RNA viruses, is inhibited by actinomycin D. This inhibition is operative only within 1 to 2 hours after adsorption of the interferon inducer. This presumably represents the period during which RNA messengers are formed. The inhibitory effect of actinomycin D on interferon-mediated protection was also noted by S. E. Grossberg. Because interferon formation is also inhibited by puromycin, protein neosynthesis is involved. This suggests that such formation is caused by the release of preformed material. While small doses of mitomycin C do not inhibit interferon formation, large doses do, but, interestingly enough, cause only slight inhibition of virus replication.

In most previous experiments interferon had been produced in vitro. Clearcut evidence that interferon can be formed in vivo, and at a rather rapid rate and in high concentration, has been provided by Samuel Baron and his associates (National Institutes of Health). When a variety of viruses was intravenously inoculated into mice, interferon was found in their serum. Circulating interferon appeared at the onset of viremia, and maximal titers varied from 30 to 400 units/ml. Interferon gave the animals significant protection against subsequent challenge with virus. Similarly, Ho attained high titers of interferon in the serum of rabbits after IV inoculation with Sindbis virus. Activity was noted as early as 1 to 7 hours after inoculation. Preliminary tests showed that blockage of the reticuloendothelial system decreased interferon formation. The presence of interferon in the blood raises the question of where the interferon action occurs. Some contentions are that interferon plays a role in controlling the infectious process at sites distant from the place of interferon production. This is contrary to previously held views that interferon would be expected to act only in the region of its production. In this connection, S. E. Grossberg (Cornell University Medical College) added further evidence in favor of the newer concept. He extended his previous observations on the protection by interferon at a distant organ site in chick embryos. Nonlethal allantoic infection with Japanese encephalitis virus induced the greatest protection against subsequent infection with the neurotropic NWS influenza virus. The protective effect was demonstrable in the brain, suggesting that interferon produced in the allantoic cavity gains access to embryonic blood and organs, including the brain. The latter is thereby protected against subsequent infection. The observations that interferon produced under experimental conditions can find its way into the circulation of the host was complemented by the clinical study of I. Gresser and K. Naficy (Children's Cancer Research Foundation, Boston, Massachusetts). They found that the cerebrospinal fluid from 28 patients in a group of 152 suffering from disease of the central nervous system contained a factor similar to interferon. The highest frequency of positive fluids, 23 out of 58, was among patients with aseptic meningitis (most likely viral). In contrast, only two positive fluids were found among 69 patients with noninfectious diseases of the nervous system; 3 of 25 patients with bacterial meningitis were positive. There was a correlation between the number of leukocytes in the cerebrospinal fluid in patients with aseptic meningitis and the presence of interferon-like substances.

An interesting concept on the mode of action of interferon was presented by R. Z. Lockart, Jr. (University of Texas). His data suggest that interferon acts as an inducer and causes sensitive cells to produce a new protein(s) which, in some yet unexplained manner, is necessary for the inhibition of virus reproduction. The production of the new protein must be of cellular origin and mediated through a DNA-dependent RNA.

The new facts and concepts discussed during the meeting make it obvious that our understanding of the mechanism of production and mode of action of interferon is far from adequate. This in itself is not too disturbing because we have always had at least one comforting feeling —we knew that interferon was made in response to virus infection. But even this bit of equanimity was shattered as the meeting progressed. Judging from the results presented by H. E. Hopps and associates (National Institutes of Health), at least one strain of rickettsiae, *Rickettsia tsutsugamushi*, caused the production of interferon in the tissue culture of chick embryo. Extending this further, J. S. Youngner, in discussing this paper, stated that he and W. R. Stinebring succeeded in producing interferon in chickens by intravenous injection of large numbers of *Brucella*. G. E. Gifford (University of Florida) presented evidence that RNA could stimulate interferon production, and, in fact, products of RNA hydrolysis were even more stimulatory. One of the most potent stimulators was adenosinemonophosphate. Gifford confirmed and extended findings of Rotem and Isaacs and of Jensen. Since many substances other than viruses seem to provoke the production of interferon, the chairman of the session asked whether anyone knew of anything that did not stimulate production of interferon-like inhibitors.

