar Process—for using elemental fluorine to prepare partially and perfluorinated organic and inorganic compounds. Thus, several polynuclear aromatic compounds (naphthalene, anthracene, tetracene, pentacene, coronene, etc.) have been converted to their perfluoro-derivatives. Aliphatic hydrocarbons (C_{12}...C_{18}... etc.) and related polymers (polyethylene, polypropylene, etc.) have been perfluorinated. Polystyrene, polycarbonates, natural rubber, adamantane, and many other materials have been fluorinated.

One can fluorinate either powders or liquids or surface-fluorinate shaped objects—thin films, beakers, bottles, tubes, etc.—and control the extent of fluorination by proper choice of parameters like temperature, flow rate, time of exposure, pressure of fluorine, etc.

Fluorination of compounds with functional groups—esters, acids, phenol and related compounds, etc.—is currently in progress. The fluorination of inorganic hydrides (MBH_4, MAIH_4, MH_2, B_{10}H_{14}, carboranes, etc.) leads to perfluoro-analogs.

When graphite is fluorinated, one produces a solid product, CF_2, which ranges in stoichiometry from CF_{0.7}(gray) to CF_{1.1}(white). This material is an outstanding solid lubricant, comparable with graphite or MoS_2. It has excellent thermal stability, oxidation resistance and retains its lubricating character under heavy loads and in vacuum.

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On the Nature of Primordial Nucleic Acids

Current research on the origin of life has advanced one-sidedly on proteinoids, while there is no agreement yet on the prebiological formation of informational macromolecules. It is not even certain that the primitive polynucleotides consisted of the presently known nucleotides. Indeed, the organic chemist is unable to polymerize common nucleotides under presumed primitive-earth conditions. Research in our laboratory proceeds now under the consideration of three new concepts which are based on the assumption of “unnatural” polynucleotide analogs.

1. The thermodynamic argument. The chemical evolution of polynucleotides had its start with prototypes of high thermodynamic stability and high entropy. The priority of the currently natural polynucleotides is ruled out since the same conditions which lead to their formation also cause their degradation. Polynucleotides containing arabinose instead of ribose or deoxyribose fulfill our criteria best. According to the observations made in the laboratory of Schramm and ours, polyarabinonucleotides are uniquely stable. This property could have ensured the continuous growth of the chain length. Poly aU is completely devoid of any secondary structure and is incapable of expressing the information that is carried in its rigidly locked uracil moieties. For this reason, poly aU represents a sort of sleeping beauty of evolution which could have been revived by a configuration change to poly rU. Several ways of arabino → ribo conversion are feasible.
2. Evolutionary role of thiophosphate. While the contemporary thinking is restricted to phosphates, thiophosphate and its polymers should have been very common in the sulfur-rich, oxygen-free, reducing atmosphere of the primitive earth. As a consequence, nucleotide analogs with sulfur substitution in both the heterocyclic and carbohydrate moieties are expected to have been a dominant type. We have obtained such a thiopolymer from thymidine and polythiophosphate.

3. The mechanism of polymerization. The mechanism via phosphate activation is widely held and is, indeed, supported by the principle of continuity. However, it is inherently inefficient in aqueous solution. We feel that a displacement reaction involving an activated primary carbon and a phosphate could be a more suitable mechanism of primitive phosphorylation, since it is possible to bring about the reaction in water and at higher temperature. The possible involvement of thiophosphate, the most powerful nucleophile hitherto known, would extend the temperature range down to 0°C. We have carried out such polymerizations and studied the prototypical poly 5’ thiodexyribonucleotides. Optical rotatory dispersion and circular dichroism data show that these polynucleotides possess secondary structures. This new approach raises the question as to the nature of prebiotic carbon activation.

Finally, our interpretation of recent results obtained in our and other laboratories suggest that pyrimidine polynucleotides could have evolved prior to the purine polynucleotides.

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**Energy Transfer in Xylene–Biacetyl Systems**

One method of measuring triplet state formation in photochemical systems is to preferentially excite emission from the triplet state of biacetyl by energy transfer from the triplet state of the donor molecule. For certain molecules, particularly simple aromatic molecules, the lowest excited singlet state lies high enough to cause excitation of biacetyl to its second excited singlet state, and biacetyl molecules excited to this state form the first excited singlet state with negligible yield. If this were not so one would necessarily have to take account of the essentially quantitative cross-over of biacetyl molecules in the first excited singlet state to the lowest-lying triplet state, thus leading to phosphorescent emission. This would, of course, invalidate calculation of triplet-state yields of aromatic molecules.

