X. Non-Antiviral Actions of Interferons

Interferons induce cells to inhibit virus replication, by an intricate system that can be described as a primary line of defense activated in cells by interferon which, by itself can likely handle translation processes of some viruses. However, if viruses are able to avoid being completely restricted by this mechanism and can begin to produce double-stranded RNA replicative forms, the cell responds more affirmatively by activating a number of more potent resistance mechanisms, such as kinases to phosphorylate (and thus inactivate) initiation factors, and an enzyme can also be activated that synthesizes a low molecular weight effector which then activates an endoribonuclease which degrades messenger RNAs. And if all else fails, the cell can abort the whole system by an interferon-induced self-destruct mechanism. It is possible that the severity of the cell's initial response may be determined by the concentration of interferon it is exposed to, and dimensions of its secondary or subsequent responses may be determined by interferon concentrations and ability of the viruses to form triggers for these reactions.

After a thorough consideration of the complex of altered reaction-sets manifested in cells by interferon treatment, it would be tempting to speculate that interferon-treated cells might be altered in their responses to substances other than viruses. For example, would the replication of other agents trying to replicate in interferon-treated cells be inhibited? Also, if one exposed interferon-treated cells to double-stranded RNA would there by cytotoxic effects not seen in cells not treated with interferon? Also, wouldn't such a series of translation obstacles and potential membrane modification be likely to alter the cells' ability to produce other products? And, would interferon-treated cells be able to carry on their own synthetic processes as well as normal cells? And if so, why wouldn't cells constitutively produce interferon so that they would be in a virus-resistant state at all times?

Interestingly, the answers to all the above questions, except the last two, are yes. The answer to the penultimate question is no, thus giving an answer to the last question. In fact these answers were revealed long before these questions were posed. It was demonstrated twenty years ago that interferons were able to alter cells in ways other than making them resistant to viruses, but, owing to the passion of the virologists who long "owned" interferon as a purely antiviral substance, it has only very recently become fashionable to recognize this reality. In this section, I shall describe several of the activities of interferons not associated with antivirus activities. The antitumor activities of

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interferon which possibly involve a composite of antiviral mechanisms and non-antiviral activities will be dealt with separately in Section XIII.

A. The Breadth of Interferon Action: The Expanding Realm of Interferonology

The coinage of the term "non-antiviral function of interferon" (Stewart II, Gosser, and Lockart, 1971a) had been preceded by repeated reports showing that interferons were not exclusively antiviral, but these were generally dismissed as artifacts, being due to impurities in the preparations, or at best, being somehow mediated by "the antiviral action". However, as it is now certain that interferons can induce a number of alterations in cells, the list of such non-antiviral activities grows at an accelerating pace. Many of the effects listed in Table 15 as interferon-induced cellular alterations are obviously related

Table 15. *Pleiotypic activities of interferons*

Activity	References
I. Antiviral	
Adenoviruses	Gallagher and Khoobyarian (1969, 1971, 1972); Barahona and Melendez (1971); Oxman, Rowe, and Black (1967)
Bovine Enterovirus	Finter (1968)
Bunyamwera	Ruiz-Gomez and Isaacs (1963 a); Isaacs, Porterfield, and Baron (1961); Baron *et al.* (1966 a); Hitchcock and Isaacs (1960)
Chikungunya	Ruiz-Gomez and Isaacs (1963 a); Friedman (1964); Gifford, Mussett, and Heller (1964)
Coronavirus	Stewart II and Desmyter (unpublished data)
Coxsackievirus	Marchenko, Povolotsky, and Krivokhatskaya (1976)
Cytomegalovirus	Glasgow *et al.* (1967); Rabson, Tyrrell, and Levy (1969); Postic and Dowling (1977)
Eastern Equine Encephalitis	Glasgow and Habel (1962 a); Vilcek and Rada (1962); Vilcek (1962)
Echo Virus	Ho and Enders (1959 a)
Encephalomyocarditis	Gresser *et al.* (1968 b); Schachter *et al.* (1970); Billiau and Schonne (1970)
Feline Leukemia	Rodgers *et al.* (1972)
Fowl Plague	Ruiz-Gomez and Isaacs (1963 a)
Fowl Pox	Asch and Gifford (1970)
Foamy Virus	Hooks *et al.* (1976)
Foot and Mouth Disease	Ahl and Rump (1976)
Friend Leukemia	Sarma *et al.* (1969); Gresser *et al.* (1967 a, b, c, d); Wheelock and Larke (1968)
GD-7	Oie *et al.* (1972 a)

Table 15 (continued)

Activity	References
Gross Leukemia	Gresser, Coppey, and Bourali (1969)
Herpesviruses	DeMaeyer and Desomer (1962); Ho and Enders (1959 a); Glasgow *et al.* (1967); Oh and Gill (1966); Vilcek (1962); Schachter *et al.* (1970); Billiau and Schonne (1970); Isaacs, Burke, and Fadeeva (1958); Fruitstone, Waddell, and Sigel (1964); Barahona and Melendez (1971)
Infectious Bovine Rhinotracheitis	Babiuk and Rouse (1976)
Influenza Viruses	Isaacs and Lindenmann (1957); Oh and Gill (1966); Burke and Isaacs (1960); Isaacs, Lindenmann and Valentine (1957); Riley, Toy and Gifford (1966); Portnoy and Merigan (1971)
Japanese Encephalitis	Stewart II, Scott and Sulkin (1969); Yamazaki (1968)
Mengo	Schachter *et al.* (1970); Billiau and Schonne (1970)
Middleburg	Bodo, Palese, and Lindner (1971)
Moloney Sarcoma/Leukemia	Sarma *et al.* (1969); Peries *et al.* (1968); Fitzgerald (1969); Berman (1970); Rhim and Huebner (1971); Declercq and Desomer (1971 a)
Newcastle Disease	Friedman (1964); Lampson *et al.* (1963); Grossberg and Scherer (1964); Isaacs, Klemperer, and Hitchcock (1961); Hallum, Thacore, and Youngner (1970)
O'Nyong-Nyong	Ruiz-Gomez and Isaacs (1963 a)
Parainfluenza-1	Balducci, Verani, and Balducci (1963); Burke and Isaacs (1960)
Parainfluenza-3 (Sendai)	Rosenquist and Loan (1967)
Poliovirus	Buckler, Wong, and Baron (1968)
Polyoma	Allison (1961); Oxman and Takemoto (1970); Dulbecco and Johnson (1970); Taylor-Papadimitriou and Stoker (1971); Atanasiu and Chany (1960)
Pseudorabies	Vilcek and Rada (1962); Vilcek (1962); Youngner, Thacore, and Kelly (1972)
Rabiesvirus	Stewart II and Sulkin (1968); Postic and Fenje (1971 a)
Radiation Leukemia	Lieberman, Merigan, and Kaplan (1971)
Rauscher Leukemia	Sarma *et al.* (1969); Gresser *et al.* (1968 a)
Reovirus	Vassef *et al.* (1974); Gauntt (1972)
Rhinoviruses	Fiala (1972)
Rous Sarcoma	Bader (1962); Strandstrom, Sandelin, and Oker-Blom (1962); Traub and Morgan (1967)
Rubella	Desmyter *et al.* (1967)
Semliki Forest	Stewart II, Scott, and Sulkin (1969); Stewart II and Lockart (1970); Buckler, Wong, and Baron (1968); Finter (1964 a, b, c, 1966 a, 1967 a, b, 1968)
Sindbis	Glasgow and Habel (1962 a); Stewart II, Scott, and Sulkin (1969); Stewart II and Lockart (1970); DeMaeyer and Desomer (1962); Ho and Enders (1959 b); Glasgow *et al.* (1967); Denys (1963)