Gifford reported that he could demonstrate an interferon-like substance in noninfected chick embryos. This activity increased with the age of the embryo and seemed to develop in the absence of an obvious stimulus. This raises the question of whether interferon can be produced spontaneously and reemphasizes the uneasy thought that much of what is referred to as interferon (because it has many properties of Isaacs's and Lindenmann's original interferon) may represent different types of inhibitors.

In order to look more closely at these questions and to discuss the nature of interferon and methods of interferon assay, the speakers and several of their guests met informally. As a result an *ad hoc* group (Interferon Reference Club) was formed to serve as a clearing house for ideas and products.

This meeting was sponsored by the Division of Virology of the American Society for Microbiology.

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Mitochondrial Structure and Function—A "Compostium"

On 1 and 2 August, immediately following the Sixth International Congress of Biochemistry, a group of approximately 150 scientists interested in mitochondrial structure and function gathered informally for a discussion meeting at Troutbeck Farm, Malvern, Pennsylvania. The purpose of the weekend was to provide an opportunity for discussion in greater depth and detail of various topics presented at the congress.

A session on the origin of mitochondrial membranes, chaired by A.

Linnane, brought vigorous discussion on the question of whether precursors exist as the preformed membranes or as smaller structures. The question of *de novo* synthesis of mitochondria as opposed to growth and division of existing mitochondrial structures was discussed by D. Luck, Th. Bücher, and P. Slonimski. Of considerable importance was recent work on anaerobic yeast by Linnane, Slonimski, and P. Chaix, particularly Chaix's finding that no hematin compounds whatsoever are present in some yeast strains if oxygen is rigorously excluded from the growth conditions. The findings of G. Schatz on the three components of the respiratory assembly or oxysome [succinic and reduced diphosphopyridine nucleotide (DPNH) dehydrogenases and oligomycin-sensitive adenosine triphosphatase] and of Slonimski on cytochrome-deficient yeast, strongly suggest membranes as the precursors of the mitochondrial structures, but obviously more studies are required. The existence of mitochondrial DNA and its role in regulation of mitochondria structures was discussed by Sanadi, Schatz, and J. Wilson. Various criteria for distinguishing mitochondrial turnover from *de novo* synthesis were discussed by C. Wilde, Slonimski, and Luck.

In the evening session, G. Palade chaired a session summarizing the present status of the electron microscopy of mitochondria, with particular emphasis on differences between the inner and outer mitochondrial membranes, on the state and content of the two mitochondrial spaces, and on the nature of the projecting subunits of the inner membrane. The frequently observed collapse of the outer mitochondrial space was discussed, and, in relation to it, the possibility of changes in the structure of the cristae under various physiological conditions. Uncertainty about the reality of such changes still hampers attempts to correlate biochemical and structural studies. The identification of the Fernandez-Moran–Green "elementary particles" or inner membrane subunits (IMS) as a distinguishing feature of the inner membrane was pointed out, particularly by W. Stoeckenius, who illustrated the presence of IMS on all parts of the inner membrane, cristae as well as envelope. The nature of the mitochondrial membranes was discussed from the standpoint of electron microscopy by W. Stoeckenius, of electrical resistivity by A. Lehninger, and of lipid content by S. Fleischer. The discrepancy between Fleischer's findings and the lipid requirements of membranes of the Danielli-Davson type was pointed out, and the differences between the cell membrane and mitochondrial membranes were stressed. Possible differences between the inner and outer mitochondrial membrane were also considered.