This method was first applied by Ishikawa (to benzene). The method has also been used for several other simple aromatic molecules and at least in some cases there is good agreement with triplet-state yields based on the isomerization of cis-2-butene as proposed by Cundall. However there began to appear cases in which the sum of fluorescent yield and triplet-state yield exceeded unity by more than the experimental error and this has led to a detailed reexamination of the biacetyl method. The method was reasonably successful for benzene because the biacetyl pressure was low, about 0.1 torr. At this pressure, excited benzene molecules in the \(^1\text{A}_\text{a}\) state transferred negligible excitation to the biacetyl because of their short lifetimes (about 75 nsec, as shown by E. K. C. Lee). It can now be shown that at higher pressures biacetyl molecules are excited to both the first and second excited singlet states, and the former cross over to the lowest triplet state and phosphoresce. The kinetics of the system have been worked out for the xylenes and will be discussed.

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Biosynthesis of dTTP in *Escherichia coli*. dCTP Deaminase, a New Enzyme Operative in the Pathway

A strain of *Escherichia coli* HD1038, isolated as a pyrimidine over-producer, was found to contain a dCTP pool 10 times as large and a dTTP pool half as large as in the parent strain. Mutant and parent strains grow with the same generation time in minimal medium. Addition of any pyrimidine deoxyribonucleoside to the growth medium resulted in a marked increase in the dCTP pool in the mutant strain and a reduction of the dCTP pool to normal levels. The addition of ribonucleosides or purine deoxyribonucleosides to the medium did not alter the anomalous pool sizes significantly.

Although thymine-stressed mutants display characteristics quite similar to the mutant described here (i.e. high dCTP, low dTTP) they differ markedly in other respects. Unlike thymine-stressed strains, HD1038 has a low DUMP pool and its pool sizes return to normal on deoxouridine addition.

A new pathway for the synthesis of dUMP was described recently for *Salmonella typhimurium* and a novel enzyme, dCTP deaminase, was isolated. In this paper we describe *E. coli* mutant deficient in this enzyme. The isolation of such a mutant allows dTTP biosynthesis to be examined more closely than was hitherto possible. The biosynthesis in the mutant and in the parent strain of *E. coli* is discussed.

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The Ages of Lunar Maria

The previously presented geomorphic index (Geol. Soc. Amer. Bull., 81, pp. 337–352) is a quantitative description of the geomorphology of lunar surfaces. In the case of the maria but not of the highlands, the geomorphic index is proportional to the age of the mare surfaces, thus permitting the determination of their relative ages. The geomorphic indices of the maria show that each mare surface is not the result of a single effusion, but of several that occurred over a span of time. From a stratigraphic point of view, there is a lateral interfingering of the effusions of different maria. Certain maria, such as Tranquillitatis, have older surfaces than other maria, meaning that effusions started earlier in some maria than in others, or that older effusions were buried by younger ones. Young effusions are lacking on the surface of certain maria, such as Humorum, indicating that the end of the effusive activity was not contemporaneous for all the maria.

A preliminary translation of the geomorphic index into age in years is attempted by using the Apollo 11 and 12 radiogenic ages. By using the range of values obtained by four models, the most recent effusion can have any age between zero and $2.6 \times 10^9$ years. Older effusions, however, can be dated with a smaller range. The oldest appear to have an age of more than $4 \times 10^9$ years. The principal conclusion of the study is that the mare effusions were not a relatively short-lived phenomenon but occurred throughout a considerable portion of the lunar geological history.

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New Methodology to Reduce the Environment-Heredity Uncertainty About Dysgenics

of racial composition, estimated from "tracer genes," on IQ for half-siblings in environments controlled to the degree of being in the same illegitimate nonwhite slum family. A new proposal combines improved accuracy in determining racial composition of populations (T. E. Reed, Science 22 Aug 70 finds 22 ± 1% Caucasian genes in Oakland, California, negroes) with population IQ differences (R. Heber describes environmentally matched Milwaukee school populations averaging 30 IQ points difference depending on mother's IQ being above or below 80). The hypothesis that the difference arises from racial mixes of 33 and 11% for the two populations respectively results in the testable prediction of 84 versus 30 instances of Reed's Caucasian gene for the two groups of 300 children each. A Negro university offers a comparable research possibility for which environmental influences of Caucasian genes might be negative rather than positive. Racial compositions could be determined for test populations selected on the basis of higher and lower performance on both nationally standardized tests and alternatively on any locally accepted measures of intellectual achievement. Resulting facts would be relevant to important national social objectives. Estimates of the variance of racial composition support the feasibility of this proposal.

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Role of Laboratory Studies in Space Science

During the past two decades space research has been greatly expanded and accelerated through the use of rockets, satellites, and ground-based telescopes. These have collectively provided the bulk of the primary data regarding the nature and composition of the earth's environment. The interpretation and understanding of these data depends on a knowledge of the collision properties of the various charged and neutral species present. These data have been provided primarily through laboratory investigations by means of a variety of experimental techniques. Of these some seek to accurately reproduce in the laboratory certain atmospheric phenomena while others isolate discrete atmospheric species, which are then studied as they react with each other. This paper will briefly but critically review the major laboratory techniques currently in use and identify major problem areas.