Table 15 (continued)

Activity	References
St. Louis Encephalitis	Stewart II, Scott, and Sulkin (1969)
SV 40	Oxman and Takemoto (1970); Todaro and Baron (1965); Oxman and Black (1966); Todaro and Green (1967); Oxman, Rowe, and Black (1967); Oxman and Levin (1971)
Vaccinia	Glasgow and Habel (1962 b); Gallagher and Khoobyarian (1969); McCullough (1972); Armstrong and Merigan (1971)
Varicella-Zoster	Armstrong and Merigan (1971)
Vesicular Stomatitis	Glasgow and Habel (1962 a); Stewart II and Lockart (1970); Rosenquist and Loan (1967); Wagner *et al.* (1963); Rabson, Tyrrell and Levy (1969); Declercq and Desomer (1971 b); Declercq, Nuwer and Merigan (1970 b)
Venezuelan Equine Encephalitis	Jordan (1973); Jahrling, Navarro, and Scherer (1976)
West Nile	Ruiz-Gomez and Isaacs (1963 a)
Western Equine Encephalitis	Vilcek and Rada (1962); Brown (1966); Balducci, Verani, and Balducci (1963)
Yellow Fever	Ruiz-Gomez and Isaacs (1963 a); Finter (1970 a)
II. Antimicrobial	
A. Chlamydiae	
C. psittacosis	Sueltenfuss and Pollard (1963)
C. trachomatis	Hanna, Merigan, and Jawetz (1966, 1967); Jenkin and Lu (1967); Mordhorst, Reinicke, and Schonne (1968); Reinicke, Mordhorst, and Schonne (1967); Kazar, Krautwurst, and Gordon (1971); Kazar, Gillmore, and Gordon (1971)
B. Protozoa	
Eperythrozoon coccoides	Suntharasamai and Rytel (1973)
Plasmodium berghei	Schultz, Huang, and Gordon (1968); Jahliel, Vilcek, and Nussenzweig (1970)
Toxoplasma gondii	Remington and Merigan (1968)
C. Rickettsiae	
R. Akari	Kazar, Krautwurst, and Gordon (1971)
D. Bacteria	
Shigella flexneri	Gober *et al.* (1972)
III. Interferon Priming	
A. *Chicken Cells*	
+ Influenza	Isaacs and Burke (1958)
+ Chikungunya	Friedman (1966 a); Levy, Buckler, and Baron (1966)
+ Venezuelan Encephalitis	Jordan (1973)
+ Polyoma	Ustacelebi and Williams (1973)
+ Adenovirus	Rosztoczy (1976 a)

Table 15 (continued)

Activity	References
B. *Mouse L Cells*	
+ Western Equine Encephalitis	Lockart (1963)
+ Newcastle Disease	Paucker and Boxaca (1967); Levy-Koenig, Golgher, and Paucker (1970 a); Stewart II, Gosser, and Lockart (1971 b, 1972); Stewart II *et al.* (1973 a); Rousset (1974); Margolis, Oie, and Levy (1972); Lobodzinska, Biernacka, and Skurska (1975)
+ MM	Giron (1969); Giron *et al.* (1971); Stewart II, Gosser, and Lockart (1971 a, 1972); Gauntt (1973); Knight (1974 b)
+ Mengo or Rhinoviruses	Stewart II, Gosser, and Lockart (1971 a)
+ poly rI·poly rC	Rosztoczy and Mecs (1970); Rosztoczy (1971, 1974, 1976 a, b, 1977); Stewart II, Gosser, and Lockart (1971 b, 1972); Stewart II *et al.* (1972, 1973 a); Stewart and Declercq (1974); Margolis, Oie, and Levy (1972); Rousset (1974); Ito and Kobayashi (1974); Ito, Suzuki, and Kobayashi (1975)
C. *Rabbit Kidney Cells*	
+ Newcastle Disease	Margolis, Oie, and Levy (1972)
+ poly rI·poly rC	Barmak and Vilcek (1973); Stewart II and Declercq (1974)
D. *Monkey Cells*	
+ MM, Mengo or Polio	Stewart II, Gosser, and Lockart (1971 a)
+ Newcastle Disease	Rousset (1974)
E. *Human Cells*	
1. Human Leukocytes + Sendai	Tovell and Cantell (1971); Goore *et al.* (1973)
2. Human Fibroblasts	Babayants, Dyuisalieva, and Marchenko (1970)
+ MM, Mengo or Polio	Stewart II, Gosser, and Lockart (1971 a)
+ Influenza	Hahon (1974)
+ poly rI·poly rC	Stewart II *et al.* (1972); Mozes *et al.* (1974); Billiau *et al.* (1977 a); Billiau, Joniau, and Desomer (1972)
IV. Cell-Multiplication-Inhibition	
A. *Mouse Cells*	
L Cells	Paucker, Cantell, and Henle (1962); Paucker and Golgher (1970); Ohwaki and Kawade (1972); Knight (1973); Borecky *et al.* (1973); Broecky, Fuchsberger, and Hajnicka (1974); Matsuzawa and Kawade (1974); O'Shaughnessy *et al.* (1974); Billiau (1975); Fuchsberger, Hajnicka, and Borecky (1975); Stewart II *et al.* (1976)
L1210 Leukemia Cell	Gresser *et al.* (1970 a, b, 1973); Maciera-Coelho *et al.* 1971); Gresser, Thomas, and Brouty-Boye (1971); Gresser, Thomas, Brouty-Boye, and Maciera-Coelho (1971); Gresser, Maury, and Brouty-Boye (1972); Fontaine-Brouty-Boye *et al.* (1969); Brouty-Boye *et al.* (1973 a, b); Gresser, Bandu, and Brouty-Boye (1974); Tovey, Brouty-Boye, and Gresser (1975); Stewart II *et al.* (1976)