Lastly the question of the reality of the IMS was taken up in detail by G. Greville, P. Whittaker, D. Parsons, P. Blair, and B. Chance. Greville showed that, in the absence of corrections for shrinking factors, the IMS head had a calculated molecular weight of 280,000, the corresponding values for the stalk and base being 108,000 and 345,000, respectively, giving a total molecular weight of 740,000; taking into account a 12 percent shrinkage during preparation and microscopy, the maximum possible estimate would amount to not more than 1×10^6, at least for the "tripartite" model of the elementary particle currently considered by D. Green, H. Fernandez-Moran, and their co-workers.

Three clear examples of projecting subunits in preparations in which cytochromes are deficient or absent were cited by B. Chance (*Ascaris mitochondria*), H. Löw (chromatophores of *Rhodospirillum rubrum*), and P. Slonimski (cytochrome-free membranes obtained from anaerobically grown yeast).

The view that the IMS demonstrated by phosphotungstate treatment are artifacts, as was recently concluded by F. Sjöstrand, was supported by Whittaker but criticized by Parsons on the basis of electron microscopical evidence, and by Chance on the basis of results obtained in collaboration with E. Racker: membranes with attached IMS can be reconstituted upon addition of a specific protein (Racker's cold labile adenosine triphosphatase) to IMS-free membrane. It was generally agreed, however, that so far the only evidence for the existence of IMS in the natural state of mitochondria is given by the recent work of H. Moor, C. Ruska, and H. Ruska [*Z. Zellforsch. Mikroskop. Anat.* **62**, 581 (1964)], in which fractured surfaces of unfixed specimens were replicated at low temperature. Alternative mechanisms for the possible physiological formation of IMS by reorganization of subunit material associated with the inner membrane were discussed by Parsons and Chance.

A number of questions remain open in current attempts to correlate biochemical and structural studies of the mitochondrion and of its respiratory chains, but the degree to which biochemical data can be used in structural studies and vice versa was found very encouraging.

L. Ernster chaired a session on the topic of pyridine nucleotide compartmentation in mitochondria. The membrane structure between cytoplasmic and mitochondrial spaces was considered from the standpoint of isotopic studies by J. Lowenstein and J. Purvis and from the aspect of reaction kinetics by Chance. It is apparent that the high level of impermeability of the mammalian cell mitochondria may not always be found in nature, since in the yeast cell there appears to be rapid communication between cytoplasmic and mitochondrial DPNH. In addition, sperm mid-pieces show a striking response to cytochrome c, resulting in an amytal-, rotenone- and antimycin-A-sensitive but nonphosphorylating oxidation of external DPNH, which Ernster suggests may involve a different type of respiratory chain in the outer membrane than in the inner membrane of the cristae. This was also suggested by Ernster for some types of muscle mitochondria, where DPNH is capable of reacting with the respiratory chains in a similar fashion.

The principles of compartmentation of enzymes and substrates were described by Bücher, who emphasized the need for determination of enzyme activity patterns in mitochondria and the influence of these patterns of the redox states in vivo. Bücher emphasized the importance of these studies in order to determine the degree of communication of various metabolic systems. The importance of distinguishing between structural and biochemical compartmentation was emphasized by R. Estabrook and O. Lindberg. J. M. Tager suggested the possibilities of an energy requirement for hydrogen transfer in the malate-ketoglutarate systems of mitochondria. C. P. Lee described the properties of the energy-linked transhydrogenase which, when supplied with an appropriate energy source, can be 500-fold out of equilibrium, favoring the formation of reduced triphosphopyridine nucleotide (TPNH). In view of the high efficiency of tritium transfer in the transhydrogenase reaction, Lee suggested a mechanism for this reaction which would

involve the formation of an intermediate between DPNH and triphosphopyridine nucleotide (TPN).

A second session, chaired by E. C. Slater, continued the discussion of mitochondrial compartmentation. Van Dam's determination of the relationship between the amplitude of the "ATP (adenosine triphosphate) jump" in rat liver mitochondria and the simultaneous appearance of diphosphopyridine nucleotide (DPN) leads to the tentative conclusion that the jump in ATP does not provide, as heretofore believed, evidence for high-energy intermediates of the respiratory chain. However, the need for verifying in terms of oxygen utilization the oxidation of DPNH and the consequent synthesis of ATP was emphasized by Estabrook. The difficulties of carrying out this experiment at low temperatures where the reactions of ADP are slow were pointed out.

