Meetings

Interferon

When Isaacs and Lindenmann first detected and described interferon some 12 years ago, they had modestly hoped that their findings would help to explain the mechanism of viral interference. Since then, much of the interest in this cell-derived protein has stemmed from the appreciation of interferon as a wide-spectrum and apparently nontoxic antiviral agent. More recently, interest in interferon has been heightened by the finding that a number of species of double-stranded ribonucleic acids can stimulate cells to produce interferon. A powerful tool has thus become available for the study of interferon production and action, along with possible new approaches to the practical therapeutic use of interferon.

This interest in interferon "inducers" was reflected in a recent symposium on interferon which was held in New York City, 5–6 December 1969, under the auspices of the New York Heart Association.

The amount of time spent on the discussion of the cellular production of interferon seems inversely proportional to the degree of understanding of this phenomenon. Most earlier studies on the production of interferon were carried out in cultures of chick embryo cells, using viruses containing RNA for the stimulation of interferon. In this system interferon production is suppressed by low concentrations of actinomycin D and by other inhibitors of cellular RNA synthesis. With actinomycin D–resistant inducing viruses, such as Sindbis, Semliki Forest, or Newcastle disease virus, interferon production gradually becomes resistant to inhibitors of RNA synthesis with time after the inoculation of cells with virus. These data, together with the results of studies with protein synthesis inhibitors, strongly indicate that interferon is coded for by the cell genome and that the virus stimulates the cell to produce a new messenger RNA which is then translated into the interferon protein. In short, interferon production in chick embryo cells superficially resembles induced enzyme synthesis in bacteria. Interferon workers have borrowed heavily from the Jacob-Monod model to explain some features of this system. However, as is the case with the synthesis of other induced enzymes in animal cells, no direct evidence has been obtained for the existence of a repressor of interferon synthesis which would be present in normal "uninduced" cells. Curiously, the only cellular product known to repress interferon production is interferon itself.

Virus-stimulated interferon synthesis in chick embryo cell cultures was discussed by R. Z. Lockart, Jr. (DuPont, Wilmington, Delaware) and D. C. Burke (University of Warwick, England). Their main goal was to characterize the molecular structure which—following the interaction of an RNA-containing virus with chick cells—is responsible for the initiation of interferon synthesis. The fact that a number of various double-stranded RNA's can stimulate cells to produce interferon has suggested that it might be the double-stranded replicative form or the replicative intermediate of the viral RNA which serves as the actual inducer molecule. However, experiments with temperature-sensitive mutants of Sindbis and Semliki Forest viruses have so far failed to confirm the notion that only double-stranded viral RNA can serve as the inducer of interferon. In fact, Burke's data suggested that Newcastle disease virus may stimulate interferon production in the absence of any detectable viral RNA synthesis. If confirmed, this finding could most likely be interpreted to mean that the single-stranded parental virus RNA can also serve as the inducer molecule. The participation of virus-specific proteins in interferon induction, while not ruled out, seems less likely.

The cellular mechanisms of interferon production in the intact animal and in some types of cell culture appear to be more complex than in chick embryo cell cultures. Interferon production in mice with bacterial endotoxin or synthetic double-stranded RNA (J. S. Youngner, University of Pittsburgh) and in rabbit kidney cell cultures with double-stranded RNA (J. Vilček, New York University) is not completely suppressed by inhibitors of protein synthesis. Cycloheximide and some other inhibitors of protein synthesis were generally found to suppress the early release of interferon, but at later stages interferon production was often increased and prolonged in the presence of inhibitors. In view of the lack of suppression by inhibitors of protein synthesis, Youngner suggested that some interferons are not de novo synthesized following "induction" but, rather, are derived from precursor protein ("preformed interferons"). Vilček suggested that the increased release of interferon in the presence of cycloheximide is the result of suppression by cycloheximide of an endogenous inhibitor of interferon production. This endogenous inhibitor probably shuts off the production of interferon and is responsible for the development of a cellular refractory state to repeated induction of interferon.

