CONFERENCE

on

IMMUNOLOGY AND CANCER

Friday, January 4 and
Saturday, January 5, 1957

THE NEW YORK ACADEMY OF SCIENCES
SECTION OF BIOLOGY
2 East Sixty-third Street
New York 21, N. Y.

and

NATIONAL INSTITUTES OF HEALTH
ALLERGY AND IMMUNOLOGY STUDY SECTION
Bethesda, Maryland

and

NATIONAL CANCER INSTITUTE
(NATIONAL INSTITUTES OF HEALTH)
Bethesda, Maryland

ALL SESSIONS WILL BE HELD AT

The Barbizon-Plaza Hotel
101 West 58th Street at 6th Avenue
New York City

Hotel Catherine Wood
45E 35th

This program will serve as a ticket of admission and is nontransferable

Conference Chairman: Jerome T. Syverton
Department of Bacteriology and Immunology
University of Minnesota Medical School
Minneapolis, Minn.

PROGRAM

FRIDAY, JANUARY 4, 1957

ANALYSIS OF CELLS

Session Chairman: Wendell Stanley
University of California, Berkeley, Calif.

9:00 A.M. –
Greetings from the Academy – A. S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

"Physical Methods for the Analysis of Cells" – Joseph W. Beard, Duke University School of Medicine, Durham, North Carolina.

"Chemical Methods for the Analysis of Cells" – Van R. Potter, University of Wisconsin School of Medicine, Madison, Wis.

"Cytochemical Approaches to the Analysis of Cells" – Arthur Pollister, Columbia University, New York, N. Y.

"Genetics of Transplantation" – George D. Snell, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

"Cytogenetic Aspects of Compatibility" – Theodore S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y.

Open Discussion

12:30 P.M. –
Luncheon – Academy Building, 2 East 63rd Street, New York, N. Y.

IMMUNOLOGIC TECHNIQUES AND THEIR APPLICATIONS

Session Chairman: Michael Heidelberger
Institute of Microbiology, Rutgers University
New Brunswick, N. J.

2:00 P.M. –

"Quantitative Precipitation" – Otto J. Plescia, Institute of Microbiology, Rutgers University, New Brunswick, N. J.

"Agar-gel Diffusion and Immunoelectrophoretic Analysis" – Pierre Grabar, Institut Pasteur, Paris, France.

"Immunochemical Analysis Based on Complement Fixation" – Maurice Rapport, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

"Virus Neutralization Tests, Implications and Interpretations" – Frank L. Horsfall, Jr., The Rockefeller Institute for Medical Research, New York, N. Y.

"Radiolabeled Antibodies" – David Pressman, Roswell Park Memorial Institute, Buffalo, N. Y.

"Purification of Tumor Localizing Antibodies" – Eugene Day and David Pressman, Roswell Park Memorial Institute, Buffalo, N. Y.

"Application of Fluorescent Antibodies to the Study of Naturally Occurring Antigens" – Albert Coons, Harvard Medical School, Boston, Mass.

"Diffusion Chamber Techniques for Studies of Cellular Immunity" – Glenn Algire, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Open Discussion

5:30 P.M. –
Cocktail Hour – Academy Building.

6:30 P.M. –
Dinner – Academy Building.

SATURDAY, JANUARY 5, 1957

RESULTS OF IMMUNOLOGICAL TECHNIQUES

Session Chairman: Renato Dulbecco
California Institute of Technology, Pasadena, Calif.

9:00 A.M. –

"Tissue-Specific Antigens" – Ernest Witebsky, University of Buffalo School of Medicine, Buffalo, N. Y.

"Acquired Tolerance in Newborn Animals" – Leslie Brent, University College, London, England.

"The Distribution and Immunochemical Properties of Human Tissue and Tumor Antigens" – Leonard Korngold, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

"Interpretation of Host Response in Quantitative Studies on Animal Viruses" – W. Ray Bryan, National Cancer Institute, National Institutes of Health, Bethesda, Md.

"Virus Neutralization" – Edward Eckert, State University of New York College of Medicine, Brooklyn, N. Y.

"Cell-Virus Relations in the Rous Sarcoma" – Harry Rubin, California Institute of Technology, Pasadena, Calif.

"Modification of the Immune Response by Radiation and Cortisone" – William Taliaferro, Department of Microbiology, University of Chicago, Chicago, Ill.

Open Discussion

12:30 P.M. –

Luncheon – Academy Building, 2 East 63rd Street, New York, N. Y.

HOST CELL RESPONSE

Session Chairman: Peyton Rous
The Rockefeller Institute for Medical Research
New York, N. Y.

2:00 P.M. –

"The Cytotoxic Effects of Antitumor Sera" – Robert W. Wissler and Martin H. Flax, Department of Pathology, University of Chicago, Chicago, Ill.

"Cytotoxins and Cytotoxic Antibodies" – John D. Ross, Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.

"Cytotoxin Studies" – Karl Habel, Basic Studies Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

"The Nature of the Antigens in Transplantation Immunity" – Leslie Brent, University College, London, England.

"Acquired Tolerance Applied to Experimental Tumors" – Hilary Koprowski, Viral and Rickettsial Section, American Cyanamid Company, Lederle Laboratories Division, Pearl River, N. Y.

"Heterotransplantability of Tumors" – Harry S. N. Greene, Yale University Medical School, New Haven, Conn.

"Immunological Problems Associated with the Heterotransplantation of Human Tumors" – Helene W. Toolan, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

SUMMARY OF CONFERENCE

Howard B. Andervont, National Cancer Institute
National Institutes of Health, Bethesda, Md.

Open Discussion

The Section of Biology provides conferences for active workers in the special fields of biology.

Attendance is limited to those invited to participate in these conferences and to interested Members of the Academy.

A. S. Gordon
Chairman, Section of Biology

Louis G. Nickell
Secretary

C O N F E R E N C E

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"PHYSICAL METHODS FOR THE ANALYSIS OF CELLS"

by
Joseph W. Beard
Duke University School of Medicine, Durham, North Carolina

Investigations of the nature and biological significance of "particulate" constituents of cells have become a principal area of interest in every field of cytological analysis. In this category of studies there are involved not only the varied functional units of the normal cell, but the specific abnormal components developing within or in association with the cells of tissues diseased by viruses. Advances in this field, as exemplified by those with the viruses, have been dependent in large part on the contribution of biophysical methods and procedures separately and in correlation with the results of chemical and biological investigations. Prominent among the widely available methods are those involving ultracentrifugation, electron micrography and electrophoresis. Such procedures provide the principal bases not only for initial fractionation, of cellular constituents but, together with other methods, for characterization and identification of the significant components. In addition electron micrography has afforded extension of the problem to direct examination of the materials within the cells by means of now well refined techniques of ultrathin sectioning. Despite the results thus far, however, critical deficiencies remain in the very basic aspects of methodology as applied to separation and definition of entities, which, though similar in principle, differ profoundly in their specific behavior. These are associated in the main with the biological attributes of component concentration and stability and with the complexity and constitution of the tissues or fluids of origin in relation to the characteristics of the component under study. The present status of these questions will be discussed particularly with respect to the study of viruses.

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SESSION I, PAPER #2

"CHEMICAL METHODS FOR THE ANALYSIS OF CELLS"

by
Van R. Potter
University of Wisconsin School of Medicine, Madison, Wis.

Chemical methods for the analysis of cells will be briefly discussed in terms of the author's experience with some current developments in the field of biochemistry. The question of choice of cells for analyses still remains a major question.

The dynamic state of cellular constituents is emphasized and it is pointed out that an analysis for any individual constituent has very little meaning unless it is examined along with the results of other analyses to provide a curve on a system of coordinates that includes a time scale. Indeed, in the case of constituents that do not change in absolute amount during a given time interval it may be more important to know the relative rates of formation and destruction than to know the absolute amount. To this must be added the Densley principle that we should "separate separable things before proceeding to their analysis", that is, the total amount of a substance in a cell may have less significance than the relative amounts in the various parts of the cell. In this connection the findings of the cytochemist and the findings based on cell fractions supplement each other. Similar comments may be made regarding the histochemist. The opportunity for combined disciplines involving immunology are apparent.

Chemical methods involving radioisotopes, chromatographic separations, cell fractionations, enzyme assays and nucleic acid metabolism will be discussed in the above terms, and the importance of the balance sheet in all analytical approaches will be stressed.

A possible relationship between immunochemistry and nucleic acid metabolism will be presented.

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"CYTOCHEMICAL APPROACHES TO THE ANALYSIS OF CELLS"

by
Arthur W. Pollister,
Columbia University, New York, N. Y.

In the broad field of study of the chemical composition of cells the cytochemical approach is generally understood to comprise those analytical methods which utilize the microscope and other tools of cytology to yield information at the level of the individual cell. Cytochemical analysis is most useful when it is applied as a complement to the orthodox chemical analysis of masses of cells or of isolated cell components. In this paper the discussion will deal briefly with the present status of the following aspects of the problem of intracellular localization and quantitative estimation of the major chemical components of cells: a. the preservation of cell structure and of chemical content; b. localization of proteins, nucleic acids, polysaccharides, and lipids; c. methods of quantitative photometric analysis (cytophotometry); d. scope of cytophotometric techniques.

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SESSION I, PAPER #4

"THE HISTOCOMPATIBILITY-1 AND HISTOCOMPATIBILITY-3 LOCI IN THE MOUSE; A PROGRESS REPORT"

by
George D. Snell
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Histocompatibility genes are the genes which determine susceptibility and resistance to transplants of normal and tumor tissue. Three histocompatibility genes, or more precisely genetic loci, H-1, H-2 and H-3 have been identified in the mouse. H-2, the most fully analyzed, is a complex locus with at least eleven identified alleles, and a "strong" locus in the sense that tumor transplants seldom grow when donor and host are separated by an H-2 difference. This report will be mostly concerned with recent work on H-1 and H-3. These loci are at present defined by means of coisogenic pairs of stocks, that is, synthesized pairs of stocks of identical genotype except for a difference at one of these loci. Thus H-3 is identified by the stocks C57BL/10 and B10.LP which are genetically identical except that one is $H\text{-}3^a$ and the other $H\text{-}3^b$. The difference in this case, unlike most H-2 differences, is a "weak" one in that some, perhaps most, tumors of one strain grow readily in untreated mice of the other. Thus C57BL/10 tumor C1498 kills 100% of untreated mice of strain B10.LP, even in small cell doses. On the other hand, if B10.LP mice are appropriately immunized with normal C57BL/10 tissues, almost 100% survive. Essentially the same situation holds for H-1. There are thus cases where there is a latent potential for immunologic resistance to tumor transplants, which, however, is manifested only where special immunization procedures are used.

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SESSION I, PAPER #5

"CYTOGENETIC ASPECTS OF COMPATIBILITY"

by
Theodore S. Hauschka and D. Bernard Amos
Roswell Park Memorial Institute, Buffalo, N. Y.

Stable antigenicity in cells with unbalanced chromosome sets would indeed be more surprising than the observation that karyotypic changes in malignant tissues are often associated with alterations in antigenic type and strength. This finding is in harmony both with immunogenetic "simplification" and with the appearance of "new" antigens in neoplasia.