Table 15 (continued)

Activity	References
Primary Mouse Embryo and Kidney	Lindahl-Magnusson, Leary, and Gresser (1971, 1972); Ohwaki and Kawade (1972)
Friend Cells	Rossi *et al.* (1975); Matarese and Rossi (1977)
3T3 Cells	Knight (1973); Sokowa *et al.* (1977); Ohwaki and Kawade (1972)
EMT6 Tumor Cells	Collyn d'Hooghe *et al.* (1977)
Granulocytic Precursor Cells	McNeill and Gresser (1973)
Regenerating Liver (in vivo)	Frayssinet *et al* (1973)
B. *Human Cells*	
Lymphoblastoid Cells	Hilfenhaus and Karges (1974); Adams, Strander, and Cantell (1975); Stewart II *et al.* (1976); Hilfenhaus *et al.* (1976); Block *et al.* (1977); Einhorn and Strander (1977); Hilfenhaus, Damm, and Johannsen (1977); Finter *et al.* (1976)
Human Transformed Cell Lines	Gaffney, Picciano, and Grant (1973)
HeLa Cells	Dahl and Degre (1975, 1976 b)
U-Amnion Cells	Dahl and Degre (1975, 1976 b)
RSa, RSb Transformed Fibroblasts	Fuse and Kuwata (1976); Finter *et al.* (1976); Kuwata, Fuse, and Morinaga (1976, 1977); Kuwata *et al.* (1976)
Osteosarcoma Cells	Strander and Einhorn (1977)
Human Diploid Fibroblasts	Lee *et al.* (1972); Dahl and Degre (1975, 1976 a); Knight (1976 b); Einhorn and Strander (1977)
Trisomic-21 Fibroblasts	Tan (1976)
V. Interferon-Blocking	
A. *Chick Cells*	
+ Tick-borne Encephalitis	Vilcek and Rada (1962)
+ Chikungunya	Friedman (1966 a)
B. *Mouse L Cells*	
+ Western Equine Encephalitis	Lockart (1963)
+ Tick-borne Encephalitis	Stancek and Vilcek (1965)
+ Newcastle Disease	Cantell and Paucker (1963 a); Paucker (1965); Paucker and Boxaca (1967); Paucker and Golgher (1969); Golgher and Paucker (1973); Stewart II, Gosser and Lockart (1971 a, b); Margolis, Oie, and Levy (1972); Borden and Murphy (1971); Borden, Prochownik, and Carter (1975); Byrd *et al.* (1973); Chadha *et al.* (1974); Youngner and Hallum (1969); Rousset (1974); Lobodzinksa, Bienacka, and Skurska (1975)
+ poly rI·poly rC	Youngner and Hallum (1969); Stewart II, Gosser and Lockart (1971 b); Rousset (1974)
(1° Mouse Kidney Cells) + Newcastle Disease	Stewart II, Gosser, and Lockart (1971 b)
C. *Rabbit Kidney Cells*	
+ poly rI·poly rC	Billiau (1970); Barmak and Vilcek (1973)

Table 15 (continued)

Activity	References
D. *Monkey Cells*	
+ Newcastle Disease	Rousset (1974)
E. *Human Fibroblasts*	
+ Newcastle Disease	Bausek and Merigan (1970 b); Mozes and Vilcek (1975)
<u>*VI. Toxicity Enhancement*</u>	
A. *Vaccinia Virus*	
Mouse L Cells	Joklik and Merigan (1966); Horak, Jungwirth, and Bodo (1971); Bodo *et al.* (1972); Jungwirth *et al.* (1972 a); Stewart II, Declercq, and Desomer (1973); Suh *et al.* (1974)
B. *Double-Stranded RNA*	
Mouse L Cells	Stewart II *et al.* (1972, 1973, 1975); Stewart II, Declercq and Desomer (1973); Stewart II and Declercq (1974); Declercq and Desomer (1975); Lackovic and Borecky (1976); Heremans *et al.* (1976)
Mouse Embryo Cells	Stewart II *et al.* (1972)
Human Fibroblasts	Stewart II *et al.* (1972); Declercq, Edy and Cassimans (1975)
C. *Influenza Virus*	
Human Conjunctival Cells	Green and Mowshowitz (1977)
D. *Vesicular Stomatitis Virus*	
Mouse L Cells	Katz, Lee, and Rozee (1974)
E. *Others*	
DEAE-Dextran, Protamine Sulfate; Methylated Albumin	Katz, Lee, and Rozee (1974)
Concanavalin A	Stewart II *et al.* (1975 b)
Arginine Starvation	Lee and Rozee (1975)
<u>*VII. Depression of Syntheses*</u>	
A. *Uninduced Syntheses*	
Protein Synthesis	Johnson, Lerner, and Lancz (1968); Falcoff *et al.* (1975)
DNA Synthesis or Thymidine Uptake	O'Shaughnessy, Lee, and Rozee (1972); Tovey, Brouty-Boye and Gresser (1975); Brouty-Boye and Tovey (1977); Fuse and Kuwata (1977); Balkwill and Oliver (1977)
B. *Induced Syntheses*	
Tyrosine Amino-Transferase (Induced in Rat HTC by Steroid)	Beck *et al.* (1974); Vassef *et al.* (1974)
Glycerol-3-Phosphate Dehydrogenase (Induced in Rat Glial Cells by Steroid)	Illinger *et al.* (1976)
Glutamine Synthetase (Induced in Chick Neural-Retina by Steroid)	Matsuno, Shirasawa, and Kohno (1976)

Table 15 (continued)