As a second topic, the site of inhibition by atractylate was discussed. M. Klingenberg and his co-workers and A. Kemp and E. C. Slater presented evidence that, unlike oligomycin, atractylate does not inhibit the phosphorylation of mitochondrial ADP by inorganic phosphate, but that it prevents the interaction of external nucleotides with the internal mitochondrial pool of the mitochondria. This conclusion, which disagrees with those presented at the Sixth International Congress by Azzone and Bruni and by P. Vignais, is in agreement with Bruni's finding that stractylate prevents the binding of adenine nucleotides to the mitochondrial membrane, and J. B. Chappell's observation that all mitochondrial reactions supported by ATP (with the exception of adenylate kinase) are inhibited by atractylate. W. C. Hülsmann presented experiments suggesting that carnitine can stimulate mitochondrial respiration by relieving a block in the Krebs cycle at the α-ketoglutarate step, caused by a high ratio of acyl-CoA to free CoA.

A session devoted to cation uptake by mitochondria was chaired by H. Rasmussen, who emphasized the similarities and differences of calcium and magnesium uptake. B. C. Pressman discussed the structures of a number of antibiotics, among them valinomycin and gramicidin, in relation to the stimulation of active transport of sodium and potassium in mitochondria. Carafoli and Lehninger described the concomitant uptake of ATP with calcium in mitochondria with an observed stoichiometry of about 1 ATP for every 10 Ca^{++}. This uptake of adenine nucleotide served to balance in part the electrostatic charges associated with calcium accumulation. Klingenberg presented similar data on the concomitant uptake of adenosine diphosphate (ADP) during calcium accumulation; apparently a difference in results exists. The possibility that other cations such as sodium or potassium were transported during oxidative phosphorylation was considered, as was the nature of the high-energy compound which contributes the energy source for ion uptake. Chappell described experiments similar to those of Pressman in which gramicidin was used to stimulate potassium uptake and hydrogen ejection, and proposed a mechanism whereby hydrogen-ion transport out of the mitochondria might be accompanied by the uptake of divalent cations such as calcium and strontium or of monovalent cations such as potassium or ammonium ion, with or without the concomitant uptake of phosphate. In the case of

ammonium ion uptake, charges are balanced and proton ejection was not to be expected, while proton ejection could be observed in the case of divalent cations. Chappell and Chance emphasized the lack of respiratory stimulation in the reaction of potassium with the mitochondrial constituents. The sites of ion accumulation were actively discussed by Chappell, L. Peachey, J. Brierley, and Klingenberg, and it was concluded that the deposits of calcium can be observed in mitochondria associated with the membrane subunits (IMS) on the membraneous part as well as in the matrix. The general hypothesis that the matrix compartment is the area where cation accumulation occurs was presented, and Peachey emphasized that the normal granules of the matrix space provide a suitable point for calcium accumulation. The possible relation between ion uptake in mitochondria and in the whole cells was discussed by A. Kleinzeller, who emphasized the possible usefulness of the concepts developed from studies with whole cells to the mitochondrial problems.

In the session on the swelling of mitochondria, chaired by A. Lehninger, a number of factors affecting large-amplitude swelling were discussed by Hunter, Azzone, and Lehninger, with particular reference to the properties of peptides such as oxytocin and gramicidin (the latter participates in cation transport). The present status of contractile protein was evaluated by Azzone and Lehninger and compared with properties of the cold labile adenosine triphosphatase and actomyosin.