Combined cytogenetic and serologic studies of representative mouse tumors have correlated chromosome constitution with graft specificity. This functional relationship was investigated particularly with respect to the iso-antigens controlled by the alleles and pseudo-alleles at the Histocompatibility-2 region. A diploid tumor

homograft (2n = 40 in the mouse) cannot grow progressively outside the rigid compatibility limits determined by the mouse-strain genotype in which it originated. Aberrations of the modal chromosome number, ranging from slight hyper-diploidy to erratic polyploidy, facilitate immunologic indifference. All degrees of non-specificity are found: Heteroploid tumors may give lethal takes in only one or two foreign strains, or they become completely indiscriminate in their host-requirements. These cytologic and transplantation results are in fair agreement with serologic tests: Significant weakening of some, but not other isoagglutinins often accompanies departures from the diploid idiogram.

No exception has as yet been met among numerous diploid tumors of inbred mouse-strains, all of which are H-2 specific serologically and in their host restrictions. An occasional balanced tetraploid may keep its rigid iso-antigenicity. The hypo-tetraploid cytologically very unbalanced MC1M fibrosarcoma poses an interesting serologic exception, for it takes across several H-2 barriers despite having strong hemagglutinins.

The existence within neoplastic cell populations of antigenic "mutants" permits experimental immunoselection, two examples of which will be presented.

Among the probable mechanisms responsible for the altered surface characteristics of heteroploid tumor cells the following possibilities exist: Loss of a chromosome or fragment carrying H-factors; structural rearrangements and consequent position effects on the synthesis of antigens; effect of polyploidy and of certain individual chromosomes on cell volume; increased diffusion distances and volume-surface ratios in enlarged cells; altered dosage relationships between genes controlling the molecular lattice of the glyco-lipo-protein cell surface and thereby the spacing of antigenic sites and the possibility of stable antibody coating.

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SESSION II, PAPER #1

"THE PRECIPITIN REACTION"

by
Otto J. Plescia
Institute of Microbiology, Rutgers, The State University, New Brunswick, N. J.

Precipitins are antibodies which are precipitated from antiserum by its corresponding antigen; the immune precipitate can be washed free of non-specific constituents and the amount of antibody determined quantitatively in weight units with the use of rigorous methods of analytical chemistry. The relationship between the quantity of antibody precipitated and the antigen added is characterized by three distinct zones; i.e., a region of antibody excess, an equivalence zone in which neither antigen nor antibody can be detected in the supernate after precipitation, and a zone of antigen excess. The amount of antibody precipitated at equivalence or slight antigen excess is a measure of the precipitin in the antiserum.

Most important for the precipitin reaction is its specificity, which can be interpreted on a chemical basis. An antigen molecule contains a number of determinent chemical groups each contributing to its immunological specificity. Therefore, two antigens having at least one determinent group in common will react to some extent with antibody directed to this grouping. The extent of reaction depends upon the number and distribution of the groups in the antigen and also upon the composition of the remainder of the molecule.

The use of the precipitin reaction for the quantitative estimation of homologous antibody in response to a pure antigen is straight forward. If the reaction is to be used to study tissue specific antigens, the mere fact that two substances react qualitatively with the same antiserum does not constitute a proof of their identity, for they may only contain multiple recurrences of the same or similar immunological determinent groups. A complete quantitative analysis of their precipitin curves is necessary.

The precipitin reaction is of limited value in the analysis of a system made up of several antigens and antibodies because it is difficult to separate each antigen-antibody reaction. Careful, exhaustive differential absorption of the antiserum is required. Relative identification of either antigens or antibodies in a mixture can often be made by means of the gel diffusion techniques of Cudin and Ouchterlony.

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SESSION II, PAPER #2

"AGAR-GEL DIFFUSION AND IMMUNO-ELECTROPHORETIC ANALYSIS"

by

Pierre Grabar
Institut Pasteur, Paris, France

Better knowledge of precipitin reactions has initiated a new development of the technique of immunochemical reactions in gels. When antigens and antibodies meet in adequate proportions in gels, they form a precipitation zone or line. In some techniques one reactant diffuses into the gel containing the other. In double diffusion methods both reactants diffuse in a gel which does not contain them. These techniques allow determinations of a minimum number of precipitating systems, and the diffusion rates. Some quantitative estimations have been attempted using simple diffusion. Double diffusion allows to compare antigenic constituents in different solutions. When the precipitating systems are numerous, the interpretation is not easy.

The immuno-electrophoretic analysis, solutions of antigens are submitted to an electrophoresis in gel, followed by a lateral diffusion of antibodies, forming arches of precitation. This method permits not only to count the antigens, even when numerous, but also to define them by their mobilities and sometimes by histochemical stains.

The main handicap of all immunological methods is the irregularity in the response of immunized animals. In the case of gels, highly precipitating antisera are needed. Other difficulties (doubling of lines, etc.) will be discussed. Results of immuno-electrophoretic analysis will be shown.

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SESSION II, PAPER #3

"IMMUNOCHEMICAL ANALYSIS BASED ON COMPLEMENT FIXATION"

by

Maurice M. Rapport
Sloan-Kettering Institute for Cancer Research, New York, N. Y.

The "grave misgivings" associated with immunochemical studies based on complement fixation arise principally from the interplay of knowledge and ignorance in three areas. In one, we superimpose fixation data obtained by an indirect method on the zonal phenomena of antigen-antibody interaction; the results frequently defy analysis. In the second, we make our measurements at the limit of sensitivity of the method, and thus subject the observations to the influence of a host of poorly understood factors which interfere through non-specific inhibition or destruction of complement activity. And in the third, we express our individual enterprise by selecting a unique set of conditions from the multiple variables available, thereby inhibiting the transmission of information and achievement of common purpose. Complement fixation will be presented with a view toward overcoming these subversive influences. Elimination of hemolysis (or fixation) data; the study of antibody-antigen interaction over the complete range of antibody excess, equivalence, and antigen excess; and the relation of complement fixation to precipitation will also be discussed.

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"VIRUS NEUTRALIZATION TESTS, IMPLICATIONS AND INTERPRETATIONS"

by
Frank L. Horsfall, Jr.
The Rockefeller Institute for Medical Research, New York, N. Y.

Neutralization tests with viruses can be a trap for the unwary or the inexperienced worker. Even for the highly skilled investigator they hold the constant threat of fallacious interpretation. They require more numerous and carefully designed controls; are more costly and time consuming than any other serological test.

Identification of a virus through neutralization by an immune serum of known potency and specificity is a common objective. More common is the estimation of the specific antibody content of a serum through neutralization of a virus of established identity and known quantity. In neither instance can it be assumed that all that neutralizes is in fact antibody.

The test is based on an arbitrary end-point selected as characteristic of the infection induced in the living host; either the intact animal or cells in culture. Displacement of the end-point relative to the quantity of serum or virus employed is the only objective finding. Interpretation is based on this observation.

The nature of the virus, the immune serum and the host all affect the neutralization test. Of special importance is their mutual effect upon the quantitative relation between virus and neutralizing antibody. Only in exceptional systems does the effect of changes in their concentration approach a one to one relationship. In most systems the relation is exponential and in some the value of the exponent is large.

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SESSION II, PAPER #5

"RADIOLABELLED ANTIBODIES"

by
David Pressman
Roswell Park Memorial Institute, Buffalo, New York

Antibodies labelled with radioactive substances can be used to yield quantitative information about the concentration of a specific antibody when it is not possible by other methods, such as in the presence of a large amount of insoluble protein or when the antibody is present in too low a concentration. Radiolabelled antibodies are especially useful for determining where anti-tumor or anti-tissue antibodies localize and also for following antibodies during certain specific reactions such as absorption by a solid absorbant. The antibodies are followed by following the radioactivity.

Antiserum prepared against a tissue usually contains antibodies which will localize in other tissues as well as in the tissue against which it was prepared. Specific antibodies can be separated from cross-reacting antibodies and also concentrated by purification procedures involving absorption of the antibody of interest. For localization studies, it is important to use a label which does not destroy antibody activity, which is not split readily from the protein, and which is not incorporated in the tissues of the recipient. I^{131} has these properties and has been used widely as a label. Moreover, it can be incorporated easily and at relatively high levels of radioactivity. Other labels are useful also.

In connection with tumor localizing anti-tumor antibodies, there is the additional problem that any foreign protein localizes in tumor to a high degree. This makes the observation of specific tumor localizing antibodies more difficult than the determination of other tissue localizing antibodies where the tissue itself does not have the large nonspecific uptake observed with tumors. In the case of studies involving animal tumors, it is possible to run a large control group of tumor-bearing animals to determine the localization of nonspecific antibody protein and to compare it with the localization

from anti-tumor preparations. In the case of investigations involving the localization of antibodies in human tumors, it is necessary to be able to determine the localization of nonspecific protein simultaneously with the localization of specific antibody. This can be accomplished by use of simultaneous label technique where antibody protein labelled with one radioisotope is injected simultaneously with control material labelled with a different isotope.

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SESSION II, PAPER #6

"PURIFICATION OF TUMOR-LOCALIZING ANTIBODIES"

by

Eugene D. Day and David Pressman,
Roswell Park Memorial Institute, Buffalo, New York

Some of the techniques involving the use of radiolabelled antibodies, which were discussed in the preceding paper, have been applied to the purification, in vitro, of tumor-localizing antibodies against the Murphy rat lymphosarcoma.

Two types of localizing antibodies are formed when homogenates of the solid Murphy tumor are injected into rabbits. The first is the cross-localizing antibody which localizes preferentially in normal tissues; the second type localizes with a high degree of specificity in the tumor. When homogenates of the ascites Murphy tumor are injected into rabbits, however, cross-localizing antibodies are not formed to any appreciable extent.

Cross-localizing antibodies cross-react in vitro with sediments of normal rat tissues, and can be concentrated by absorption on and elution from these tissues or solid tumor sediment but not ascites tumor sediment. They can also be concentrated when solid tumor sediment, pretreated with trypsin, is used. Radioiodinated antibodies of this type, when so concentrated, have been found to localize in rat liver in vivo as much as 20 percent of the injected radioactive dose.

Tumor-localizing antibodies do not cross-react in vitro to any appreciable extent with sediments of normal tissues, and can, in fact, be purified by absorption with these sediments (which remove cross-localizing antibodies). They can be concentrated by serial absorptions on and elutions from solid or ascites tumor sediments, but not those pretreated with trypsin. Radioiodinated anti-tumor antibodies, thus purified and concentrated, have been found to localize specifically in tumor in vivo as much as 30 percent of the injected dose.

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SESSION II, PAPER #7

"APPLICATION OF FLUORESCENT ANTIBODIES TO THE
STUDY OF NATURALLY OCCURRING ANTIGENS"

by

Albert H. Coons
Harvard Medical School, Boston, Mass.

The use of fluorescent antibody as a method for the histochemical study of antigenic substances normally present in tissue cells will be described and illustrated by the few cases in the literature to which it has thus far been applied. These are the demonstrations of ACTH in the pituitary (Marshall), antigens common to glomeruli and reticulum (Hill and Cruickshank), chymotrypsinogen and procarboxypeptidase in the pancreas (Marshall), Forssman antigen (Tanaka and Leduc), and liver mitochondria. The possibilities and pitfalls of its use in the investigation of neoplasia will be discussed.

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"DIFFUSION CHAMBER TECHNIQUES FOR STUDIES OF CELLULAR IMMUNITY"

by
Glenn H. Algire
National Cancer Institute, National Institutes of Health, Bethesda, Md.

Methods have been developed for the culture of cells implanted within diffusion chambers in mice. The chambers were constructed using filters of cellulose derivatives of average pore diameter ranging from 10 mu to 0.8 u. Filters of suitable porosity exclude host cells, but permit the entry of extracellular fluids for nutrition of the implanted cells. These chambers have been used in studies of immunity to homografts and to heterografts.