Progress in the study of structural characteristics of synthetic polynucleotide inducers was reported by T. C. Merigan (Stanford University). Of interest was the finding that the interferon-inducing and antiviral activities of the alternating copolymer polyriboadenylic-uridylic acid (poly AU) could be considerably increased by the substitution of thiophosphate for phosphate groups. This substitution was tried because it had increased ribonuclease resistance of the polymer. The results of this study revealed several characteristics essential for the interferon-inducing activity of polynucleotides. The important characteristics include the number of hydrogen-bonded polynucleotide strands, thermal stability, and resistance to ribonuclease.

E. DeMaeyer (Institut du Radium, Orsay, France) reviewed his and his wife's radiobiological and genetic studies of interferon production in mice. In terms of resistance to x-irradiation, interferon production with various viral and nonviral stimuli could be grouped into three categories. The highly radiosensitive interferon response to, for example, Newcastle disease virus was shown to be produced by lymphocytes. To demonstrate the role of lymphocytes, the authors used an ingenious technique of transplanting rat bone marrow to x-irradiated mice. When injected with Newcastle disease virus, such mice produced only rat interferon and no mouse interferon. Other genetic studies demonstrated that the high or low interferon-producing capacity, inherent with certain imbred mouse lines, is controlled by a single gene.

The purification and physicochemical characterization of interferons was discussed by K. H. Fantes (Glaxo Research, England) and K. Paucker (Chil-

dren's Hospital, Philadelphia). The former speaker reviewed the known properties of interferon, concentrating on examples of electric charge heterogeneity of various interferons. Paucker discussed recent attempts at the radioactive labeling of Newcastle disease virus-induced mouse L cell interferon. This is an important subject, but since complete purification of interferon has so far not been achieved, the conclusions of these studies are still somewhat vulnerable. The results did suggest quite strongly that labeled amino acids were incorporated into interferon, because radioactivity remained associated with interferon during numerous purification steps including a final separation by polyacrylamide gel electrophoresis or isoelectric focusing. However, a component of control cells could not be separated from interferon by any of the techniques employed. Quite similar observations were made by S. Yamazaki (University of Virginia, Charlottesville), who studied the labeling of rabbit kidney cell interferon. Both Paucker and Yamazaki speculated that the protein from normal cells, which appears to be indistinguishable from interferon in its size and electric charge, might represent an inactive precursor of interferon. If so, then these two interferon species studied might be mixtures of de novo synthesized and preformed activated interferons, but it seems wise to defer judgment on this subject until more hard facts become available.

Incidentally, Yamazaki's most purified—albeit probably still not quite pure—rabbit cell interferon had a stunning specific activity of 4.8×10^7 units per milligram of protein.

The two papers concerned with the mechanism of interferon action (R. M. Friedman, National Institutes of Health; J. A. Sonnabend, Mt. Sinai School of Medicine, New York) reflected the current skepticism toward the Marcus-Salb hypothesis. This once almost universally accepted model had postulated that an interferon-induced cellular protein, named translation inhibitory protein, brings about a specific alteration in cellular ribosomes. Such altered ribosomes were said to be unable to bind or translate the viral messenger RNA while retaining their capability to translate cellular messenger RNA. It is fair to say that no definite evidence disproving the Marcus-Salb hypothesis has been presented. Rather, current criticism is based on the failure to reproduce the experiments forming the basis of this model. Alas, no alternative experimental evidence is available to explain the mechanism of interferon action which, as repeatedly demonstrated, results in a selective suppression of viral protein synthesis.

The role of interferon in the pathogenesis of viral infections and the interrelationships of interferon and specific immune reactions were the subjects of papers by S. Baron (National Institutes of Health) and L. A. Glasgow (University of Rochester). Recent developments have suggested that interferon and specific immunity are not fully independent. In particular, it has become clear that lymphocytes not only play an important role in the production of antibody and in the cell-mediated types of immune response, but they also are an important source of interferon. Moreover, as demonstrated by Glasgow, immune lymphocytes respond to a homologous viral stimulation with increased interferon production.