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Observability of Single Atoms in Biological Molecules

To form an image of a single molecule in which the images of the individual atoms are resolved, the wavelength of the illuminating radiation must be similar to or smaller than the interatomic spacing (\( \lambda \approx 1 \text{ Å} \)), and each atom imaged must scatter (or absorb) at least one quantum of radiation. Although the effects of elastically scattered quanta on molecular structure may be ignored, the inelastic collisions will lead (with a certain probability) to molecular dissociation or rearrangement, and the "image" formed will be a composite picture of the molecule over the history of its irradiation and may bear little relation to its original structure. On the basis of estimates of the molecular damage caused in the observation process we conclude that "molecular microscopy" of biological molecules in which the individual atoms are resolved is impossible with an electron or x-ray microscope. Similar conclusions are reached with respect to microscopes using all other possible illuminating radiations, with the possible exception of neutrons.

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Isozyme Activity During Early Development of Diploid and Androgenetic Haploid and Hybrid Frog Embryos

Cross fertilization of geographic races of the frog, *Rana pipiens*, produces viable diploid hybrids. Starch gel electrophoresis of extracts from the different races of frogs and their hybrid progeny reveal different forms of certain isozymes. Distinct isozyme bands identifiable as maternal in origin are present from the very beginning of embryonic development, while the paternal and hybrid forms first appear as discrete bands in the extracts of hybrids at various post-neurula stages of development. Thus, paternal and “hybrid” molecules of phosphogluconate dehydrogenase (EC 1.1.1.-43) are first detected at the tail bud stage; the B subunit of lactate dehydrogenase appears slightly later, at the muscular response stage, while gene expression for soluble isocitrate dehydrogenase and mitochondrial malate dehydrogenase becomes evident still later, when heart beat is detected in the embryo.

Androgenetic haploid hybrids can be produced by removal of the nucleus from the egg of one race after artificial insemination with sperm from another race. Such haploids display different degrees of abnormalities during embryogenesis: some arrest at about the neurula stage, while others develop abnormally through hatching. We determined the paternal (nuclear) and the maternal (cytoplasmic) contributions to the various isozyme forms in these androgenetic haploid hybrids. The maternal and paternal forms of dehydrogenases are evident at the same time in embryonic development as the diploid controls, even though the haploids are grossly abnormal and retarded in morphogenesis. However, the “hybrid” isozymes, composed of maternal and paternal subunits, are not obtained with extracts from androgenetic haploid hybrids.

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Heats of Hydrogenation of cis- and trans-2-Methylhexatriene: Resonance or Sigma Bond Energy?

The heats of hydrogenation (acetic acid solution, 25°C) of cis- and trans-2-methylhexatriene have been measured in order to test the suggestion of M. J. S. Dewar and N. H. Schmeising that conjugative stabilization in butadiene results not from resonance interaction, but from a strengthening of the sp²–sp² sigma bond relative to sp³–sp² sigma bonds in model systems.

Cis- and trans-2-methylhexatriene were selected since the cis compound, but not the trans, should be nonplanar, a view derived from extensive previous work in the carotenoid and vitamin A fields.

The heat of isomerization (cis → trans) obtained for the methylhexatriene pair is −4.7 kcal/mol. The corresponding value for the unsubstituted hexatrienes (planar) is −1.1 kcal/mol (unpublished results of R. B. Turner and W. von E. Doering). If the conformational postulates are correct, planarity seems to be an important factor in conjugative stabilization. The results are thus consistent with the resonance hypothesis.

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Should the Science-Based Food Industry Be Expected to Advance?

In all other science-based industries (transportation, textiles, communication, electronics, etc.) we take periodic if not revolutionary advance for granted. Should not the food industry be providing us continuously with food of better and better quality?

That this is not being done in the crucial milling and baking industry is shown by the following experiment, 128 weanling rats of four strains were placed on two bread diets. Half of them received commercial “enriched” bread, the others received the same bread supplemented, in accordance with modern nutritional knowledge, with small amounts of minerals, vitamins, and one amino acid (lysine). The two breads were undistinguishable to the baker. The supplemented bread would cost perhaps 5% more.

After about 2 weeks the least responsive strain of rats was growing almost five times as rapidly on the supplemented bread; the most responsive strain grew eight times as fast. After 12 weeks two-thirds of the animals on the “enriched” bread had died of malnutrition while practically all the other animals were alive and growing.

Because of the consistency observed with four strains of rats, it is readily inferred that many other mammals, including man, would yield similar results.