Homografts usually are destroyed because of the immune response of the host. However, homografts within cell-impenetrable diffusion chambers survive indefinitely. They not only do not initiate immunity in control mice but survive in an immunized host. When filters with pores large enough to allow cells to enter were used, homografts were destroyed in immunized mice, whereas non-immune controls were not affected. From these results it appeared that antibodies cytotoxic to homografts are associated with cells. Histologic evidence suggested that the cells which destroyed the homografts were lymphocytes.

In contrast to the results with homografts, heterografts of the HeLa strain of human carcinoma in cell-impenetrable diffusion chambers induced immunity in the host and degeneration of the HeLa cells occurred within 15 days. Destruction of HeLa cells occurred more rapidly in previously immunized hosts, using cell-impenetrable filters. Evidence was obtained that cytotoxic antibodies to heterografts were present not only in extracellular fluid of immune animals but also were associated with cells.

The advantages and pitfalls of using diffusion chambers in studies of cellular immunity are discussed in relation to differences in the capacity of various cell types to escape through the filters; to the permeability of filters to plasma proteins, and to haemagglutinating or hemolytic antibodies; to changes in porosity of filters in contact with living tissues; and to the degree of immunity of the host.

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SESSION III, PAPER #1

"TISSUE-SPECIFIC ANTIGENS"
(Thyroid-Specific Autoantibodies)

by
Ernest Witebsky and Noel R. Rose
University of Buffalo School of Medicine, Buffalo, New York

Rabbits were injected intradermally with crude saline extracts of pooled rabbit thyroid glands incorporated into Freund adjuvants. Antibodies produced in this way were perfectly specific for the thyroid extract and did not react with extracts of any other rabbit organ tested. The most sensitive method for the demonstration of thyroid antibodies proved to be the tanned cell hemagglutination test of Boyden. The thyroid extracts of various species, such as beef and hog, also gave considerable reaction with the rabbit thyroid antibodies. In addition to rabbits, dogs were injected with pooled dog thyroid extract and antibodies against the dog thyroid could be produced in this way. The dog thyroid antibodies also cross-reacted with saline extracts of the thyroid glands of other species but not with extracts of any other dog organs nor with dog serum. Finally, out of 11 patients with a history of past or present chronic thyroiditis, 4 revealed the presence of circulating antibodies against extracts of human thyroid gland by means of the hemagglutination technic. Only one serum gave a trace of precipitation and complement fixation. The human thyroid antibodies cross-reacted with the extracts of thyroid glands of other species without reacting with extracts of various human organs or human serum. A connection between the presence of thyroid antibodies in human and the pathogenesis of thyroiditis is proposed. Antisera prepared in rabbits against extracts

of the human thyroid gland (heteroantibodies) usually give strong cross-reaction with saline extracts of human thyroid cancer tissue and, conversely, rabbit antisera against cancer tissue of the human thyroid strongly react with normal human thyroid extracts. The availability of naturally-occurring human thyroid autoantibodies in thyroiditis patients, because of their extreme specificity, offers a new reagent for the study of antigens which are shared by both the normal and malignant thyroid gland or which are specific for either of them.

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SESSION III, PAPER #2

"ACQUIRED TOLERANCE IN NEW-BORN ANIMALS"

by
L. Brent and R. E. Billingham
University College, London, England

It has been possible to induce tolerance of skin homografts in a high proportion of experimental subjects by the intravenous injection of homologous spleen cells into new-born mice. However, not all mouse strains have been found to be equally amenable to such treatment: tolerance appears to depend not only on the immunological maturity of the new-born animal, but also on the antigenic relationship between donor and recipient. It has been demonstrated that, in the mouse, the route of injection is of crucial importance: intraperitoneal (as opposed to intravenous) inoculation was found to be only moderately effective, whilst the subcutaneous route yielded wholly negative results. Experimental data throwing further light upon the 'neutral' period are reported.

There is evidence that mice injected at birth with homologous spleen cells become true tissue cell chimaeras, the foreign cells being filtered out in the host's lymph modes and spleen. Such mice frequently exhibit a marked atrophy or deficiency of their lymphoid tissues, and it is suggested that this state of affairs is the consequence of an immunological reaction on the part of the donor cells. In extreme cases a 'graft-versus-host' reaction of this kind appears to have brought about an almost entire depletion of the host's lymph nodes, and with it the death of the host. It is argued that these findings will have to be borne in mind in any attempts to restore normal immunological reactivity to agammaglobulinemic patients by the injection of homologous blood leucocytes or other tissues capable of immunological response.

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SESSION III, PAPER #3

"THE DISTRIBUTION AND IMMUNOCHEMICAL PROPERTIES OF HUMAN TISSUE AND TUMOR ANTIGENS"

by
Leonhard Korngold
Sloan-Kettering Institute for Cancer Research, New York, N. Y.

The introduction of the double gel diffusion technique by Ouchterlony has facilitated the study of soluble tissue and tumor antigens. Our objectives in studying these antigens are:

1. To enumerate the various soluble antigens of human tissues and tumors and to define them by immunological and physico-chemical means.
2. To determine the distribution of these antigens in different tissues and tumors.
3. To search for antigens that are specific for certain tissues or tumors.

Our studies to date have shown that some tissue antigens are widely distributed in all tissues and tumors examined. Other antigens are limited to the tissues of certain individuals (i.e., they are group specific), and still others show a limited degree of tissue specificity.

Soluble tissue and tumor proteins were fractionated by ammonium sulfate or zone eletrophoresis and the resulting fractions were analyzed immunologically and spectrophotometrically.

Most of the antigens have electrophoretic mobilities corresponding to those of serum beta and alpha-2 globulins.

A few examples of abnormal protein synthesis in neoplasia will be presented.

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SESSION III, PAPER #4

"INTERPRETATION OF HOST RESPONSE IN QUANTITATIVE STUDIES ON ANIMAL VIRUSES"

by

W. Ray Bryan
National Cancer Institute, National Institutes of Health, Public Health Service,
Department of Health, Education and Welfare
Bethesda, Md.

Until recent years, strictly quantitative investigations on animal viruses were confined to a few favorable agent-host systems in which the quantitative approach could be patterned after certain well-known and long established methods developed initially for the enumeration of bacteria, but later adapted to quantitative work with bacterial viruses and with some plant and animal viruses. Many animal viruses could not be successfully approached with these methods, however, and it became necessary for biologists in the virus field to search for other more appropriate procedures.

The methods found to be most suitable in situations in which virus-particle enumeration is not practical are adaptations of principles and analytical procedures developed during recent years in the fields of pharmacology and biological standardization. Many biological reactions, representing quantitative responses of both the quantal and graded types, have proven useful for bioassays of virus activity by these methods. Of growing significance also is their increasing use as research tools for investigating the reactions of living animals (or of other units of living matter), per se, in their responses to viruses, under varied experimental conditions.

The addition to the armamentarium of experimental virologists of a relatively few biometric tools (of proven value in other areas of biology) greatly extends not only the number of virus-host systems which can be investigated, but also the types of problems which can be approached quantitatively. The principles and uses of some of these tools for interpreting host responses to viruses will be illustrated and discussed.

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SESSION III, PAPER #5

"VIRUS NEUTRALIZATION"

by

Edward A. Eckert
State University of New York College of Medicine, Brooklyn, N. Y.

The immunological characteristics of many viruses have been defined quite adequately, while there has been a noticeable lack of similar studies of the tumor viruses. Indeed, the question has often been raised whether tumor viruses possess the attributes making such a study valid. An investigation of the viruses of myeloblastosis and erythroblastosis has shown that the classical serological techniques and approach could be applied. The success of this study was due to the fulfillment of certain basic requirements necessary for immunological studies of all viruses and not to the elucidation of any unique immunological properties of the leukosis viruses.

In the case of these two viruses, antisera were produced in both homologous and heterologous animals by inoculation of the viruses, and the techniques of complement fixation, the precipitin reaction, and neutralization were used.

The utilization of these techniques, when coupled with studies of the biological, physical, and chemical properties of the viruses, made it possible to classify the etiological agents of these two leukoses on a broader basis than that usually available.

In addition, the presence of normal tissue antigens as an integral part of the virus particle has been verified.

The general application of these immunological techniques to the tumor viruses is indicated when the essential preliminary requirements can be met.

###

SESSION III, PAPER #6

"APPARENT NEUTRALIZATION OF ROUS SARCOMA VIRUS BY ANTISERUM TO NORMAL CHICKEN EMBRYO"

by
Harry Rubin
California Institute of Technology, Pasadena, Calif.

Various investigators have claimed and denied the neutralization of the Rous sarcoma virus by antibodies to normal tissue components. The present report is a reexamination of these claims, using the chorioallantoic membrane of the developing chick embryo to assay the virus. The results show that anti-chick serum does indeed reduce the number of tumors on the C.A.M. if mixed with the virus, plus complement and inoculated without further dilution. In contrast to neutralization by a specific anti-viral serum obtained from a convalescent chicken however, the effect required complement and was completely reversed by diluting the serum-virus mixture before inoculation. In addition, the anti-chick serum was effective long after the virus had penetrated host cells, again in contrast to anti-viral serum. The anti-chick serum could be largely absorbed with chick embryo homogenates and to a lesser extent with sheep red cells, while these had no effect on the anti-viral serum. Forssmann antibody simulated the action of the anti-chick serum. These and other supporting findings led to the conclusion that the anti-chick serum reduced the apparent titer of the virus by preventing cell multiplication rather than by neutralizing the virus directly.

###

SESSION III, PAPER #7

"MODIFICATION OF THE IMMUNE RESPONSE BY RADIATION AND CORTISONE"

by
William H. Taliaferro
Department of Microbiology, University of Chicago, Chicago, Ill.

This review describes the radiosensitivity of different parts of the immune response and as far as possible relates X-ray damage to different stages of antibody synthesis. The antibody process can be divided into a preinduction period, an induction period and a production period. All phases of the antibody response are radiosensitive but those of the preinduction period are the most sensitive. When the latter are subjected to heavy doses of X-rays, the antibody response is completely blocked. As far as the formation of normal amounts of antibody is concerned, damage by X-rays during the preinduction period can be averted by shielding sufficient lymphatic tissue or counteracted by injecting various homologous or heterologous (e.g., HeLa) cells or yeast autolysate with the antigen. The liberation of material having a similar physiological action at a strategic point in the immune process may account for the enhancement of antibody formation as a result of heavy doses of total body or local irradiation given at sharply delimited times. Marked X-ray damage to the processes in the induction and production periods is associated with a delay in formation but not necessarily with a decrease in the amount of antibody formed.

X-radiation and cortisone probably affect the same phases in antibody formation.

###

"CYTOTOXIC EFFECTS OF ANTI-TUMOR SERUM"

by
Robert W. Wissler, and Martin H. Flax
Department of Pathology and the Argonne Cancer Research Hospital,
The University of Chicago, Chicago 37, Ill.

The immunologic and pathologic principles governing cell damage and death due to antigen-antibody reactions will be summarized. Specifically, the pathologic characteristics of the Arthus and Auer phenomena will be contrasted with the delayed, tuberculin-type of tissue hypersensitivity and the Shwartzman reaction. The histological characteristics of these classical immunological processes will be compared briefly to the pathological effects of antibodies against normal organs and tissues including those resulting from heterologous antikidney serum, and those observed following autoimmunization with normal tissues given with adjuvants.