The final part of the symposium was devoted to two recently recognized activities of interferon—the inhibitory effect on tumor growth and the suppression of intracellular multiplication of some protozoal parasites. H. B. Levy (National Institutes of Health) discussed his findings on the suppression of the growth of various malignant tumors in mice by a double-stranded polynucleotide interferon inducer polyinosinic-polycytidylic acid (poly I · poly C). It is still unclear whether all of this antitumor effect is interferon-mediated. However, the involvement of interferon seems likely in view of I. Gresser's independent demonstration of the suppression of mouse tumor growth by the passive administration of interferon. Since both poly I · poly C and interferon exert an inhibitory effect on tumors not known to be of viral origin, this activity cannot be the result of the antiviral action of these agents. Similar problems are posed by the demonstrated protective effect of interferon and of several interferon inducers against *Plasmodium berghei* mouse malaria (R. I. Jahiel, New York University). These findings indicate that interferon can no longer be considered a strictly antiviral agent.

Research on interferon has come a long way since its beginning some 12 years ago. Recent developments have suggested that the clinical applications of this research may be forthcoming. But our understanding of the mechanisms of interferon production and action has remained quite rudimentary.

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Forthcoming Events

May

17–18. Modern Methods of **Chemical Separations** Symp., Buffalo, N.Y. (L. B. Church, Dept. of Chemistry, State Univ. of New York at Buffalo, Buffalo 13214)

17–20. American Inst. of **Chemical Engineers** and Puerto Rican Inst. of Chemical Engineers, 3rd joint mtg., San Juan, Puerto Rico. (P. Santiago, Caribbean Gulf Refining, San Juan)

17–21. **Pulp Bleaching**, 5th intern. conf., Atlanta, Ga. (R. A. Joss, Canadian Pulp and Paper Assoc., 2300 Sun Life Bldg., Montreal, P.Q., Canada)

17–30. International **Electrotechnical** Commission, Washington, D.C. (D. Hogan, U.S.A. Standards Inst., 10 E. 40 St., New York 10016)

18–20. National **Aerospace Electronics** Conf., Dayton, Ohio. (Inst. of Electrical and Electronics Engineers, Dayton Office, 134 E. Monument St., Dayton 45402)

18–20. American **Gastroenterological** Assoc., Boston, Mass. (H. D. Janowitz, Mt. Sinai Hospital, 11 E. 100 St., New York 10029)

18–20. **Neonatal Enteric Infections** Caused by *Escherichia coli*, conf., New York, N.Y. (L. R. Neville, New York Acad. of Sciences, 2 E. 63 St., New York 10021)

18–20. Instrument Soc. of America, **Power Instrumentation** Symp., 13th, Kansas City, Mo. (R. A. Russell, Box 8405, Kansas City 64114)

18–20. **Steels for Dynamic Loading**, Cleveland, Ohio. (W. M. Mueller, American Soc. for Metals, Metals Park, Ohio)

18–21. **Photosynthetic** Unit, intern. conf., Gatlinburg, Tenn. (R. M. Pearlstein, Oak Ridge Natl. Lab., P.O. Box Y, Oak Ridge, Tenn. 37830)

18–22. **Air Force Materials** Symp. '70, Miami Beach, Fla. (J. Shipp, Executive Director, AFMS '70, P.O. Box 38, Dayton, Ohio 45420)

18–22. **Medical Library** Assoc., New Orleans, La. (H. B. Schmidt, Executive Secretary, MLA, 919 N. Michigan Ave., Chicago, Ill. 60611)

18–22. Society of **Photographic Scientists and Engineers**, 23rd annual conf., New York, N.Y. (F. Brown, Logetronics, 7001 Louisdale Rd., Springfield, Va. 22150)

19–20. International Conf. on **Magnet Technology**, Hamburg, Germany. (W. Jentschke, German Hamburg Electron Synchrotron, Notkeskieg 1, D-2, Hamburg 52)

19–22. Society for **Experimental Stress Analysis**, Huntsville, Ala. (B. E. Rossi, 21 Bridge Sq., Westport, Conn. 06880)

20–22. Conference on **Fracture Control**: Theory and Application, Chicago, Ill. (A. M. Mueller, American Soc. for Metals, Metals Park, Ohio 44073)

20–22. **Teratology** Soc., 10th annual, Annapolis, Md. (R. W. Miller, 402 Wisconsin Bldg., Bethesda, Md. 20014)

22–29. International **Cancer** Congr., 10th, Houston, Tex. (M. M. Copeland, Univ. of Texas, P.O. Box 20465, Houston 77025)

24–28. Institute of **Food Technologists**, San Francisco, Calif. (C. L. Willey, IFT, 221 N. LaSalle St., Chicago, Ill. 60601)