“Enrichment” started in the baking and milling industry nearly thirty years ago. In the intervening years there has been no nutritional improvement or advance whatever. The quality of the nutrition furnished children and adults who consume “enriched” products as well as many processed foods is far below what scientific advance should demand.

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Mechanism of Carcinogenesis by RNA Tumor Viruses, I. An RNA-Dependent DNA Polymerase in Murine Sarcoma Viruses*

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Abstract. A highly active and stable DNA polymerase was found in purified preparations of two murine sarcoma viruses. Enzyme activity is not detected in most virus preparations unless they are treated with low concentrations of a nonionic detergent such as Nonidet P-40. The incorporation of labeled thymidine triphosphate requires all four deoxyribonucleoside triphosphates and either Mg²⁺ or Mn²⁺. Enzyme activity is proportional to virus concentration and is linear with time up to 90 min. That the template is RNA is suggested by the reduction in polymerase activity upon treatment of murine sarcoma virus with RNase, and by the absence of detectable amounts of DNA in the virus. That the product is DNA is shown by the incorporation of all four deoxyribonucleoside triphosphates into an acid-insoluble product which is stable in alkali, is destroyed by DNase, sediments in alkaline sucrose gradients with a sedimentation coefficient of 7 S, and bands in isopycnic CsCl gradients with a mean buoyant density of 1.700.

The RNA-containing leukemia and sarcoma viruses possess two unique properties which distinguish them from other RNA-containing animal viruses and bacteriophages: (1) DNA synthesis is required early during infection for virus replication and for cell transformation, i.e., if DNA synthesis is inhibited during the first 8–12 hr of infection viral replication and cell transformation are irreversibly aborted; this requirement can be fulfilled in the presence of cycloheximide, an inhibitor of protein synthesis. (2) Actinomycin D, an inhibitor of DNA dependent RNA transcription, blocks virus replication at all times after infection, suggesting that functioning DNA is required continuously for the synthesis of viral progeny.

In a recent review, we concluded on the basis of the above inhibition studies and other recent experiments that only two mechanisms are consistent with all the data: (1) the virus particle (virion) contains an enzyme which copies DNA from viral RNA, or (2) cellular DNA binds viral RNA, perhaps stabilizing the viral genome. Based mainly on inhibitor studies with Rous sarcoma virus, Temin² has proposed that DNA that contains viral information is synthesized early after infection, presumably by a reversal of genetic transcription, i.e., viral RNA → DNA, and that newly synthesized viral DNA is a template for the synthesis of viral RNA.
We report here evidence for mechanism (1): purified preparations of two murine sarcoma viruses (MSV), the Harvey isolate, MSV(H), and the Moloney isolate, MSV(M), contain a highly active and stable DNA polymerase that incorporates all four deoxyribonucleoside triphosphates into DNA. The properties of the DNA polymerase, the nature of the template, and the properties of the DNA product are described. The possible role of transcribed viral DNA in virus replication and in cell transformation is discussed in this paper. Very recently, DNA polymerase activity was detected in Rous sarcoma virus by Temin and Mizutani, in Rauscher murine leukemia virus by Baltimore, and, more recently, in feline leukemia virus by Spiegelman.

**Materials and Method.** Materials: The following items were purchased: dATP, dGTP, dCTP, and dTTP from the Sigma Chemical Co., St. Louis; [3H]dTTP from the New England Nuclear Corp. and from Schwarz Biochemicals; α-32P-labeled dATP, dGTP, dCTP, and dTTP from the International Chemical and Nuclear Corp.; Nonidet P-40 from the Shell Chemical Co.; polyethylene glycol (avg mol wt 6,000–7,500) from Matheson, Coleman and Bell, Inc.

**Virus and cells:** A seed culture of MSV(H), twice focus purified, was kindly provided by Dr. H. Temin. We transformed mouse embryo cultures (NIH-Swiss) with MSV(H), deriving the MEH cell line, which grows in suspension after 60 passages in monolayer in Eagle's MEM medium supplemented with 10% tryptose phosphate broth and 10% fetal calf serum. The MSV(M) transformed rat cell line, 78A1, kindly provided by Drs. M. Benyesh-Melnick and N. Biswal, was grown as monolayers in Eagle's MEM medium supplemented with 10% fetal calf serum. Media harvested from MEH and 78A1 cells were frozen at −35°C until used for purification of virus.

**Virus purification:** Virus was isolated and purified from MEH or 78A1 growth media by the procedure of Duesberg and Robinson with a slight modification. Six liters of cell media were stirred for 1 1/2 hr at 4°C after the addition of polyethylene glycol (10%) and sodium chloride (0.5 M). The virus-containing precipitate was centrifuged at 2700 × g for 20 min, resuspended in buffer, and further purified as described.