Activity	References
DNA Synthesis Induced by:	
a. Lectins	Lindahl-Magnusson *et al.* (1972); Rozee, Lee, and Ngan (1973); Blomgren, Strander, and Cantell (1974); Pacheco *et al.* (1976); Heine and Adler (1977)
b. Antigens	Thorbecke, Friedman-Kien, and Vilcek (1974); Cupples and Tan (1977)
c. Erythropoietin	Smith *et al.* (1976)
VIII. Enhanced Syntheses	
A. *Uninduced Products*	
Hyaluronic Acid	Yaron *et al.* (1976)
Prostaglandin E	Yaron *et al.* (1977); Karmazin *et al.* (1977)
B. *Induced Products*	
tRNA methylase	Rozee, Katz and McFarlane (1969)
Aryl Hydrocarbon Hydroxylase Induced by Benzanthracene	Nebert and Friedman (1973)
Histamine Induced by Ragweed Antigen E	Ida *et al.* (1977)
IX. Surface Alterations	
A. *Toxicity Depression*	Yabrov (1966, 1967); Moehring, Moehring, and Stinebring 1971); Boquet (1975)
B. *Surface Charge*	Knight and Korant (1977)
C. *Increased Concanavalin A Binding*	Huet *et al.* (1974)
D. *Increased Surface Antigen Expression*	Lindahl, Leary, and Gresser (1973, 1974); Lindahl *et al.* 1976 a, b); Killander *et al.* (1976); Skurkovich *et al.* (1976 a, b); Vignaux and Gresser (1977); Lonai and Steinman (1977)
E. *Enhanced Cytotoxicity of Lymphocytes for Target Cells*	
1. Specific	Lindahl, Leary, and Gresser (1972); Lindahl (1974)
2. Non-specific	Svet-Moldavsky and Chernyakovskaya (1967); Chernyakovskaya, Slavina, and Svet-Moldavsky (1974); Slavina and Svet-Moldavsky (1973); Borecky, Lackovic, and Waschake (1970)
F. *Enhanced Sensitivity of Target Cells to Cytotoxic Antibody*	Skurkovich, Klinova, and Eremkina (1976); Skurkovich *et al.* (1976 a)
G. *Enhanced Phagocytosis*	
Mouse Macrophages + Carbon	Huang *et al.* (1971); Donahoe and Huang (1973, 1976)
Human Monocytes + Latex	Imanishi *et al.* (1975)

Table 15 (continued)

Activity	References
H. *Macrophage „Activation"*	Khesin *et al.* (1973); Schultz, Papamatheakis, and Chirigos (1977)
I. *Reversible Metamorphosis of Human Amnion Cells*	Gresser (1961 c)
X. Toxicity of Interferons	
A. *In Vitro*	
Human Bone Marrow	Nissen *et al.* (1977)
Human Granylocytic Progenitor Cells	Greenberg and Mosny (1977)
B. *In Vivo*	
Lethality (Liver Degeneration in Newborn Mice)	Gresser *et al.* (1975)
Glomerulonephritis (in Mice)	Gresser *et al.* (1976 a)
Febrile Skin Toxicity (in Human)	Desomer, Edy, and Billiau (1977)
XI. Immunodepression	
A. *Antibody Production*	
1. In Vivo	Braun and Levy (1972); Brodeur and Merigan (1974); Chester, Paucker, and Merigan (1974); Merigan, Chester, and Paucker (1975)
2. In Vitro	Gisler, Lindahl, and Gresser (1974); Johnson, Smith, and Baron (1974, 1975); Johnson, Bukovic, and Baron (1975); Johnson and Baron (1976 a, b); Booth, Booth, and Marbrook (1976); Sonnenfeld, Mandel, and Merigan (1977)
B. *Delayed-Type Hypersensitivity*	DeMaeyer, DeMaeyer-Guignard, and Vandeputte (1975); DeMaeyer-Guignard, Cachard, and DeMaeyer (1975); DeMaeyer (1976)
C. *Heterologous Adaptive Cutaneous Anaphylaxis*	Ngna, Lee, and Kind (1976)
D. *Graft-vs-Host Reaction*	Hirsch *et al.* (1973, 1974); Hirsch, Black, and Proffitt (1977); Mobraaten, DeMaeyer, and DeMaeyer-Guignard (1973); DeMaeyer, Mobraaten, and DeMaeyer-Guignard (1973); Imanishi *et al.* (1977)
(Enhanced Graft-vs-Host Reaction)	Skurkovich *et al.* (1973 a, b); Chernyakhovskaya and Slavina (1972)
E. *Complement Fixation*	Strander *et al.* (1973, 1977); Aho *et al.* (1976)
XII. Antitumor Activity	(Section XIII and XIV)

phenomena, and some may prove to not be induced by interferons, as only a few have been ascertained with the most highly purified interferons. In this section, emphasis will be placed on proving whether these effects are due to interferons rather than to non-interferon materials in the preparations. It should soon be possible to determine how many types of alterations are induced by interferon preparations and how many of these are induced by pure interferons. Then we shall begin to decipher which effects mesh into the schemes of regulatory mechanisms involved in interferon-induced antiviral activity and which alterations are mechanistically unique. Then perhaps the "true" function(s) of interferons will begin to be more apparent.

B. Inhibition of Non-Viral Agents

Interferon preparations have been shown to inhibit the growth of several non-viral agents (Table 15) both in animals and in cell cultures. All are obligate intracellular parasites. Little information has accumulated on this aspect of interferon action since it was reviewed by Vilček and Jahiel (1970). However, with the availability of highly potent and purified interferon preparations, these activities should be further explored, and evaluation of the effect of antiserum to interferons on the replication of these agents *in vitro* and *in vivo* should be informative.

Where the sensitivities of these microorganisms have been compared to those of viruses, they have generally been found to be less sensitive (Kazar, Gillmore, and Gordon, 1971; Hanna, Merigan, and Jawetz, 1966; Kazar, Krautwurst, and Gordon, 1971; Remington and Merigan (1968), at least in relation to vesicular stomatitis virus. This has been interpreted to imply that these agents are less dependent on the host cell metabolism (Kazar, Gillmore, and Gordon, 1971; Kazar, Krautwurst, and Gordon, 1971). However, as illustrated in Table 14, many viruses can also be much less sensitive than vesicular stomatitis virus to interferon action, so such evaluations are mechanistically meaningless.

Interferon inducers have been reported to inhibit multiplication of *Klebsiella pneumoniae* (Weinstein, Weitz, and Came, 1970), *Pasteurella tularensis* or *Diplococcus pneumoniae* (Giron *et al.*, 1972) and *Mycobacterium leprae* (Levy and Merigan, 1977); however, as interferon had no such effect itself, it is likely these actions are not mediated by interferons. Inducers of interferon have also been shown to enhance infections with fungi (Worthington and Hasenclever, 1972), *Trypanosoma cruzi* (Kumar *et al.*, 1971) and *Listeria monocytogenes* (Gruenewald and Levine, 1976), again without reason to suspect interferon involvement.