The effect of a soluble relaxing-factor preparation upon mitochondrial contraction caused by the addition of ATP and magnesium was discussed by H. Baltscheffsky, and experiments illustrating the temporary inhibition of contractility were presented. Lastly, the physical changes that occur in mitochondria in large-amplitude swelling were described by Lehninger, with particular emphasis on the fact that the basic lipid bi-layers of which the cristal membrane may well be composed cannot stretch to the degree required to explain large-amplitude swelling. The general question of the shape of the cristae in nature was evaluated critically, and it was pointed out that fewer cristae are observed in 0.44*M* than in 0.32*M* sucrose. A number of properties of mitochondria prepared in a high concentration of sucrose differ from those prepared in low sucrose concentration, particularly their high content of endogenous substrate and the relatively small response to added ADP. Chance presented the hypothesis that cristae may well be in the "collapsed" state as observed by Stoeckenius. The cristal structure would be held in this condition by cross-links between adjacent crista, which may well involve portions of the projecting subunits, that is, either a zipper-like interlocking of the heads or (in view of the evidence suggesting that the subunits project into the matrix space) an extension of these cross-links, allowing for a reasonable amount of swelling and shrinkage; in large amplitude swelling the cross-links may be entirely broken.

All in all the "compostium" a (polyglot term created by Bücher) appeared to be highly successful. The presence of experts on morphology as well as on enzymology helped to focus on the major areas currently under active investigation. A greater appreciation of the two approaches was achieved. In brief, the

meeting, although loosely organized, served as a forum for the extensive discussion in depth of current hypotheses dictating the direction of research in mitochondrial structure, biological oxidations, and associated energy-transfer reactions.

The organizers of the Malvern meeting are deeply indebted to Mrs. L. S. L. Chance and to the panel chairmen, Drs. Linnane, Palade, Ernster, Slater, Rasmussen, and Lehninger. The ability of these discussion leaders to set the rapid tempo of the meeting was the direct factor responsible for the success of this postcongress discussion session.

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Forthcoming Events

November

21–22. American **Geological** Inst., Miami Beach, Fla. (L. Hoover, 1444 N St., NW, Washington, D.C. 20005)

21–24. American **Speech and Hearing** Assoc., San Francisco, Calif. (K. O. Johnson, 1001 Connecticut Ave., NW, Washington, D.C.)

23–24. **Water** for Texas, 9th annual, Texas A&M Univ., College Station. (E. T. Smerdon, Water Resources Inst., Texas A&M Univ., College Station)

23–25. American Physical Soc., **Fluid Dynamics** Div., Pasadena, Calif. (R. J. Emrich, Dept. of Physics, Lehigh Univ., Bethlehem, Pa.)

23–27. **Dosimetry of Irradiations from External Sources**, intern. symp., Health Physics Soc., French section, Paris, France. (M. Gras, 5, rue Armand, Gauthier, Paris 18e)

23–27. Use of **Radioisotopes** in Animal Nutrition and Physiology, symp., Intern. Atomic Energy Agency, Food and Agriculture Organization of the UN, Prague, Czechoslovakia. (Symp. Secretariat, Kärntnerring 11, Vienna 1, Austria)

23–28. **Internal Medicine**, 8th intern. congr., Buenos Aires, Argentina. (Secretariat, Melo 2081, Buenos Aires)

24. **Manufacturing Chemists'** Assoc., 14th conf., New York, N.Y. (Manufacturing Chemists' Assoc., 1825 Connecticut Ave., NW, Washington, D.C.)

26–28. Central Assoc. of **Science and Mathematics Teachers**, 64th annual, Detroit, Mich. (Sister Mary Ambrosia, Gesu Convent, 17180 Oak Drive, Detroit 48221)

27–28. National Council for **Geographic Education**, Minneapolis, Minn. (L. Kennamer, Univ. of Texas, Austin)

29–1. Applications of **Fundamental Thermodynamics** to **Metallurgical Processes**, conf., Pittsburgh, Pa. (G. R. Fitterer, Engineering Research Div., Schools of Engineering and Mines, 405 Engineering Hall, Univ. of Pittsburgh, Pittsburgh)

29–1. Association for Research in **Oph-**