The cytotoxic effects of heterologous antitumor sera observed by various investigators in the past as well as recent work in this and other laboratories will be presented in the light of these more general immunological phenomena. Neoplastic cytotoxic reactions will be considered in relation to the duration of treatment, the quantity of antibody administered, the cellular metabolic alterations produced, the requirement of complement, the specificity of the reaction, and the present limitations of this approach to therapy of cancer.

Suggestions for further investigation of the immunological aspects of tumor suppression utilizing the principles of delayed tissue hypersensitivity and other immunological techniques will be outlined and related to the results of preliminary experiments by the authors and other recent reports.

###

SESSION IV, PAPER #2

"CYTOTOXINS AND CYTOTOXIC ANTIBODIES"

by
John D. Ross
Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.

On reviewing approaches to immunotherapy of cancer in 1952, Hauschka concluded that "although passive transfer of experimental immunity by means of cell-free sera... or therapeutic trials with antisera 'specific' to various tumors and homologous normal tissues...have generally met with failure, a hopeful attitude relative to direct serologic therapy persists to this day in some quarters." Now, four years following this less than optimistic view, sufficient new study of cytotoxic antibody has been done to warrant re-evaluation. Survey of the field from 1952 to date imparts somewhat increased hope, but hardly less confusion. Answers to fundamental questions still require better definition. 1). Can cytotoxic antibodies be produced? Here serum cytotoxins must be differentiated from cytotoxic antibodies. Cytotoxins have been demonstrated in serum from antigen-injected rabbits, guinea pigs, mice, rats, chickens, and horses in 18 of 26 reported studies. In fewer instances has the toxic property been associated with a specifically absorbable serum globulin with the properties of antibody. The necessity for control tests with adequate numbers of pre-immunization sera is emphasized by: a) the common experience of cell culturists that variations in the toxicity of sera may be wider between homologous than heterologous samples, b) the demonstration that tissue cells contain blood-group antigens, and c) reports of cytotoxicity exerted by normal sera of some species. 2). Can cytotoxic antibodies act in vivo? Reported negative findings in tests for cytotoxic antibody are about as frequent when in vitro neutralizations were done as when passive protection tests were employed, provided that protective antisera were administered soon after challenge. In vivo action appears to depend on antibody dosage and penetration. 3). In what sense are cytotoxic antibodies specific? Commonly qualitative or quantitative tissue specificity has been observed. Although methods of antigen preparation have varied widely, and possible denaturation of antigens has not

always been duly considered, it has been shown that specificity can extend to cell type. The nature of the specificity is not clear because quantitative data are lacking. Some studies indicate that quantitative relations could prevent in vivo realization of in vitro specificity. Conversely, reports of antibody to distinctive cell constituents suggest the possibility of exploiting extraordinary specificity. 4). What effect do cytotoxic antibodies have on cells? While cytopathologic findings generally agree, loss of cell viability and lysis are not always associated. In this respect the influence of antibody and complement relations have not been resolved. Recent finding of interference with cellular metabolism by antibody action may provide an alternative explanation of apparent antibody specificity, or at least define a biochemical basis of cytopathology.

Summary consideration of the present state of research on cytotoxic antibody again suggests that work should be continued, because: a) evidence is not sufficient to discard the possibility of cancer immunotherapy as an adjuvant to surgery at least, and b) use of cytotoxic antibody as an investigative tool in cell biology has barely been attempted.

###

SESSION IV, PAPER #3

"CYTOTOXIC EFFECTS OF ANTISERA AGAINST TISSUE CULTURE GROWN HUMAN EPITHELIAL CELLS"

by

Karl Habel

U. S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Infectious Diseases, Bethesda, Md.

Antisera were produced in rabbits by immunization with cells grown in a rabbit serum medium. Cell lines used included HeLa, normal skin (Earle), KB (Eagle), and amnion. Cytotoxic effects were determined by inoculation of antisera into roller tubes containing sheets of the various cell types. In general, antisera tended to have slightly higher cytotoxic titers against the homologous cell type, but there was some cross reactions in most instances.

Highest titers were obtained when the antisera were used against human fibroblasts. Multiple adsorption of the anti-skin serum with human red blood cells caused a reduction in the cytotoxic titer against both homologous and heterologous cells. No cytotoxic action was demonstrated when rabbit antiserum against human serum was employed. Titers of the antisera were usually at a low level even against homologous cells averaging 1:40 to 1:80 final dilution. Adding antisera to the growth media at the time of planting cell suspensions did not enhance the cytotoxic titers over that obtained when antisera were added to cells already grown out in sheets.

The low titers of cytotoxic antibodies make it difficult to investigate the effects of adsorbing the antisera with different cell suspensions.

###

SESSION IV, PAPER #4

"THE NATURE OF THE ANTIGENS IN TRANSPLANTATION IMMUNITY"

by

Leslie Brent

University College, London, England

It has recently been demonstrated (R. E. Billingham, L. Brent and P. B. Medawar, Nature 178, 514-519, 1956) that tissue cells need not be viable or structurally intact in order to be able to elicit transplantation immunity in homologous hosts. Mouse spleen cells suspended in certain media such as normal citrate-saline or isotonic sucrose can be totally disintegrated by ultrasound and yet retain the capacity to immunize mice from other strains against skin grafts from the donor strain. Cellular fractionation procedures have revealed that the antigens responsible for transplantation immunity are present in the nuclei of cells, but not in their cytoplasm. They may be extracted in water

or strong salt solutions, and although they are inactivated or destroyed by desoxyribonuclease they retain their activity after treatment with trypsin and ribonuclease. The evidence at present available suggests that the transplantation antigens are desoxyribonucleoproteins. They are highly unstable and are inactivated by overnight storage at 5 deg. C., freezing and thawing three times, heating to 48.5 deg. C. for 20 minutes, or drying from the frozen state.

The relationship between these tissue antigens and those eliciting the formation of haemagglutinating antibodies is discussed.

###

SESSION IV, PAPER #5

"ACQUIRED TOLERANCE APPLIED TO EXPERIMENTAL TUMORS"

by

Hilary Koprowski
Viral and Rickettsial Section, American Cyanamid Company,
Lederle Laboratories Division, Pearl River, N. Y.

During the past two years, two mouse and two rat ascites tumors have been grown in mice of a stock previously resistant to the tumors. After one or more passages in fetuses of the Swiss ICR mouse strain, one rat and two mouse tumors became transplantable into adult ICR mice, causing ascites and death of the animals. The other rat tumor underwent numerous intracerebral passages in infant mice before its transplantation specificity changed.

Studies are now being made in an attempt to identify the "derived" tumors and to elucidate the relationship between the adapted and original lines.

###

SESSION IV, PAPER #6

"HETEROTRANSPLANTATION OF TUMORS"

by

Harry S. N. Greene
Yale University Medical School, New Haven, Conn.

Under this title, the results of a long series of heterologous transplantation experiments will be discussed. Particular emphasis will be placed on the factors influencing heterotransplantability and their mode of action.

##

SESSION IV, PAPER #7

"IMMUNOLCGICAL PROBLEMS ASSCCIATED WITH THE HETEROTRANSPLANTATION OF HUMAN TUMORS"

by

Helene W. Toolan
Sloan-Kettering Institute for Cancer Research, New York, N. Y.

The resistance engendered by implants of heterologous tissues has been studied in weanling and adult rats. Such animals, injected with uniform suspensions of transplantable human tumor cells produce both humoral cytotoxins and a cellular type immunity. Each of these systems is enhanced by subsequent injections of the human neoplasms. An attempt will be made to evaluate their relative importance in the total resistance phenomenon.

Localization of cellular immunity and the comparative results of immunization with whole and cell free fractions will be discussed.

###

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PROGRAM

MONDAY, JANUARY 7, 1957

9:00 A.M.-12:30 P.M.

POLIOMYELITIS

Session Chairman: J. ØRSKOV
State Serum Institute,
Copenhagen, Denmark

9:00 A.M.

Welcoming Remarks—Ross F. Nigrelli, President of The New York Academy of Sciences; New York Zoological Society, Zoological Park, The Aquarium, New York, N. Y.

"Mechanisms of Infection with Polioviruses"—David Bodian, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md. (30 minutes)

Discussion:

Dorothy M. Horstmann, Yale University School of Medicine, New Haven, Conn. (10 minutes)

"Control of Poliomyelitis with a Noninfectious Vaccine"—Jonas Salk, Municipal Hospital, Pittsburgh, Pa. (30 minutes)

Discussion: (10 minutes each)

R. D. Defries, Connaught Medical Research Laboratories, University of Toronto, Toronto, Ont., Canada.

James H. S. Gear, South African Institute for Medical Research, Johannesburg, South Africa.

Alexander D. Langmuir, Epidemiology Branch, Communicable Disease Center, Atlanta, Ga.

Herdis von Magnus, State Serum Institute, Copenhagen, Denmark.

Joseph E. Smadel, National Institutes of Health, Bethesda, Md.

"Facts and Perspectives of a Vaccine Field Trial"—Thomas Francis, Jr., School of Public Health, University of Michigan, Ann Arbor, Mich. (30 minutes)

Discussion: (10 minutes each)

William G. Cochran, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

A. Bradford Hill, The London School of Hygiene and Tropical Medicine, London, England.

POLIOMYELITIS

Session Chairman: A. BRADFORD HILL
The London School of Hygiene and Tropical Medicine, London, England

2:00 P.M.

"Properties of Attenuated Polioviruses and Their Behavior in Human Beings"—A. B. Sabin, The Children's Hospital Research Foundation, Cincinnati, Ohio. (30 minutes)

Discussion: (10 minutes each)

Hilary Koprowski, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

G. W. A. Dick, Queen's University, Belfast, Northern Ireland.

Renato Dulbecco, California Institute of Technology, Pasadena, Cal.

R. E. Shope, The Rockefeller Institute for Medical Research, New York, N. Y.

"Immunization with Killed Poliovirus Followed by Induced Infection with Attenuated Poliovirus"—John R. Paul, Dorothy M. Horstmann, Joyce V. Deutsch, and Joseph L. Melnick, Yale University School of Medicine, New Haven, Conn. (20 minutes)

Discussion: (10 minutes each)

P. Lépine, Institut Pasteur, Paris, France.

C. H. Stuart-Harris, Royal Hospital, Sheffield, England.

John P. Fox, Tulane University School of Medicine, New Orleans, La.

"Physical and Chemical Characteristics of Purified Poliomyelitis Virus"—Carlton E. Schwerdt, University of California, Berkeley, Cal. (30 minutes)

Discussion: (10 minutes each)

Manfred Mayer, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

Jesse Charney, Merck Institute for Therapeutic Research, West Point, Pa.

NUCLEIC ACIDS

Session Chairman: V. R. POTTER
The Medical School, University of Wisconsin, Madison, Wis.

8:00 P.M.

"The Structure of the Nucleic Acids and Related Substances"—F. H. C. Crick, Cambridge University, Cambridge, England. (30 minutes)

Discussion: (10 minutes each)

M. H. F. Wilkins, King's College, London, England.

Alexander Rich, National Institutes of Health, Bethesda, Md.

"The Biosynthesis of Ribopolynucleotides"—Severo Ochoa, New York University-Bellevue Medical Center, New York, N. Y. (30 minutes)

Discussion: (10 minutes each)

Arthur Kornberg, Washington University School of Medicine, St. Louis, Mo.

John D. Smith, University of California, Berkeley, Cal.

TUESDAY, JANUARY 8, 1957

9:00 A.M.-12:30 P.M.