Interferon did not seem to inhibit growth of *Coxiella burneti* (Kazar, 1969), and, whereas rabbit interferon was inhibitory for *Toxoplasma gondii* in rabbit cells, mouse interferon did not exert such an effect in mouse cells (Schmunis *et al.*, 1973). Interference with replication of *Plasmodium gallinaceum* malaria in chickens was demonstrated, which presumably involved interferon (Herman, Shiroishu, and Buckler, 1973; Herman, 1972). And one study demonstrated inhibition of extracellular multiplication of *Shigella flexneri*

by cell-free lysates prepared from mouse or human cells pretreated with interferons (Green, 1973).

C. Priming

Isaacs and Burke (1958) observed that chick cells treated with interferon were able to produce more interferon than untreated cells when exposed to influenza virus; they referred to this effect of interferon pretreatment as "priming". This phenomenon was subsequently observed in numerous other virus-cell systems (Table 15). Priming can also be demonstrated in a number of systems where cells are repeatedly exposed to interferon inducers (Burke and Isaacs, 1958; Ho and Breinig, 1962; Mahdy and Ho, 1964; Billiau, 1970; Goorha and Gifford, 1970a, b). The apparent explanation of this effect would be that interferon pretreatment inhibited viral functions in cells that prevented the virus from shutting off cellular functions involved in interferon synthesis, and there seemed to be support for this interpretation in the findings that treatment of cells with low concentrations of interferon enhanced their ability to make interferon when subsequently induced, while with pretreatment higher concentrations of interferon inhibited ("blocked") their ability to produce interferon (Lockart, 1963; Friedman, 1966a); the larger amounts of interferon presumably induced enough antiviral activity to not only prevent virus from shutting-off cellular synthesis (of interferon) but also prevented virus from producing the "inducer molecule" (Bausek and Merigan, 1970a, b).

However, on the basis that certain evidence cannot be satisfactorily rationalized into this comforting blanket of interferon acting solely as an antiviral, Stewart II, Gosser, and Lockart (1971a) reported priming to be a "non-antiviral function of interferon". It has been a consistent finding that interferon-treated cells are able to produce interferon more quickly, whether their total production is quantitatively enhanced or depressed ("blocked") by the interferon pretreatment (Fig. 18; Paucker and Boxaca, 1967; Stewart II, Gosser, and Lockart, 1971a, b; Rosztoczy and Mecs, 1970; Rosztoczy, 1971; Stewart II *et al.*, 1972; Ustacelibi and William, 1973; Friedman, 1966a; Levy, Buckler, and Baron, 1966; Stewart II and Declercq, 1973; Levy–Koenig, Golgher, and Paucker, 1970). It is difficult to imagine that the supposed viral replicative event requisite for interferon induction could occur more quickly in interferon-treated cells than in normal cells.

Also, it was found that interferon-treated cells produce more interferon than do normal cells when they are induced with poly rI·poly rC (Rosztoczy and Mecs, 1970; Stewart II, Gosser, and Lockart, 1971b; Table 15).

The requirments for cells to produce interferon after priming are similar to those of normal cells, requiring induction of RNA and protein. However, the interferon response of primed cells becomes resistant to inhibition by actinomycin D much sooner than the response of normal cells (Friedman, 1966a; Levy, Buckler, and Baron, 1966; Stewart II, Gosser, and Lockart, 1971a; Rosztoczy, 1976b), suggesting they produce interferon messenger RNA more quickly than do normal cells, though this has not been directly determined by messenger RNA isolations. Interferon treatment seemingly sensitizes cells to

induction processes and shortens the induction process in those cells having a long induction lag-phase (Fig. 7), making these responses occur with kinetics similar to those already seen in cells with short lag-phase. In fact, cells with normally short induction lag-phases do not show quicker responses when primed (Stewart II, Gosser, and Lockart, 1971b; Tovell and Cantell, 1971; Barmak and Vilček, 1973). These data suggest that interferon priming removes some restriction on the induction process that normal cells with long induction-lags must perform before producing interferon messenger RNA (Stewart II, Gosser, and Lockart, 1972). A partial induction of a sequential induction process would also explain the quantitative and temporal aspects of priming. However, a more direct shunt-mechanism of interferon induction in primed cells can be envisioned, as interferon priming also removes the requirement of mouse cells for DEAE-dextran to be induced by poly rI·poly rC (Rosztoczy, 1971; Stewart II *et al.*, 1972), suggesting a direct surface membrane alteration by interferon treatment.

Development of priming differs from development of antiviral activity in a number of respects. Priming can develop rapidly (less than one hour) in cells treated with high concentrations (100 to 1000 units/ml) of interferon, whereas development of antiviral activity is delayed (Giron *et al.*, 1971; Stewart II,

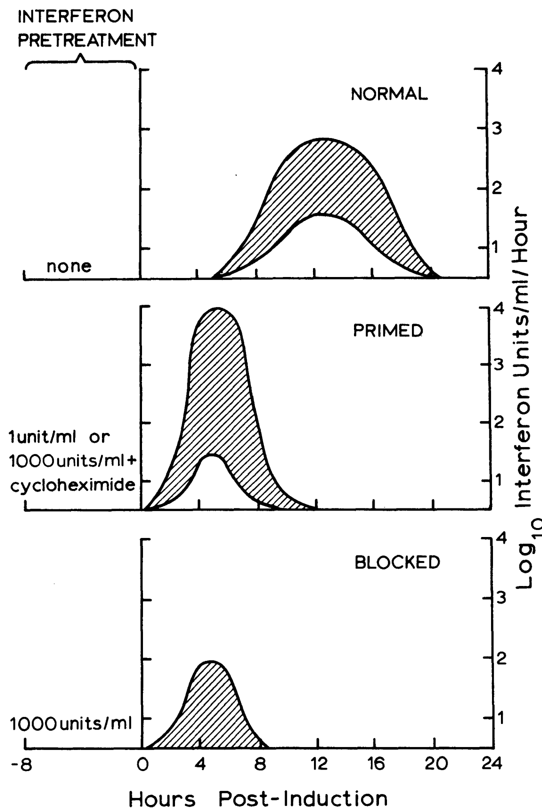

Fig. 18. Requirements for development of blocking and priming

Gosser, and Lockart, 1971a). Pretreatment for longer periods with high concentration of interferon can give both priming and blocking; that is, the total amount of interferon produced can be decreased but it will be made sooner than normal (Stewart II, Gosser, and Lockart, 1971b). The development of priming can also vary in different cell types (Havell and Vilček, 1972).