**DNA polymerase assay:** The procedures described previously were used with slight modifications. The incubation mixture (100 μl) contained 40 mM Tris, pH 8.1; 5 mM dithiothreitol; 2.5 or 5 mM MgCl₂; 0.1 mM dATP, dGTP, dCTP; 10 μCi of [3H]dTTP (4.3–11.4 Ci/mmol) and 2–20 μg of viral protein. NaCl (30 mM) was included in the assay but does not appear to be necessary for enzyme activity. Purified MSV was dialyzed against 500 volumes of 0.01 M Tris, pH 8.1 for 3 hr before assay for polymerase. Nonidet P-40 (0.01%) was added to the virus at the time of assay.

After incubation at 37°C for various times (usually 1 hr), the reaction was stopped by the addition of 150 μl of cold 1 N perchloric acid (PCA) and 100 μl of carrier calf-thymus DNA (1 mg/ml). Radioactive DNA was isolated as described previously, and counted in a scintillation spectrometer.

**Results.** Incorporation of [3H]dTTP into an acid-insoluble form by purified MSV (DNA polymerase activity of MSV): All ten preparations of purified MSV(H) that we tested for DNA polymerase activity were able to incorporate [3H]dTTP into an acid-insoluble form upon treatment with 0.01% Nonidet P-40 (NP-40). Other nonionic detergents, including Triton X-100, Tween 40, and Tween 80, also were effective in unmasking DNA polymerase activity (Table 1). One MSV(H) preparation incorporated [3H]dTTP without added detergent but the addition of 0.01–0.025% NP-40 increased incorporation to 3000–3500 cpm (Fig. 1). Electron micrographs of detergent-treated virion after the polymerase reaction revealed strand-containing structures, probably cores with viral RNA.
MSV(H) and MSV(M) have very active DNA polymerases which are activated to different extents by NP-40 (Table 2). The DNA polymerase of purified virus is stable. No activity of dialyzed virus was lost after storage at 4°C for two weeks, and not more than 50% loss occurred after 2 months.

Requirements for the DNA polymerase reaction: The conditions for optimal incorporation of [H]-dTTTP into an acid-insoluble product were determined using NP-40 to activate enzyme activity. Polymerase activity requires all four deoxyribonucleoside triphosphates and a divalent cation, is proportional to virus concentration, and is linear for at least 90 min.

![Graph showing the effect of different concentrations of Nonidet P-40 on the incorporation of dTTP by the MSV DNA polymerase. The standard assay was used with 10 µCi of [H]dTTP (11.4 Ci/mmol) and 10 µg of viral protein.]

**Fig. 1.**—Effect of different concentrations of Nonidet P-40 on the incorporation of dTTP by the MSV DNA polymerase. The standard assay was used with 10 µCi of [H]dTTP (11.4 Ci/mmol) and 10 µg of viral protein.

**Table 1.** Effect of detergents on MSV DNA polymerase activity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incorporation of [H]dTTP (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>188*</td>
</tr>
<tr>
<td>NP-40 (0.01%)</td>
<td>2909</td>
</tr>
<tr>
<td>Triton X-100 (0.01%)</td>
<td>4310</td>
</tr>
<tr>
<td>Tween 40 (0.01%)</td>
<td>2804</td>
</tr>
<tr>
<td>Tween 80 (0.01%)</td>
<td>2817</td>
</tr>
</tbody>
</table>

*Average of duplicate enzyme assays.

Standard assay conditions were used with 10 µCi of dTTP (11.4 Ci/mmol), approximately 14 µg of viral protein, and 1 hr of incubation.

**Table 2.** DNA polymerase activity of murine sarcoma virus (Harvey) and murine sarcoma virus (Moloney).

<table>
<thead>
<tr>
<th>Virus</th>
<th>Time (min)</th>
<th>Incorporation of [H]dTTP (cpm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSV(H)</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>MSV(M)</td>
<td>0</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>15026</td>
</tr>
</tbody>
</table>

*Average of duplicate enzyme assays.

Standard assay conditions were used with 10 µCi of [H]dTTP (11.4 Ci/mmol) and 9.2 µg of MSV(H) or 9.6 µg of MSV(M).

(1) **Relationship between DNA synthesis and virus concentration:** The amount of DNA synthesized by NP-40-activated virus is proportional to the concentration of viral protein from 2.4 to 19.2 µg of protein per assay (Fig. 2).

(2) **pH and temperature:** The pH optimum for the DNA polymerase from MSV(H) is about 8.1 (Fig. 3). Maximal enzyme activity for NP-40-activated MSV(H) occurs at 37°C (Fig. 4). Incorporation is decreased at 40, 45, and 50°C (Fig. 4).