NUCLEIC ACIDS

Session Chairman: W. M. STANLEY
University of California, Berkeley, Cal.

9:00 A.M.

"Electron Microscopic Studies of Tobacco Mosaic Virus and of Nucleic Acids"—Robley C. Williams, University of California, Berkeley, Cal. (30 minutes)

"The Infectivity of Tobacco Mosaic Virus Nucleic Acid"—H. L. Fraenkel-Conrat, University of California, Berkeley, Cal. (30 minutes)

Discussion: (10 minutes each)

George E. Palade, The Rockefeller Institute for Medical Research, New York, N. Y.

R. D. Hotchkiss, The Rockefeller Institute for Medical Research, New York, N. Y.

"Investigations on the Ribonucleic Acid of Tobacco Mosaic Virus"—Gerhard Schramm, Max-Planck Institute for Virus Research, Tübingen, Germany. (30 minutes)

"The Biological Activity of Tobacco Mosaic Virus Components"—Barry Commoner, Washington University, St. Louis, Mo. (30 minutes)

Discussion: (10 minutes each)

N. W. Pirie, Rothamsted Experimental Station, Harpenden, Herts, England.

F. C. Bawden, Rothamsted Experimental Station, Harpenden, Herts, England.

NUCLEIC ACIDS

Session Chairman: M. DEMEREC
Carnegie Institution of Washington,
Cold Spring Harbor, N. Y.

2:00 P.M.

"Experimental Problems Concerning the Role of Nucleic Acid in Growth of T_2"—A. D. Hershey, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. (30 minutes)

Discussion: (10 minutes each)

André Lwoff, Institut Pasteur, Paris, France.

Max Delbrück, California Institute of Technology, Pasadena, Cal.

E. L. Tatum, The Rockefeller Institute for Medical Research, New York, N. Y.

Colin M. MacLeod, University of Pennsylvania School of Medicine, Philadelphia, Pa.

Seymour Cohen, University of Pennsylvania, Philadelphia, Pa.

"The Role of Nucleic Acid in Protein Synthesis, with Special Reference to the Cell Nucleus"—Alfred E. Mirsky, The Rockefeller Institute for Medical Research, New York, N. Y. (30 minutes)

Discussion: (10 minutes each)

Paul Zamecnik, Massachusetts General Hospital, Boston, Mass.

Fritz A. Lipmann, Massachusetts General Hospital, Boston, Mass.

"Possibilities of Metabolic Interferences with Nucleic Acids"—George B. Brown, Sloan-Kettering Institute for Cancer Research, New York, N. Y. (30 minutes)

Discussion: (10 minutes each)

Howard E. Skipper, Southern Research Institute, Birmingham, Ala.

John M. Buchanan, Massachusetts Institute of Technology, Cambridge, Mass.

TUESDAY EVENING
DINNER BY INVITATION

WEDNESDAY, JANUARY 9, 1957

9:00 A.M.-12:30 P.M.

PROPERTIES ACQUIRED BY CELLS MAINTAINED IN CONTINUOUS CULTURE

Session Chairman: E. W. GOODPASTURE
Armed Forces Institute of Pathology,
Walter Reed Army Medical Center,
Washington, D. C.

9:00 A.M.

"The Mammalian Cell as an Independent Organism"—Theodore T. Puck, University of Colorado Medical Center, Denver, Colo. (25 minutes)

Discussion: (10 minutes each)

Harry Eagle, National Institutes of Health, Bethesda, Md.
André Lwoff, Institut Pasteur, Paris, France.

"Altered Cell Strains in Continuous Culture: A General Survey"—Raymond C. Parker, Connaught Medical Research Laboratories, University of Toronto, Toronto, Ont., Canada. (20 minutes)

"Indirect Evidence of Changes in Nutrient Requirements of Human Epithelial-like Cells in Continuous Culture"—R. S. Chang, Harvard University School of Public Health, Boston, Mass. (20 minutes)

"Tumor Formation by Cultured Cells Derived from Normal and Cancerous Individuals"—Alice E. Moore, Sloan-Kettering Institute for Cancer Research, New York, N. Y. (20 minutes)

"Comparative Studies of Normal and Malignant Cells in Continuous Culture"—Jerome T. Syverton, University of Minnesota, The School of Medicine, Minneapolis, Minn. (20 minutes)

"Criteria for Determining Malignancy in Tissue Culture Cell Lines in the Albino Rat"—Lewis L. Coriell, South Jersey Medical Research Foundation, Camden, N. J. (20 minutes)

Discussion: (10 minutes each)

Robert F. Parker, Western Reserve University, School of Medicine, Cleveland, Ohio.

Harry Eagle, National Institutes of Health, Bethesda, Md.

J. Leighton, University of Pittsburgh, School of Medicine, Pittsburgh, Pa.

H. S. Ginsberg, Western Reserve University, School of Medicine, Cleveland, Ohio.

Shields Warren, New England Deaconess Hospital, Boston, Mass.

ROLE OF TISSUE CULTURES IN THE ISOLATION AND STUDY OF VIRUSES

Session Chairman: JOHN R. PAUL
Yale University School of Medicine,
New Haven, Conn.

2:00 P.M.

"ECHO Viruses"—Joseph L. Melnick, Yale University School of Medicine, New Haven, Conn. (30 minutes)

Discussion: (10 minutes each)

A. J. Rhodes, Director, School of Hygiene, University of Toronto, Toronto, Ont., Canada.

H. A. Wenner, University of Kansas Medical Center, Kansas City, Kans.

D. T. Karzon, The University of Buffalo, Buffalo, N. Y.

G. J. Dalldorf, State of New York Department of Health, Albany, N. Y.

"The Significance of Adenoviruses as Agents of Respiratory and Ocular Diseases"—Robert J. Huebner, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. (30 minutes)

"Measures for Control of Adenovirus Diseases"—Maurice R. Hilleman, Walter Reed Army Institute of Research, Washington, D. C. (30 minutes)

Discussion: (10 minutes each)

H. S. Ginsberg, Western Reserve University, School of Medicine, Cleveland, Ohio.

J. E. Smadel, National Institutes of Health, Bethesda, Md.

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John M. Cotton, Chairman
50 East 63rd Street
New York, New York

Raymond H. Barrows
Executive Director
The National Foundation for Infantile Paralysis, Inc.
New York, New York

Margaret Eagan
2 Fifth Avenue
New York, New York

Eunice Thomas Miner
Executive Director
The New York Academy of Sciences
New York, New York

H. Latkowitz
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C O N F E R E N C E

on

CELLULAR BIOLOGY, NUCLEIC ACIDS AND VIRUSES

MONDAY, JANUARY 7, TUESDAY, JANUARY 8,
AND WEDNESDAY, JANUARY 9, 1957

THE NEW YORK ACADEMY OF SCIENCES
2 East Sixty-Third Street
New York 21, New York

CONFERENCE - CELLULAR BIOLOGY, NUCLEIC ACIDS AND VIRUSES

SESSION I, PAPER #1

"MECHANISMS OF INFECTION WITH POLIOVIRUSES"

by
David Bodian
School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

The consequences of infection with polioviruses depend largely on the characteristics of growth and genetic variation of the virus, and on the modes of physiologic expression of genetic variables in both virus and host. Poliovirus infections with virulent, invasive strains consist of one or more of a sequence of stages of infection, beginning with implantation and multiplication in upper and lower alimentary tract. Invasion of associated lymph nodes is sometimes followed by blood vascular dissemination to final sites of multiplication in other lymph nodes, in brown fat, and in central nervous system in chimpanzees, and apparently in a wider range of tissues in cynomolgus monkeys. Viral multiplication in non-neural tissues poses problems of the precise cells of origin and mode of shedding of virus inwardly into vascular channels or outwardly into the lumen of the alimentary tract. The time course of these processes must be considered in relation to immune responses, and probably as well to damping factors unrelated to antibody. Viral dissemination within the host further raises the controversial problem of whether spread to the central nervous system is by way of nerve fibers or by way of the blood stream. Both modes of spread can be demonstrated experimentally, but assessment of their role in natural infections involves consideration of such problems as the failure of spread of non-invasive strains, and of host factors such as the enhancement of nervous system invasion by peripheral trauma. The infection in nervous tissue requires consideration of the nature of "virulence", the relation of viral multiplication to cell injury and destruction, the role of the inflammatory process, the relation of pathologic to clinical events, and the nature of the variation in host cell susceptibility in different parts of the central nervous system. Host factors such as the role of antibody and of corticosteroids in influencing various stages of the infection also are involved in any comprehensive understanding of the determinants of this disease.

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SESSION I, PAPER #2

"VIRUS & CELLULAR FACTORS OF IMPORTANCE FOR THE CONTROL OF POLIOMYELITIS WITH A NON-INFECTIOUS VACCINE"

by
Jonas E. Salk
Municipal Hospital, Pittsburgh, Pa.

A general consideration will be presented concerning the prospect for the control of paralytic polio with a non-infectious vaccine. This will be done from the view point of the virus and cells involved and the properties and behavior of each. Factors to be considered are the relatively small size of the mass of specific virus nucleoprotein that is capable of inducing an immune response. The factors of importance for retention of this property are each considered, and data are to be presented on the degree of dissociation between infectivity and antigenicity and on the kinetics of each reaction. The influence of effective concentration of tissue culture cells for the production of antigen, and the factors conducive to the most effective use of the tissue culture method, will be cited. The importance of inactivation, of the relative purity of the specific antigen, of filtration, and of the preservative used for maintaining stability for retention of antigenic activity will be indicated. The significance of the relative instability of certain strains will be cited and the possible relation of this to the differences in antigenic capacity of the different strains will be considered.

The quantitative aspect of the immune reaction will be stressed, especially the relationship between antigenic mass and primary response, and of response of the booster as it is influenced by antigenic mass used for primary stimulation. The mechanism of immunity to paralysis and the probable role of the immunologically hyper-reactive state of the antibody forming cells will be discussed.

The possible implication of the prevention of CNS invasion with polio virus by means of a non-infectious vaccine is to be discussed, not only from the view point of poliomyelitis but of certain other diseases.

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SESSION I, PAPER #3

"FACTS AND PERSPECTIVES OF A LARGE SCALE FIELD TRIAL"

by
Thomas Francis, Jr.
School of Public Health, University of Michigan, Ann Arbor, Mich.

The evaluation of a proposed preventive measure in the human population is an epidemiological experiment.

Therefore, before a field trial of significant size in a general population is undertaken, certain scientific and social requirements must be met. The subsequent planning should be influenced by considered judgment as to the suitability in time and scope, and the probability that decisive data can be obtained. The possible influence of negative or positive results upon current concepts and future lines of investigation should be visualized. Moreover, the influence of the study upon research in other areas should be considered. The 1954 Field Trial of inactivated poliomyelitis virus vaccine will be discussed from these points of view.

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SESSION II, PAPER #1

"PROPERTIES OF ATTENUATED POLIOVIRUSES AND THEIR BEHAVIOR IN HUMAN BEINGS"

by
Albert B. Sabin
Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio

Attenuated polioviruses are viruses with decreased neurotropism for primates, but the neurotropic spectrum of such viruses covers an extremely wide range. Strains which are only partly attenuated for monkeys are completely nonparalytogenic when millions of infective particles are injected directly in the lumbar cord of chimpanzees. Virus populations which are attenuated a million or more times by the intracerebral test in monkeys may further vary over an almost 100,000 or million-fold range when tested intraspinally. Strains which are not paralytogenic for cynomolgus monkeys on spinal inoculation of approximately 1 million tissue culture infective particles have now been obtained for each of the 3 immunologic types after an extensive series of tests on the progeny of a large number of individual virus particles derived from previously selected highly attenuated virus populations.