The major distinction between requirements for development of antiviral activity and priming is protein synthesis. Whereas protein synthesis is always required for cells to become resistant to viruses, it is not required for at least some cells to become primed. Friedman (1966a) reported that chick cells failed to become primed by treatment with interferon in the presence of protein synthesis inhibitors. However, Stewart II, Gosser, and Lockart (1971a) found that L cells treated with interferon in the presence of cycloheximide, puromycin or p-fluorophenylalanine developed no virus resistance yet became fully primed. This observation was confirmed in L cells by Knight (1974b) and Rosztoczy (1974) and in rabbit kidney cells by Barmak and Vilček (1973). Curiously, Ito, Suzuki, and Kobayashi (1975) claimed a requirement for newly synthesized protein for priming activity of interferon; their data do not, however, support this interpretation. In fact, they looked only at amounts of interferon made, not when it is made; even so, the amount of interferon produced by cells treated with interferon in cycloheximide was more than that made by normal cells, though slightly less than that made by cells primed with interferon alone, and no data indicate the efficiency of reversal of protein synthesis inhibition, which could diminish interferon production. Similar problems of interpretation are involved in the data of Saito and Kohno (1977), in which case interferon yields were enhanced in cells exposed to interferon and protein synthesis inhibitors, though less so than in cells exposed to interferon alone; however, complete reversal of the protein inhibition was not achieved, thereby further obscuring the results. Where these complications are considered, it is evident the protein synthesis inhibition during interferon treatment does not prevent priming.

It was reported by Rosztoczy (1974) that whereas antiviral activity induced by interferon decayed slowly over a period of over 72 hours, cells had reverted to the unprimed state within 24 hours after removal of interferon.

Priming is, as pointed out previously (Section VI. B. 3.), of considerable practical importance, as it can increase interferon yields significantly, and often exerts a cooperative enhancement with superproduction.

Studies on the mechanism of priming by determining the fate of polynucleotide inducers have added little to our understanding. Stewart II and DeClercq (1974) found that primed cells had the same molecular size requirements for poly rI·poly rC molecules to trigger interferon production. DeClercq, Stewart II and Desomer (1973c) and DeClercq and Stewart II (1974a) reported that priming did not alter the degradation of poly rI·poly rC by cells.

It is also important to note that while there are a number of substances that can enhance the ability of cells to produce interferons, none of these can be classified as able to prime cells, in terms of the cells' ability to make more interferon than normal and to make it sooner than normal. Rosztoczy (1977) was unable to prime cells by pretreatment with cyclic AMP, norepinephrine or

DEAE-dextran (though the latter can enhance the ability of cells to respond to induction with poly rI·poly rC) or ethanol. Similarly, the enhancement of interferon production in L cells induced with poly rI·poly rC after amphotericin B also did not alter the production kinetics (Borden, Booth, and Leonhardt, 1978).

In contrast to all the other non-antiviral actions of interferons, the interpretation that priming activity resides in interferon itself rather than in impurities in the interferon preparations has never been much doubted, perhaps because priming was initially interpreted as resulting from antiviral activity. Priming activity has been found in every species that has been tested, with each interferon preparation assayed. Priming activity exhibits the same defined host-range of activity as the antiviral activity, though often being a more sensitive assay system than induction of antiviral action (Levy–Koenig *et al.*, 1970; Stewart II, Gosser, and Lockart, 1971a; Ito and Kobayashi, 1974; Rousset, 1974). Interferon was found to maintain constant ratios of antiviral unit/priming unit through more than a 1000-fold purification (Stewart II, Gosser, and Lockart, 1971a), and a 1,000,000-fold purification (Stewart II *et al.*, 1973); it was also demonstrated that each of the distinct molecular species of mouse interferons were identical in this respect (Stewart II, 1975b; Fig. 13); and we have recently found that mouse interferon purified to 10^9 units/mg of protein is able to prime cells in the same ratio/antiviral unit as "crude" (10^4 units/mg protein), "semi-crude" (10^5 units/mg), "partially–purified" (10^6 units/mg), "purified" (10^7 units/mg) and "highly purified" (10^8 units/mg) preparations. Further, the low molecular weight mouse interferoid (Fig. 15) is identical in this respect to native mouse interferon (Stewart II, Lin, and Wiranowska–Stewart, 1978). The priming factor fulfills all the criteria for acceptance as an interferon.

Additionally, priming activity in human cells is, in spite of the claim to the contrary (DeClercq *et al.*, 1975), apparently determined by chromosome 21 (Frankfort *et al.*, 1978).

D. Blocking

As demonstrated in Fig. 18, pretreatment of cells with interferon can either increase interferon yields (priming), or it can diminish interferon responsiveness of cells (blocking). This blocking effect of interferon on interferon production was first reported by Vilček and Rada (1962), and has since been observed in many cell-interferon systems (Table 15). However, the early exclusively antiviral attitude of interferonologists dictated only two possible interpretations of this phenomenon:

1. The antiviral action induced by interferon prevented the virus from performing "the viral function responsible for initiation of interferon production" (Bausek and Merigan, 1970b); or,

2. Blocking was due to an impurity in interferon preparations (Baron and Levy, 1966).

The former interpretation was comfortable until it was reported that interferon responses induced by poly rI·poly rC were also blocked in interferon-

treated cells (Youngner and Hallum, 1969; Stewart II, Gosser, and Lockart, 1971b; Golgher and Paucker, 1973; Rousset, 1974; Barmak and Vilček, 1973). These data make it difficult to rationalize blocking in terms of antiviral action. In fact, as illustrated in Fig. 18, when cells are blocked and not able to produce normal levels of interferon in response to inducers, the residual interferon they make is made earlier. For this to be interpretable as an antiviral manifestation it would be necessary to imagine that the production of the viral event necessary for interferon induction was performed sooner in interferon-treated cells. More likely the interferon-treated cells are primed by interferon (to produce interferon earlier) and blocked at a post-transcriptional step (to translate less of the induced interferon message). It could be imagined that the message translation obstacles imposed in interferon-treated cells as part of "the antiviral mechanism" could as well turn to the attack of newly induced interferon messenger RNA, thus restricting the production of new interferon in cells previously exposed to "enough" of it.