(3) **Deoxyribonucleoside triphosphates:** All four deoxyribonucleoside triphosphates are required for polymerase activity. Omission of one of the four deoxyribonucleoside triphosphates reduced incorporation of dTTP nearly completely (Table 3). The incorporation of α-32P-labeled dTTP, dATP, dGTP, and dCTP is linear for at least 90 min (Fig. 5) (the different incorporation rates reflect different concentrations of labeled nucleotides in the assay mixture and,
(Left) Fig. 2.—Linear relationship between virus concentrations and MSV DNA polymerase activity. The standard assay was used with 0.01% NP-40 and 10 μCi of [3H]dTTP (11.4 Ci/nmol).  

(Right) Fig. 3.—Effect of pH on the incorporation of dTTP by the MSV DNA polymerase. The standard assay was used with 0.01% NP-40, 10 μCi of [3H]dTTP (4.3 Ci/nmol) and 2.96 μg of viral protein. The following buffers, at a concentration of 40 mM, were used: Tris-HCl buffer at pH 7.0, 7.5, 8.10, 8.45; phosphate buffer at pH 6.15, and Tris acetate buffer at pH 9.0.

Fig. 4.—Effect of temperature on NP-40-activated incorporation of dTTP by the DNA polymerase from MSV. The standard assay was used with 10 μCi of [3H]dTTP (11.4 Ci/nmol) and 7.26 μg of viral protein.

Fig. 5.—Incorporation of α-32P-labeled deoxyribonucleoside triphosphates into DNA by the MVA DNA polymerase. The standard assay was used with 0.01% NP-40, 0.1 mM of three unlabeled deoxyribonucleoside triphosphates, and one labeled with 32P, and 6.3 μg of viral protein in 300 μl final volume. Aliquots (50 μl) were assayed at 0, 20, 40, 60, and 90 min. The following concentrations of labeled precursors was used: (1) [α-32P]dATP, 2.5 μCi/50 μl (0.88 mCi/mmole); (2) [α-32P]dGTP, 2.5 μCi/50 μl (4 Ci/mmole); (3) [α-32P]dTTP, 1.78 μCi/50 μl (3.8 Ci/mmole); (4) [α-32P]dCTP, 3.9 μCi/50 μl (4.0 Ci/mmole).
perhaps, the base composition of the template). Polymerase activity is not affected by 10 mM phosphate but is fully blocked by 10 mM pyrophosphate.

(4) Metal ions and reducing agent: Divalent cations are essential for polymerase activity. Omission of Mg$^{2+}$, or the addition of 10 mM EDTA, completely inhibits incorporation of dTTP (Table 4). The optimal concentration of Mg$^{2+}$ is 1–2.5 mM. Mn$^{2+}$ effectively replaces Mg$^{2+}$, increasing incorporation 50% (Table 4, Expt. 2). This is surprising since Mn$^{2+}$ is generally a poor substi-

<table>
<thead>
<tr>
<th>Table 3. Deoxyribonucleoside triphosphate requirement for DNA synthesis.</th>
<th>Table 4. Requirements for divalent cations for DNA synthesis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incorporation of [H]$d$TTP</strong></td>
<td><strong>Incorporation of [H]$d$TTP</strong></td>
</tr>
<tr>
<td><strong>Components</strong></td>
<td><strong>Components</strong></td>
</tr>
<tr>
<td>Complete system*</td>
<td>Complete system* (5 mM</td>
</tr>
<tr>
<td>Minus dATP</td>
<td>Mg$^{2+}$, 5 mM dithiothre-itol)</td>
</tr>
<tr>
<td>Minus dGTP</td>
<td>2.5 mM Mg$^{2+}$</td>
</tr>
<tr>
<td>Minus dCTP</td>
<td>– Mg$^{2+}$</td>
</tr>
<tr>
<td></td>
<td>+ 10 mM EDTA</td>
</tr>
<tr>
<td></td>
<td>2.5 mM dithiothreitol</td>
</tr>
<tr>
<td></td>
<td>– dithiothreitol</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Standard assay conditions were used with 0.01% NP-40, 10 μCi of [H]$d$TTP (11.4 Ci/ mmol), and 12.6 μg of viral protein.
† Average of duplicate enzyme assays. Zero time incorporation, 140 cpm was subtracted.

<table>
<thead>
<tr>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system (2.5 mM Mg$^{2+}$)</td>
<td>Complete system (2.5 mM Mg$^{2+}$)</td>
</tr>
<tr>
<td>1.0 mM Mg$^{2+}$</td>
<td>193</td>
</tr>
<tr>
<td>0.5 mM Mg$^{2+}$</td>
<td>250</td>
</tr>
<tr>
<td>– Mg$^{2+}$ + 5.0 mM Mn$^{2+}$</td>
<td>116</td>
</tr>
<tr>
<td>– Mg$^{2+}$ + 2.5 mM Mn$^{2+}$</td>
<td>296</td>
</tr>
<tr>
<td>– Mg$^{2+}$ + 1.0 mM Mn$^{2+}$</td>
<td>311</td>
</tr>
<tr>
<td>– Mg$^{2+}$ + 0.5 mM Mn$^{2+}$</td>
<td>382</td>
</tr>
<tr>
<td>– Mg$^{2+}$ + 0.5 mM Mn$^{2+}$</td>
<td>351</td>
</tr>
</tbody>
</table>

*Standard assay conditions were used with 0.01% NP-40, 5 mM MgCl$_2$, 0.023 mM [H]$d$TTP (4.3 Ci/mmol), and 2.96 μg of viral protein.
† Average of duplicate enzyme assay. Counts after 1 hr were 1018 cpm above a zero time incorporation of 148 cpm.