Highly attenuated poliovirus can still combine with the specific substance in the nerve tissue of susceptible primates but is limited in its capacity for multiplication and spread to other neurons. The yield of infective virus per monkey kidney epithelial cell was also found to be much lower in vitro at low concentrations of bicarbonate and acid pH with all attenuated polioviruses. Among the type 2 and type 3 strains the more attenuated the virus the smaller was the size of the plaque on monkey kidney epithelial cells, but this did not obtain for the type 1 viruses.

Polioviruses of varying degrees of attenuation readily multiplied in the more susceptible alimentary tract of chimpanzees and humans but only poorly or not at all in the more resistant alimentary tract of cynomolgus monkeys. Absence of demonstrable viremia with many of the attenuated strains in chimpanzees and humans is indicative of an inability to multiply in other extraneural tissues. Simultaneous multiplication of two and even three types of poliovirus has been demonstrated in the human alimentary tract. No interference was encountered when the 3 types of poliovirus were fed at 3-week intervals, even when the preceding type was still multiplying at the time the next type

was fed. A previous natural or experimental oral infection prevented or greatly modified the alimentary multiplication of homotypic virus, particularly in the case of type 1, while antibody produced by two doses of formalinized vaccine had no effect either on the duration of virus excretion or the amount of virus per gram of stool. When partly attenuated or demonstrably mixed populations of virus were fed, the stool frequently contained virus of somewhat greater neurotropism, but no evidence was found that the alimentary tract selectively favored the multiplication of more neurotropic virus. Multiplication in the alimentary tract was invariably associated with development of neutralizing antibody.

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SESSION II, PAPER #2

"IMMUNIZATION AGAINST POLIOMYELITIS: KILLED VACCINE FOLLOWED BY INDUCED INFECTION WITH LIVE VIRUS"

by

John R. Paul, Dorothy M. Horstmann, Joseph L. Melnick, and J. Niederman
Yale University School of Medicine, New Haven, Conn.

In the observations to be reported from our Poliomyelitis Study Unit the purpose has been to determine the ease with which infection or re-infection could be induced in individuals who already possessed naturally acquired type III poliovirus antibodies versus an individual whose antibodies had been induced with formalinized vaccine.

Using a type III strain (KP-34) of poliovirus, kindly supplied by Dr. Sabin, a trial was carried out in January/February 1956 in 13 individuals all of whom were remained under isolation precautions during the period of observation. The group consisted almost entirely of adults - but included one child of 9. Virus isolation studies and antibodies (both neutralizing and complement fixing) were followed.

Five of these individuals were fed virus using two dosages: 1 ml containing $10^{7.5}$ T.C. (tissue culture) doses, and one containing $10^{4.5}$ T.C. doses. Four of these five individuals developed brief inapparent infections as evidenced by virus excretion and a rise of antibodies. At no time did illness occur nor did any spread of the infection to 8 close associates who were living in the same hospital ward. Seven of these associates already had Type III antibodies, one did not.

The vaccinated child in whom an inapparent infection was readily induced with a $10^{4.5}$ dose continued to excrete virus in the feces for at least 41 days, and her response over a period of 6 weeks or more followed the pattern seen in a primary natural infection in persons with no antibodies. This was in some contrast to that of individuals who were older but who had acquired their antibodies naturally, and in whom the carrier state was much shorter.

Our results are similar in many respects to those of Sabin who has had a more extensive experience with this type of observation.

These preliminary results may indicate that there may well be two aspects of human immunity to poliomyelitis which are measurable, whereas only one (antibody determinations) has hitherto been extensively studied. Here is a field in which further trials should point the way to a better understanding of immunity in poliomyelitis.

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CONFERENCE - CELLULAR BIOLOGY, NUCLEIC ACIDS AND VIRUSES

SESSION II, PAPER #3

"PHYSICAL AND CHEMICAL CHARACTERISTICS OF PURIFIED POLIOMYELITIS VIRUS"

by

Carlton E. Schwerdt
Virus Laboratory, University of California, Berkeley, Cal.

A method of purifying representative strains of the three immunological types of human poliomyelitis virus from large volumes of monkey kidney tissue culture fluid is presented. The characteristic physical particles isolated in a highly purified state are identified as virus and the numerical relationship between particles and units of infectivity is discussed. The size, shape, density and mass of the virus particles are estimated experimentally by electron microscopic and hydrodynamic methods. Data on the chemical nature of the virus particles are somewhat limited because of the difficulty in amassing sufficiently large quantities of purified virus for destructive chemical analyses. Crystallizability and various physical properties are presented as evidence for the homogeneity and high degree of purity of the suspensions of virus particles studied. The results of preliminary investigations on the antigenic nature of poliomyelitis virus particles are summarized briefly.

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SESSION III, PAPER #1

"THE STRUCTURE OF NUCLEIC ACIDS AND RELATED SUBSTANCES"

by

F. H. C. Crick
Cambridge University, Cambridge, England

The paper will summarise in a non-technical way the various structures proposed for these materials, and will point out the general features they have in common.

Some of the limitations and requirements of the X-ray studies of nucleic acid polymers are briefly described: it is necessary to know the chemical formula: only that part of the material which repeats regularily in space can be studied effectively; information from other techniques is often extremely valuable; X-ray work is mainly restricted to materials outside their biological context.

The salient features of the structures proposed for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and the RNA-like synthetic polyribonucleotides are briefly set out. The fact that no natural material is known which has both ribose and deoxyribose in the same chain is underlined. A short description is given of the very recent work on polyadenylic acid and also the structure formed by mixing it with polyuradylic acid.

It is noted that so far there is no evidence for any regular configuration for a single polynucleotide chain, and that all the regular structures so far suggested contain two chains intertwined about a common axis. Moreover the helical screws of these structures are very similar, all being right-handed and having very similar parameters for the screw axis. No case is as yet known in which two intertwined backbones come very close together, though perhaps this might happen if divalent cations were used in place of sodium. Finally the need for more physical-chemical studies, especially on base-pairing, is emphasised.

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SESSION III, PAPER #2

"BIOSYNTHESIS OF RIBOPOLYNUCLEOTIDES"

by

Severo Ochoa,
New York University-Bellevue Medical Center, New York, N. Y.

Polynucleotide phosphorylase, an enzyme catalyzing the synthesis of ribopolynucleotides of high molecular weight from nucleoside-5'-diphosphates with liberation of orthophosphate, is widely distributed in bacteria. The reaction is reversible and the enzyme also catalyzes a phosphorolysis (i.e., fission by orthophosphate) of polynucleotides

to yield nucleoside-5'-diphosphates. Data on the purification of the enzyme of Azotobacter vinelandii, the stoichiometry of the reaction, and the properties of biosynthetic polynucleotides will be presented.

From equimolar mixtures of the 5'-diphosphates of adenosine, guanosine, uridine, and cytidine, polynucleotide phosphorylase catalyzes the synthesis of RNA; but, it can also bring about the synthesis of polynucleotides containing but one nucleotide unit, for example, adenylic acid or uridylic acid, when incubated with only ADP or UDP. Data on structure, molecular weight, X-ray diffraction patterns, biological activity, and nucleotide composition show that the biosynthetic polynucleotides are closely related to RNA, and that indeed the polynucleotides containing all four nucleotides present in RNA are indistinguishable from the latter.

The availability of polynucleotides containing only one kind of nucleotide unit has disclosed important features in the physical chemical behavior of nucleic acids. Thus, the adenylic and uridylic polynucleotides interact with one another to form stable complexes of much higher molecular weight than that of the parent compounds. There are indications, through X-ray diffraction studies, that fibers drawn from such a complex possess a double stranded helical structure like that proposed by Watson and Crick for DNA. The interaction of polynucleotide chains in RNA, and in synthetic polynucleotides containing two or more kinds of nucleotide units, is reflected in molecular weights which are much higher than expected from their chain-length. The complexes (which occur also in natural RNA) are but slowly phosphorolyzed by polynucleotide phosphorylase. Therefore, the formation of these complexes should favor the synthesis of RNA by shifting the equilibrium of the reaction in favor of polymerization.

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SESSION IV, PAPER #1

"ELECTRON MICROSCOPIC STUDIES OF TOBACCO MOSAIC VIRUS AND OF NUCLEIC ACIDS"

by
Robley C. Williams
Virus Laboratory, University of California, Berkeley, California

The electron microscope has been used by the author, and others, to study the length distribution, the external shape, and the internal structure of the particles of tobacco mosaic virus. In some cases the results can be related to those from X-ray analysis. TMV particles have a highly uniform length distribution. Their diameter, in packed arrays, agrees with the X-ray results, but is larger for isolated particles. Electron microscopy has yielded equivocal answers regarding the external shape, since it has been reported to be that of a six-sided prism, a cylindrical rod, and a grooved helix (the shape inferred from X-ray investigation). The causes of the conflicting results will be discussed.

The approximate localization of the ribonucleic acid of TMV has been secured by electron microscopic observation. Partial degradation of the native virus, and of reconstituted virus, shows the RNA to be situated concentrically with the axis of the virus rod. Particles of RNA-free protein (the A-protein) of the virus, obtained by alkali treatment, have also an appearance implying the same localization of the RNA. X-ray analysis indicates that the axial region of the virus rod is hollow; a conclusion now confirmed by electron microscopy.

Electron microscopy of purified DNA has been difficult, owing to its structural lability under conditions combining extreme dilution and desiccation. Recent techniques have allowed significant micrographs to be obtained of DNA purified from calf thymus and herring sperm. The micrographs show the DNA as long fibers, with a diameter consistent with the X-ray measurements and with the two-strand model. The length distribution is very broad, while the contour of the dried fibers is best described as sinuous. Preparations of purified RNA, and of artificial ribonucleotide polymers, have been microscopically examined, but the results are disappointing as yet.

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"THE INFECTIVITY OF TMV-NUCLEIC ACID"

by
H. L. Fraenkel-Conrat
University of California, Berkeley, Cal.

The fact that the nucleic acid of TMV is per se infective was recognized simultaneously in our laboratory and that of Professor Schramm's. Although the nucleic acid preparations were obtained by different methods and differed in spectrophotometric and sedimentation behavior, both, unlike intact TMV, were very sensitive to ribonuclease and salts and resistant to anti-sera, and both were almost free from virus rods upon electron microscopic search.

Further studies have shown that the infectious material in our preparation is of the same molecular weight as the bulk of the material (about 200,000). It is sensitive to 0.1 M salts, but not to 0.001 M salts; it is also sensitive to certain divalent metals. The pH-stability and the effect of enzymes have been studied in detail.

Control experiments were performed to establish the properties of those virus rods which had resisted disintegration by the hot detergent treatment used in preparing the nucleic acid. This material, like intact TMV, was resistant to ribonuclease and to 0.1 M salts, and inhibited by anti-TMV-sera.

Studies of the chemical reactivity of the infectious nucleic acid have been initiated.

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SESSION IV, PAPER #3

"INVESTIGATION OF THE RIBONUCLEIC ACID OF TOBACCO MOSAIC VIRUS"

by
Gerhard Schramm
Max-Planck Institute for Virus Research, Tübingen, Germany

Degradation experiments and X-ray analysis have shown that the tobacco mosaic virus (TMV) consists of a ribonucleic acid (RNA) core surrounded by a protein cylinder. Assuming a molecular weight of 40.10^6 for the virus, the molecular weight of the RNA would be $2,3.10^6$. Previously we observed that by incubating the virus for a short time at pH 10,3 the protein can be removed at least partially and that the particles containing more RNA than the original virus were still infectious. By extraction with phenol the protein could be completely removed and an infectious RNA was obtained with no detectable protein. By several different experiments it was found that the activity is due to the RNA rather than to a contamination with intact virus. Comparing the activities weight by weight, that of the RNA is 5-10% of the original virus.