Whereas development of priming requires only brief exposure to low concentrations of interferon, during which time no protein synthesis is required (see above), development of blocking is in each case different: blocking requires several hours of exposure to relatively high concentrations of interferon (Paucker and Boxaca, 1967; Stewart II, Gosser, and Lockart, 1971b; Barmak and Vilček, 1973; Rousset, 1974; Lobodzinska, Biernacka, and Skurska, 1975), and during this exposure protein synthesis is required (Stewart II, Gosser, and Lockart, 1971b; Barmak and Vilček, 1973). In fact, if protein synthesis is arrested during prolonged exposure of cells to high concentrations of interferons, the cells not only do *not* become blocked, they become primed (Stewart II, Gosser, and Lockart, 1971b; Barmak and Vilcek, 1973). Thus blocking must develop under conditions allowing development of a pronounced antiviral state, perhaps even sufficient to activate the restrictions imposed on transcripts that avoid the first line of interferon-induced defenses. In this regard it should be recalled that Chany and Vignal (1968, 1970) reported an interferon-resistant cell line that did not develop hypo-responsiveness (blocking?) to repeated induction (Section VI. 3.).

Kleinschmidt (1972) offered an intriguingly elaborate explanation of blocking based on the formation of complexes of the monomeric interferon "subunits"; however, as the "subunit hypothesis" (Carter, 1970, 1971) has been debunked, so has this model (Section VII. A.).

There have been three papers on induction of cultures with virus and poly rI·poly rC in which the virus induced "late" interferon while the poly rI·poly rC induced "early" interferon (Bausek and Merigan, 1970a; Stewart II, Gosser, and Lockart, 1971b; Mozes and Vilček, 1975). In these systems the early interferon responses were not markedly inhibited by interferon pretreatment and could be repeatedly stimulated, whereas the late responses were quite sensitive to blocking and could not be restimulated. However, when these cultures were simultaneously induced with virus and double-stranded RNA, the "early" response did not restrict the "late" response.

So, again we have an effect that does not allow us to attribute it to restriction of virus replication (though it may reflect a similar altered cellular state).

Can it then be attributed to impurities in the interferon preparations? This interpretation was favored previously, as it was claimed that the antiviral component could be separated from the blocking component by chromatography (Paucker and Boxaca, 1967; Borden, Prochownik, and Carter, 1975), or that interferons collected at different intervals after inductions exhibited different degrees of blocking activity per antiviral unit (Borden and Murphy, 1971; Chadha *et al.*, 1974). However, as described earlier (Section VI. A. 3.), all these claims seem to err in interpretation, the former claim of chromatographic separation having been withdrawn (Golgher and Paucker, 1973), and the claimed enrichments being insignificant. It is not possible to reliably distinguish between interferon responses that are blocked by about 95% and those blocked about 90% (Borden and Murphy, 1971), nor is it reasonable to rely on reports claiming assay differences of 5% (Chadha *et al.*, 1974). Also, where more highly purified interferon preparations have been evaluated for ability to block, they have been found to do so (Stewart II, Gosser, and Lockart, 1971b; Golgher and Paucker, 1973), and recently we have found blocking activity in mouse interferon preparations purified to about 10^9 units/mg of protein and have found mouse interferoid (Fig. 15) to possess this property (Stewart II *et al.*, 1978).

E. Cell-Multiplication-Inhibition

In this Section I shall discuss those phenomena which seem to relate to the ability of interferons to inhibit cell multiplication, those measured directly by cell counts, those inferred from reduced incorporation of precursors, and those repressing cell-stimulating effects or depression of syntheses of uninduced and induced cell products. The antitumor action of interferon which is likely, at least in part, to be attributable to this anti-proliferative effect will be discussed in Section XIII.

1. Evidence That Interferon Is the Active Component Inhibiting Cell-Multiplication

The controversy concerning the ability of interferons to inhibit or not to inhibit normal cell replicative events and cell-multiplication has been by far the most long-standing argument over the interferon system. Paucker, Cantell, and Henle (1962) first reported that interferon preparations restricted the growth of cells in culture, but Cocito, Schonne, and Desomer (1965), Baron, Merigan, and McKerlie (1966), and Levy and Merigan (1966) attributed this effect to "non-interferon" impurities in interferon preparations since they were unable to measure inhibitory activity on cell synthesis with partially-purified (i. e., somewhat less crude) interferon preparations. The intervening decade has seen constant confusion on the issue, with some investigators presenting evidence that interferon is itself the factor restricting replication (Fontaine–Brouty–Boye *et al.*, 1969; Gresser *et al.*, 1970b, c, 1973; Gresser, Bandu, and Brouty–Boye, 1974; Lindahl–Magnusson, Leary, and Gresser, 1971; Tovey, Brouty–Boye, and Gresser, 1975; Knight, 1973; Hilfenhaus and Karges, 1974; Adams, Strander, and Cantell, 1975; Tan, 1975; Gaffney, Picciano, and Grant, 1973; Hil-

fenhaus *et al.*, 1976; Lee *et al.*, 1972; O'Saughnessy, Lee, and Rozee, 1972; McNeill and Gresser, 1973; Kishida *et al.*, 1971, 1973; Ohwaki and Kawade, 1972), while others either failed to observe any inhibition or observed inconsistencies in the ratios of antiviral and cell-multiplication-inhibitory activity in given preparations (Baron and Isaacs, 1962; Marcus and Salb, 1966; Moehring and Stinebring, 1971; Kishida *et al.*, 1971; Borecky *et al.*, 1972; Fuchsberger, Hajnicka, and Borecky, 1975; Matsuzawa and Kawade, 1974).

The evidence for or against interferon exerting cell-multiplication-inhibitory activity has hinged on ability to accurately demonstrate cell-multiplication-inhibition and on the question of purity of interferon preparations.

The conditions for demonstration of interferon cell-restriction delineated by Lindahl–Magnusson, Leary, and Gresser (1971) have been helpful in reproducing this effect. Cultures must be initiated at sufficiently low density to allow several days of observations before control cell cultures have reached saturation (Fig. 19).