<table>
<thead>
<tr>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>Complete system</td>
</tr>
<tr>
<td>1.0% NP-40, 2.5 mM MgCl$_2$, 0.025 mM [H]$d$TTP (4.8 Ci/mmol)</td>
<td>916</td>
</tr>
</tbody>
</table>

*Standard assay conditions were used with 0.01% NP-40, 2.5 mM MgCl$_2$, 0.025 mM [H]$d$TTP (4.8 Ci/mmol) (this lot of [H]$d$TTP is different from that in Expt. 1), and 2.96 μg viral protein.

Table 5. Effect of RNase on the template.

<table>
<thead>
<tr>
<th>Incorporation of [H]$d$TTP (cpm*)</th>
<th>Incorporation of [H]$d$TTP (cpm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system†</td>
<td>3652</td>
</tr>
<tr>
<td>+ RNase (100 μg/ml)</td>
<td>991</td>
</tr>
</tbody>
</table>

*Average of duplicate enzyme assays with 0.01% NP-40, 10 μCi of [H]$d$TTP (11.4 Ci/mmol) and 9.6 μg of viral protein.
† The virus was incubated with pancreatic RNase for 45 min in the presence of NP-40 before the standard DNA polymerase assay.

Table 6. Nature of the template: Two lines of evidence suggest that viral RNA is the template for the DNA polymerase of MSV: (1) RNase destroys polymerase activity: Incubation of NP-40-treated MSV(H) with RNase reduced incorporation by 73% (Table 5).

(2) RNA tumor viruses contain no DNA? RNA tumor viruses contain 70S viral RNA which is converted to 35S RNA by various denaturing agents; the
molecular weight of viral RNA estimated from these S values ranges from 3 to $13 \times 10^6$. Experiments which could exclude the presence of a small amount of DNA, e.g., 250,000–500,000 daltons, have not been reported. We attempted to determine whether MSV may contain a DNA template for the DNA polymerase reaction. Four millicuries of $^{3}H$thymidine (17.8 Ci/mmole) was added to 2 liters of MEH cells in suspension (2.0 x $10^8$ cells/ml). After 24 hr at 37°C, when the cell number had doubled, the culture was centrifuged, virus was isolated from the medium, and cell DNA was extracted from the pellet. From the specific activity of the cell DNA (10,960 cpm/µg), and if we assume a doubling of the DNA content, we estimate that newly synthesized DNA would have a specific activity of about 22,000 cpm/µg. Virus (1.5 ml) isolated from the medium contained 926 µg of viral proteins/ml and 6984 cpm/ml, thus a specific activity of 7.5 cpm/µg of viral protein. The specific activity was reduced to 3 cpm/µg by treatment with DNase, sucrose density gradient centrifugation, and two cycles of solubilization in alkali and precipitation with perchloric acid. From the values of 3–7 cpm/µg, and if we assume a particle weight of 450 x $10^6$ daltons, we estimate that not more than 50,000–100,000 daltons of DNA can be present in a MSV particle.

**Properties of the product**: The following experiments show that the product has properties of DNA with regard to DNase digestion, stability in alkali, sedimentation in alkaline sucrose gradients, and buoyant density in isopycnic CsCl gradients. (1) *The product is hydrolyzed by DNase but not by RNase*: The $[^{3}H]dTTP$ labeled polymerase product was rendered acid-soluble by DNase or snake venom phosphodiesterase (70–80%) but not by RNase (Table 6). (2) *The product is stable in alkali*: There is no reduction in the acid-precipitable $^{3}H$-product after treatment with 0.2 N alkali for 20 min at 80°C, conditions which convert RNA, but not DNA, to an acid-soluble form. (3) *The product sediments in alkaline sucrose gradients*: The labeled product was centrifuged in a 5–20% alkaline sucrose gradient together with a 34S adenovirus type 7 DNA marker. The product synthesized by NP-40-activated MSV(H) sediments at 7 S (Fig. 6), corresponding to single-stranded DNA of molecular weight 200,000–250,000. (4) *The product has a buoyant density like that of DNA*: Preparations of labeled product were denatured and centrifuged in CsCl density gradients (Fig. 7). The $^{3}H$MSV product has a much broader peak than marker adenovirus type 7 $^{32}P$DNA, as expected from the differences in molecular weight. Based on the known buoyant density of