Thus for the first time a RNA was obtained showing a definite biological activity and studies were possible as to how this activity depends on the physical and chemical structure. These studies are complicated by the observation that the RNA is extremely unstable. At 20°C a considerable loss of activity was observed within 1-2 hours, while at -15°C the inactivation was slower. The active preparation of RNA had a molecular weight of the same order as the RNA core of the intact virus as estimated from the sedimentation constant and the intrinsic viscosity, s_{20}=20 S(c=0,1%). After inactivation we found a sedimentation constant of s_{20}=5 S (c=0,1%). In the same time the viscosity decreased. The degradation product was inactive. Several experiments were carried out to estimate the smallest active subunit of the RNA.

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"THE BIOLCGICAL ACTIVITY OF TOBACCO MOSAIC VIRUS COMPONENTS"

by
Barry Commoner
Washington University, St. Louis, Mo.

This paper is a report of recent investigations designed to help elucidate the problems developed by the recent observations that dissociated, uninfectious, tobacco mosaic virus (TMV) protein and nucleic acid can be reconstituted to form biologically active nucleoprotein, and that freshly prepared TMV nucleic acid is inherently infectious itself.

The relation between the inherent infectivity of TMV nucleic acid, and its role in the infectivity of reconstituted nucleoproteins has been studied. To some degree, reconstitution appears to reactivate nucleic acid which has lost its inherent infectivity. Slightly infectious nucleoproteins can also be formed by copolymerization of TMV protein and DNA prepared from healthy tobacco leaf. It has been found that RNA blocks the participation of DNA in this unusual reconstitution process, the two nucleic acids apparently competing for the same sites in the nucleoprotein. These observations suggest that the virus protein exerts some type of secondary influence on the biological activity of virus nucleic acid.

The inherent infectivity of TMV nucleic acid preparations has been studied. Although infectivity is usually rapidly lost on aging of nucleic acid preparations, significant increases in infectivity can be induced under some conditions. These effects appear to be associated with changes in the physical configuration of the RNA. Corresponding, but smaller, changes in the infectivity of intact TMV appear to be possible.

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SESSION V, PAPER #1

"EXPERIMENTAL PROBLEMS CONCERNING ROLE OF NUCLEIC ACID IN GROWTH OF T2."

by
A. D. Hershey
Carnegie Institution of Washington, Cold Spring Harbor, N. Y.

This paper will itemize evidence, and deficiencies in the evidence, that the deoxyribonucleic acid of T2 is an authentic example of the hypothetical autosynthetic substances that the facts of genetics seem to require.

Two new kinds of information can be cited. First, as a result of work in several laboratories, it now appears likely that functionally and physically intact molecules of nucleic acid pass from parental to offspring phage during viral growth. Second, a recently detected polypeptide present in small amounts in T2, which from its properties was suspected to be associated with the viral nucleic acid, proves not to accompany nucleic acid en route to viral offspring, and is probably not formed stoichiometrically with viral precursor nucleic acid in the presence of chloramphenicol.

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SESSION V, PAPER #2

"THE ROLE OF NUCLEIC ACID IN PROTEIN SYNTHESIS, WITH SPECIAL REFERENCE TO THE CELL NUCLEUS"

by
Alfred E. Mirsky
The Rockefeller Institute for Medical Research, New York, N. Y.

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CONFERENCE - CELLULAR BIOLOGY, NUCLEIC ACIDS AND VIRUSES

SESSION V, PAPER #3

"POSSIBILITIES OF METABOLIC INTERFERENCES WITH THE NUCLEIC ACIDS"

by

George Bosworth Brown
Sloan-Kettering Division of Cornell University Medical College

Considerable information has now accumulated on the biosynthetic pathways which lead to nucleotides and polynucleotides. In a wide variety of biological systems it has been demonstrated that unusual purine and pyrimidine derivatives can behave as antimetabolites, and may exert specific toxicities. The available evidence indicates that the metabolic interferences can involve competition of abnormal metabolites with the normal intermediates in polynucleotide synthesis, or can involve interferences with the functional balance of nucleotide coenzymes or nucleotide-like "active intermediates". The best examples of incorporation of purine and pyrimidine antimetabolites into polynucleotides stem from studies of virus nucleic acid formation.

Possibilities of chemotherapeutic or chemoprophylactic approaches to virus inhibition will be considered.

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SESSION VI, PAPER #1

"THE MAMMALIAN CELL AS AN INDEPENDENT ORGANISM"

by

Theodore T. Puck
University of Colorado Medical Center, Denver, Colo.

The development of a simple technique for plating mammalian cells in a petri dish containing nutrient solution so that each single cell multiplies indefinitely to form a macroscopic colony makes possible study of these cells as independent organisms. The concepts and methods of quantitative microbiology thus become applicable to the study of the biology of the mammalian cell. This plating procedure has been found applicable to cells from every human organ on which it has been tested, including skin, spleen, bone marrow, amnion, kidney, appendix, conjunctiva and liver. Cells of fibroblast-like and epithelial-like morphology behave similarly. Equally good success has been obtained with cells taken from adults as well as newborns, and from normal as well as neoplastic tissues. Large numbers of clonal cell lines from various human organs have been readily established.

This technique permits rapid and quantitative construction of growth curves for single human cells, which closely parallel those obtained for bacteria. Agents have been found which exert differential actions on the growth process, some affecting the proportion of cells able to initiate multiplication, others the duration of the lag period required for adaptation, and still others the generation time in the final logarithmic growth phase.

The problem of variation in human cells has been attacked by means of this technique. Adaptive changes in clonal cell stocks are under study. Gradual acquisition of the ability of a cell to multiply in media of successively decreasing nutritional complexity has been observed and the underlying mechanisms are in analysis. One particularly interesting change in phenotype with constant genotype has been found which affects the morphology of cells grown in vitro. Material present in human serum can transform a closely packed, polygonal-shaped, columnar growth of epithelial-like cells to a migratory, highly stretched, spindle-shaped, fibroblast-like, colonial morphology. This change is completely reversible, and appears to be completely determined by the molecular environment, with certain clonal cell strains.

Genetic changes in human cells have been found to occur spontaneously in laboratory cell stocks. Many more of these mutations have been produced by the action of high energy irradiation. The cell-lethal action of this agent has been demonstrated to stem predominantly from its action on the cellular genetic apparatus.

Both morphologic and biochemical mutations have been produced, and while some of the genetic markers appear to be unstable, others have persisted as reliably as those which have been most useful in studies of microbiological genetics. One mutation has been of particular interest: A change in genetic constitution of the S3 clone of the HeLa cell has destroyed its ability to respond to the substance in human serum which alters morphology from epithelial-like to fibroblast-like morphology. Thus, it would appear that morphological characteristics of human cells are under control of both genetic and molecular-environmental influences.

These studies offer hope of providing new tools for exploration of some of the classical problems involved in understanding embryonic cellular differentiation, carcinogenesis, ageing, and virus interaction with mammalian cells.

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SESSION VI, PAPER #2

"ALTERED CELL STRAINS IN CONTINUOUS CULTURE: A GENERAL SURVEY"

by

Raymond C. Parker, LaRoy N. Castor and Ernest A. McCulloch

Connaught Medical Research Laboratories and Department of Medicine,

University of Toronto, Toronto, Ont., Canada

Down through the years, there have been numerous instances of the development of strains of altered cells that cannot readily be identified with any cell type existing in the animal body. These altered cells are characterized mainly by their ability to multiply with great rapidity (e.g., 20- to 30-fold in 7 days) to yield uniform populations of free-living cells that can be propagated continuously under conditions quite unfavorable to the cells from which they were derived. Sometimes, the altered cells appear during sudden bursts of activity in old cultures undergoing marked cellular degeneration. At other times, healthy cultures become altered. Sometimes, the alterations take place in tissues only recently explanted from the body; at other times, the cells affected have been cultivated for months or years. In our laboratory, strains of altered cells have been obtained from cultures of bone marrow, kidney, liver and peripheral blood, from several species, and from various long-established strains of cells from other sources. More recently, cell alterations have been observed in clones derived by single cell isolation from cultures of monkey kidney epithelium. The single cells are isolated in capillary drops of medium on a coverslip, under a layer of oil in a Petri dish, according to a technique devised by deFonbrune and adapted to animal cell work by Lwoff and collaborators (Virology 1955, 1: 128-139). When clonal populations containing both altered and unaltered monkey kidney cells are treated with viruses, the two cell types respond quite differently, just as they also respond differently to the medium in which they are cultivated. A description will be given of efforts being made to determine the nature of these cell changes and the conditions under which they occur.

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SESSION VI, PAPER #3

"INDIRECT EVIDENCE OF CHANGES IN NUTRIENT REQUIREMENTS OF HUMAN EPITHELIAL-LIKE CELLS IN CONTINUOUS CULTURE"

by

R. Shih-Man Chang

Harvard University School of Public Health, Boston, Mass.

A strain of human conjunctival cell had been established and propagated satisfactorily for 65 successive subcultures from Feb. 1954 to Nov. 1955 in a medium consisting of 20% human serum, 5% chick embryo extract and 0.005% crystalline soybean trypsin inhibitor diluted in Hank's balanced salt solution. The same cell strain, after further continuous cultivation in 20% human serum diluted in Eagle's basal medium since Dec. 1955, shows marked degenerative changes whenever the original medium is used again. Experimental results suggest that the chick embryo extract is detrimental to this conjunctival cell; this detrimental effect was not apparent prior to Nov. 1955.

A strain of Hela cell, obtained from the Microbiological Associates, Inc. in March 1956 and since maintained in 20% human serum diluted in Eagle's basal medium, fails to propagate in 5% to 20% dialyzed human serum in Eagle's basal medium. However, when serum dialysate or meso-inositol is added to the dialyzed serum in Eagle's basal medium, this same strain of Hela cell shows satisfactory multiplication. This finding is in sharp contrast to the published works of Dr. Eagle on the nutritional requirement of the Hela cell; it is presented as evidence of a possible difference in the nutritional requirement of a cell strain maintained independently in two laboratories.

These results indicate that certain changes in the nutritional requirements of human epithelial-like cell in prolonged continuous culture could occur. Since the exact nature of such changes is not known, the evidence presented is considered indirect, and the changes, nutritional.

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SESSION VI, PAPER #4

"TUMOR FORMATION BY CULTURED CELLS DERIVED FROM NORMAL AND CANCEROUS INDIVIDUALS"

by

Alice E. Moore

Sloan-Kettering Institute for Cancer Research, New York, N. Y.

It is a well recognized fact that cells grown in tissue culture, although obtained from normal sources, have on numerous occasions acquired the ability to produce tumors in susceptible animals. Dr. Chang, who was successful in establishing in continuous culture four different cell lines from normal individuals, kindly supplied us with transplants of his conjunctiva and liver after it had been trypsinized 50-60 times. Because of the morphological appearance of the cells as they grew rapidly in our laboratory, studies were undertaken to try to determine whether they could still be considered normal. We do not believe they are for the following reasons: (1) Morphological appearance had varied from the original culture so that now the cells, nucleus, and nucleoli show a great difference in size, shape, and staining properties. (2) Chromosomal studies showed high chromosome numbers--roughly double the normal--and the idiograms differed from the normal in distribution of chromosome types. (3) Inoculation into irradiated and cortisone-treated rats and into volunteer patients with far advanced cancer resulted in the appearance of nodules which, when examined by the pathologists, were said to show cellular changes of the type associated with cancer cells. (4) Manometric studies done to determine the respiration of these cells have shown that they resemble human cancer cells rather than the control normal fibroblasts.

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SESSION VI, PAPER #5

"COMPARATIVE STUDIES OF NORMAL AND MALIGNANT CELLS IN CONTINUOUS CULTURE"

by

Jerome T. Syverton

University of Minnesota, The School of Medicine, Minneapolis, Minn.

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SESSION VI, PAPER #6

"CRITERIA FOR DETERMINING MALIGNANCY OF TISSUE CULTURE CELL LINES IN THE ALBINO RAT"

by

Lewis L. Coriell

South Jersey Medical Research Foundation, Camden, N. J.

The criteria for distinguishing a normal cell from a malignant cell cannot be defined in the present state of knowledge. Modern methods of tissue culture permit the simultaneous comparison of normal and malignant cells by many disciplines. Pure cultures

of cells isolated from their complex normal environment offer many opportunities to learn the minute details of their inner structure, organization and metabolism. These new facts as they are developed may well contribute useful criteria for distinguishing between malignant and normal cells. Indeed, the essence of all cancer research is to learn the precise ways in which cancer cells differ from normal cells.

The criteria developed by generations of clinical pathologists for diagnosis of and prognosis in cancer serve a useful function to the clinician but are of little value to the tissue culture cytologist because they are based on information peculiar to the patient. Some of these are: the age of the patient, location of tumor, tissue of origin, speed of growth, invasion of blood vessels, extension through connective tissue septa, invasion of the basement membrane in epithelial tumors, necrosis of adjacent muscle fibers and, of course, the appearance of the individual cells. The latter is the only one of these categories applicable to tissue culture cells. The pathologist's definition of a malignant cell embraces all these criteria but places greatest emphasis on the patient, i.e., independent growth at expense of normal tissue, invasion and destruction of tissue and ability to metastasize to new locations. Stripped of verbiage a malignant tumor is one which kills the patient. This definition cannot be used or put to test in tissue culture work and, therefore, we tend to coin other definitions. Many of these will be discussed and their shortcomings pointed out.

Finally the available in vitro and in vivo comparison of five tissue culture cells will be presented, namely, HeLa, human conjunctiva and kidney (Chang), human intestine (Henle), and normal monkey kidney.

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SESSION VII, PAPER #1

"ECHO VIRUSES"

by

Joseph L. Melnick

Yale University School of Medicine, New Haven, Conn.

The expanding use of tissue culture methods has led to the recovery of large numbers of cytopathogenic agents from the human intestinal tract. In the process of sorting these agents, a new group of viruses was discovered, and already 15 antigenically distinct types have been established. These agents had been referred to in the literature as orphan viruses or human enteric viruses, but through joint action* they are now classified as the enteric cytopathogenic human orphan, or echo, viruses. They were brought to recognition chiefly in laboratories using cell cultures derived from primary human or monkey sources. Such cells have proven to be more susceptible than HeLa or other lines carried in continuous passage.

Echo viruses are not related to any of the other groups of viruses which may be recovered from the alimentary tract (throat or intestine) by inoculation of primate tissue cultures. Differing from the enteric cytopathogenic monkey orphan (ecmo) viruses, the echo viruses are neutralized by human sera or gamma globulin, indicating that they produce infections in man.

The viruses may be identified by cross-neutralization or cross-complement fixation tests. Except for type 10, those that have been measured are about 30 millimicrons in diameter, similar in size to other enteric viruses (poliovirus, Coxsackie). On the basis of differential rates of inactivation by irradiation with high energy electrons, the unit bearing CF antigenicity was found to be smaller in diameter than the infectious unit.

The plaque sizes and patterns produced by the echo viruses on rhesus kidney monolayer cultures have allowed them to be placed into two groups: (A) types producing plaques with irregular, diffuse boundaries (types 1, 3, 4, 6', 9, 13, 14); and (B) types producing

*Committee on the Echo Viruses, Science 122: 1187(1955)

circular plaques resembling those of the polioviruses (types 7, 8, 12).

Because cells of different monkey species vary in their viral susceptibilities, they can be used as differential media in the isolation and presumptive classification of enteric viruses. For example, cells from the African monkey, Erythrocebus patas, were found to be highly susceptible to polioviruses and the group B echo viruses, but resistant to plaque production when exposed to the group A echo viruses.

This assembly of viruses, first encountered in the course of epidemiological studies on poliomyelitis, has been found widespread in population groups studied here and abroad. The incidence of healthy carriers has been found to be greater among children living in poorer surroundings than in those living in higher income districts with good environmental sanitation. Like other enteric viruses (poliomyelitis, Coxsackie), echo viruses were found with high frequency during the summer and fall but only rarely during the winter and spring.

Although several types have been recovered from patients with aseptic meningitis clinically indistinguishable from nonparalytic poliomyelitis, the epidemiological criteria for regarding all of these viruses as etiological agents of this syndrome have not been met. However, certain members - notably types 4, 6, 9, and 14 - have been associated with aseptic meningitis outbreaks. Moreover, types 5, 6, and 14 have been isolated from the cerebrospinal fluid of such cases.

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SESSION VII, PAPER #2

"THE SIGNIFICANCE OF ADENOVIRUSES AS AGENTS OF RESPIRATORY AND OCULAR ILLNESS"

by

R. J. Huebner, Joseph A. Bell, and Wallace P. Rowe

National Institute of Allergy and Infectious Diseases,

National Institutes of Health, Bethesda, Md.

The adenovirus family includes more than 14 immunologically distinct human agents with common biological, chemical, and serologic properties. Closely related agents also occur in other primates. Some of the human agents appear, from urban surveys at least, to be almost universally prevalent, and because of the multiplicity of independent serotypes, they approach the Coxsackie and ECHO groups in total prevalence. The prevalence and disease manifestations, however, of the different serotypes vary considerably according to several epidemiologic factors, the most important of which appear to be age, geographic locality, and environmental influences. Thus, types 1, 2, and 5 appear to be most prevalent, occurring endemically in infants and very young children, most children in urban study populations showing evidence of infection with one, and usually more than one, agent prior to grade school. They persist in adenoids and tonsils, and perhaps other lymphoid tissues, for long periods. Data bearing on the diseases associated with these types will be presented and discussed.

Types 3, 4, and 7 usually occur in epidemic or outbreak form; type 3 and possibly type 7, most commonly in general population groups, causing pharyngoconjunctival fever, febrile pharyngitis, and catarrhal conjunctivitis. Types 4 and 7 have been shown to be important causes of "acute respiratory disease" (ARD) and viral pneumonitis (atypical pneumonia), particularly as these illnesses are observed in military recruits. Type 8 has been shown to cause illnesses identical with epidemic keratoconjunctivitis. Experience with the other types is insufficient to establish them as causes of human illness.

Finally, the published data on adenoviruses thus far available will be critically reviewed, with particular consideration to the significance of adenoviruses in the total problem of undifferentiated respiratory illness.

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"MEASURES FOR THE CONTROL OF ADENOVIRUS DISEASE"

by
Maurice R. Hilleman
Walter Reed Army Institute of Research, Washington, D. C.

The acute respiratory illnesses caused by viruses of the adenovirus (RI-APC-ARD) family are a major medical problem to the Armed Forces. These agents cause up to 90% of all hospitalized cases of acute respiratory illness which occur among new recruits during winter training. On a yearly average, 1 of every 10 recruits who trains at Fort Dix, N. J., a typical training base in a northern climate, is hospitalized for an average of 8 days for respiratory illness caused by these agents. This effects great losses in the cost for hospital care, reduction in manpower, lowered operating efficiency and the consequences of disruption of the troop training program, especially in time of war.

Numerous non-specific measures for the prevention and control of bacterial and viral respiratory infections of man have been employed in the past aimed toward "sterilizing" the atmosphere by ultraviolet light irradiation or by triethylene glycol vapor or by dust suppression. All of these have been disappointingly ineffective in practice.

The discovery of the adenoviruses and their successful propagation in the laboratory made possible, for the first time, control of adenovirus infections by vaccination. A formalin-killed adenovirus vaccine prepared from infected monkey kidney tissue cultures has been developed by our laboratory and evaluated in a controlled field trial among newly recruited soldiers at Fort Dix, N. J. This vaccine effected a 98% reduction in the expected incidence of adenovirus cases. The vaccine is safe and caused no untoward effects in persons who received it. Preliminary reports by Dr. Bell and his associates of tests of a commercially prepared vaccine have indicated a substantial reduction in occurrence of respiratory disease among naval recruits.

Studies to date have indicated a relatively low incidence of adenovirus-caused respiratory illness among adult civilians. There is no indication for general use of adenovirus vaccine in this group at present. The need for the vaccine in children remains to be clarified.

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SESSION VI, PAPER #5

"COMPARATIVE STUDIES OF NORMAL AND MALIGNANT HUMAN CELLS IN CONTINUOUS CULTURE"

by
Jerome T. Syverton
University of Minnesota, The School of Medicine, Minneapolis, Minn.

The needs of virologists, which ten years ago shifted emphasis in cell culture from cells to viruses, now are returning the focus of interest back to the cell. Experience accumulated at the University of Minnesota in the maintenance of older stable strains of mammalian cells in continuous culture, in the derivation of new varieties of normal and malignant human cell strains, and in the preparation of clonal strains by modification of the plating technic of Puck and associates, led recently to coordinated attempts at biological characterization of continuously cultivated human cells. Strains being studied include lines of palate, esophagus, cervix, lung, liver and conjunctiva cells. Various criteria for differentiation of cells are proposed: specific origin, growth characteristics, nutritional responses, metrical properties, metabolic character, ploidy, response to environmental stress, response to irradiation, susceptibility to viruses and transplantability to normal or prepared animals.

Several cell lines have been subjected to chromosomal analysis. Monkey kidney and various human primary cell cultures, and a human palate fibroblast strain, were found

diploid; long cultivated normal and malignant cells varied from hypertriploid to hypotetraploid. Biokinetic analysis of cell culture growth by use of manometry revealed distinct differences among a number of cell types cultivated in standard medium, in growth lag, dependence of lag on inoculum, and rate of growth. Further comparison of two strains, HeLa and Chang's liver epithelium, showed differences also with respect to recovery after standard trypsin treatment, oxygen consumption rate, sensitivity to medium depth, and capacity to grow or maintain in media containing various serums. The comparative capacity of 7 stable or clonal human cell strains to reproduce each of 5 cytopathogenic viruses was determined. Tube titrations revealed little variation in titers of reproduced viruses between the cell lines, or between clonal isolates from successive passages of a single cell strain. Use of plaque counts, however, indicated a two-fold greater capacity of strain Maben to produce herpes and poliovirus.

With regard to current interest in malignant transformation of cells in continuous culture, it is felt that variations in the morphologic or biochemical behavior of cultivated human cells should be interpreted conservatively until more definitive study has been made of cells at each stage of cultivation from the initial explant to the final clonal strain.

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Herbert Marcuse
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Tue 2 pm
Kinne Hall 209

M. H. Trytten
Pres. Cttee
Nat. Acad. of Sci.
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