### Table 6. Effect of nucleases on the product of the MSV(H) polymerase reaction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incorporation of $[^{3}H]dTTP$ (cpm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3287</td>
</tr>
<tr>
<td>RNase, 100 µg/ml</td>
<td>3358</td>
</tr>
<tr>
<td>RNase, 1 mg/ml</td>
<td>3224</td>
</tr>
<tr>
<td>DNase, 100 µg/ml</td>
<td>1100</td>
</tr>
<tr>
<td>DNase, 1 mg/ml</td>
<td>993</td>
</tr>
<tr>
<td>Snake venom phosphodiesterase, 100 µg/ml</td>
<td>1037</td>
</tr>
<tr>
<td>Snake venom phosphodiesterase, 400 µg/ml</td>
<td>1037</td>
</tr>
<tr>
<td>RNase, 100 µg/ml + DNase, 100 µg/ml</td>
<td>658</td>
</tr>
<tr>
<td>RNase, 1 mg/ml + DNase, 1 mg/ml</td>
<td>1263</td>
</tr>
</tbody>
</table>

* Average of duplicate enzyme assays with 10 µCi of $[^{3}H]dTTP$ (11.4 mCi/mmole) and 9.6 µg of viral protein. After 1 hr of incubation at 37°C, nucleases were added and incubation was continued for 1 hr at 37°C.
RNA-DEPENDENT DNA POLYMERASE

adeno virus type 7 DNA (\( \rho = 1.713 \)), we estimate that the mean buoyant density of the product shown in Fig. 7 and in other determinations of 30- and 90-min products is 1.736-1.738, a value consistent with the product being DNA.

Discussion. The presence of a unique enzyme, an RNA-dependent DNA polymerase, in RNA tumor viruses raises many provocative questions concerning the mechanism of enzyme action, the nature of the template and the product, the function of the DNA product in virus replication and cell transformation, and the possible role of information flow from RNA to DNA in normal cell function.

Evidence that viral RNA is the template is provided by the decrease in polymerase activity of disrupted virions upon treatment with RNase and the absence of an appreciable amount of DNA in the MSV virion. But decisive proof that viral RNA is the template requires the demonstration of (1) RNA template dependency of purified MSV polymerase and (2) hybridization of the DNA product with viral RNA (see Note added in proof).

The product of the reaction is alkali-stable DNA, Digestible by DNase, sedimentating at 7 S in alkaline sucrose gradients, and possessing a mean buoyant density of 1.736 in CsCl. Although isolated MSV DNA is much smaller than viral RNA, the possibility exists that the entire viral genome is copied, and
that the smaller DNA molecules are produced by contaminating nucleases, or possibly represent intermediates in DNA synthesis.14

What is the biological function of the viral DNA product? We speculate on several possibilities. (1) As a DNA-RNA hybrid, viral DNA protects the genetic information of viral RNA from nucleases. (2) Viral DNA may bind to specific regions of cellular DNA and modify the activity of cellular genes involved in virus replication. It might be significant that 4% of the RNA sequences in RNA tumor viruses hybridizes with the DNA of normal uninfected cells.1 (3) Viral DNA may be copied from the entire viral RNA genome and be integrated into cellular chromosomes where it serves as a template for the synthesis of progeny viral RNA, and as a continuous source of information for the maintenance of the neoplastic phenotype of the cell.

Possibly information flow from RNA to DNA plays an important role in normal cell development. Huebner15 proposes that cellular genes carrying genetic information of RNA tumor viruses are expressed in carcinogenesis, and recent studies indicate that some RNA tumor virus information is also expressed during embryonic development in the mouse.16 Further studies on virus replication and cell transformation by RNA tumor viruses offer unique opportunities to understand molecular mechanisms involved in cellular growth and neoplasia, and may provide important leads towards an understanding of normal gene expression during embryonic development and cell differentiation.

**Note added in Proof.** We have recently described two lines of evidence which show that viral RNA is the template for the MSV DNA polymerase: (1) viral RNA-DNA hybrid molecules are formed by the MSV DNA polymerase and (2) the DNA product formed by the MSV DNA polymerase hybridizes with viral RNA (Rokutanda, Rokutanda, Green, Fujinaga, Ray, and Gurgo, *Nature*, in press).

We thank Mary Beranek and Maria Caertas for excellent technical assistance.

Abbreviations: MSV, murine sarcoma virus; NP-40, Nonidet P-40.

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† Research Career Awardee (5-K6-4739), National Institutes of Health, Public Health Service.

‡ On leave from the Aichi Cancer Center Research Institute, Nagoya, Japan.


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