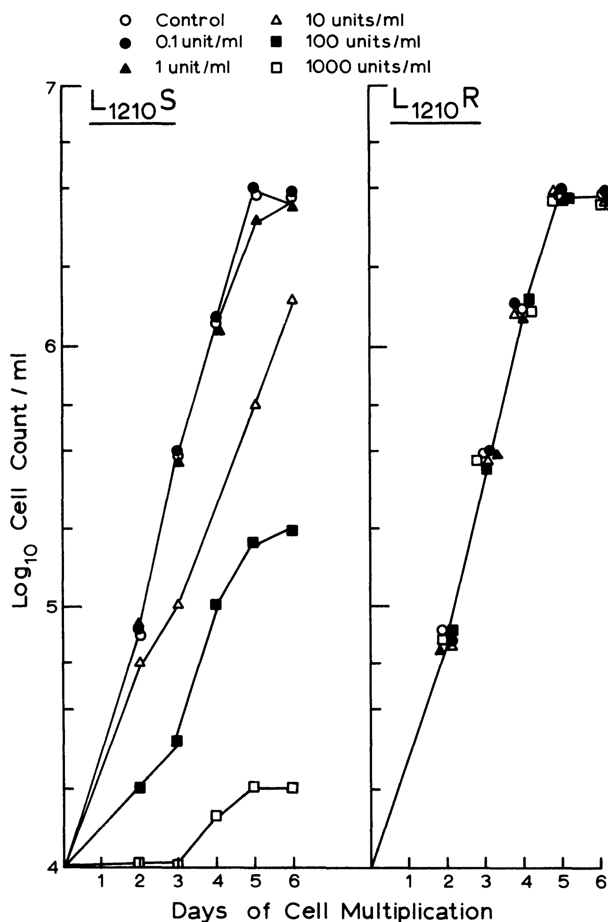

Fig. 19. Cell-multiplication-inhibitory activity of interferon. An assay of mouse interferon in mouse leukemia L1210S and L1210R cells. (Data courtesy of T. Chudzio and E. Chou)

The evidence for interferon involvement in this effect has evolved with each increase in interferon purification. Clearly this issue was decided mostly on emotional basis during the years of stagnation of interferon purification studies. For each claim there were counter-claims. The first demonstration of inhibition of cell growth (Paucker, Cantell, and Henle, 1962) being countered by claims that less crude preparation did not do so (Baron, Merigan, and McKerlie, 1966); some workers demonstrated inhibition with yet higher purity preparations (Paucker and Golgher, 1970; Gresser *et al.*, 1970b, c) and others claimed separation of the activity from the antivirally active component (Borecky *et al.*, 1972). More highly purified interferon seemed to have the activities at constant ratios (Gresser *et al.*, 1973; Knight, 1973), but some interferon preparations were reported to be devoid of cell-multiplication-inhibitory action (Dahl and Degre, 1975; Dahl, 1977a, b; Dahl and Degre, 1976a, b).

The evidence now available is clearly overwhelmingly in favor of the interpretation that interferon inhibits cell multiplication:

a) Crude and purified interferon preparations, regardless of their cell sources or inducing agents, exhibit constant ratios of antiviral and cell-multiplication-inhibitory activity, and the defined host-range of the activities are identical (Gresser *et al.*, 1970, 1971, 1973; Stewart II *et al.*, 1976; Babiuk and Rouse, 1977).

b) The component responsible for cell-multiplication-inhibition fulfills all the interferon criteria (VII. A. 7.).

c) Mouse L1210 cells resistant to the antiviral action of interferon (Gresser *et al.*, 1970a) and which are unable to bind interferon (Gresser, Bandu, and Brouty–Boye, 1974), are also resistant to the cell-multiplication-inhibitory action of interferon preparations (Gresser *et al.*, 1970b; Lindahl–Magnusson, Leary, and Gresser, 1971; Gresser, Bandu, and Brouty–Boye, 1974). And the reverse situation, human cells trisomic for chromosome-21 which are more sensitive to human interferon (Tan *et al.*, 1973) and which bind more interferon (Wiranowska–Stewart and Stewart II, 1977) are also more sensitive to the cell-multiplication-inhibitory effect of the interferon preparations (Tan, 1976, 1977; Cupples and Tan, 1977).

d) Both human (leukocyte or fibroblast) interferons and mouse interferon preparations electrophoresed in SDS-polyacrylamide gels are resolved into each of the profiles of activities presented in Fig. 13 and 14; the distribution of activities in these profiles are identical whether assayed for antiviral activity or cell-multiplication-inhibitory activity (Stewart II *et al.*, 1976; Knight, 1976b).

e) Mouse interferon purified to about 10^9 units of antiviral activity/mg of protein (Gresser, 1977a; Stewart II, Lin, and Wiranowska-Stewart, 1978), human fibroblast interferon purified to apparent homogeneity (at least 2×10^8 units/mg of protein) (Knight, 1976b), and apparently pure human leukocyte interferon purified to a single spot in two-dimensional gel electrophoresis (Stewart II, Lin, and Wiranowska-Stewart, 1978) are equally as able to induce cell-multiplication-inhibition as crude interferon preparations of various specific activities.

f) Mouse interferoid (Fig. 15) purified to about 10^9 units/mg of protein is equally able to perform this function.

2. *Cellular Systems Inhibited by Interferon*

The interferon-induced cell-restriction is a reversible event (Gresser *et al.*, 1970b, c; Paucker, Cantell, and Henle, 1962; Gaffney, Picciano, and Grant, 1973; O'Shaughnessy *et al.*, 1974). When cells are washed free of interferon they return to their normal growth rate. As viruses can vary several hundred-fold in sensitivities to interferon restriction, cells also can so vary. The multiplication of mouse L1210 cells can be inhibited almost completely by 100 units/ml, and can be inhibited by about 90% by 10 antiviral unit/ml (Stewart II *et al.*, 1976; Gresser *et al.*, 1970b).

Certain human lymphoblastoid cell lines are very sensitive [Daudi cells being markedly inhibited by about one unit or less (Stewart *et al.*, 1976)], whereas others are resistant to several thousand units (Adam, Strander, and Cantell, 1975; Hilfenhaus, Damm, and Johannsen, 1977; Hilfenhaus and Karges, 1974). Primary cell cultures have usually been reported to be markedly less sensitive than established cultures (Lindahl–Magnusson, Leary, and Gresser, 1971, 1972; Knight, 1973; Lee, O'Shaughnessy, and Rozee, 1972). However, Ohwaki and Kawade (1972) found no difference in susceptibility of transformed or untransformed 3T3 cells and mouse embryo fibroblasts to cell-multiplication-inhibition. Strander and Einhorn (1977) found that several lines of osteosarcoma cells were all inhibited by human leukocyte interferon, and were more sensitive than non-tumor-derived lines. These authors (Einhorn and Strander, 1977) have also interpreted that osteosarcoma cells are more sensitive to human fibroblast interferons than to human leukocyte interferons, suggesting tissue specificities of interferons. However, the unusual method employed to gauge cell-multiplication-inhibition (cells grown in interferons and cultures split once/week × 2 to 4 weeks), the differences in specific activities of the interferon preparations, and the narrow margins of the differences reported, make the validity of this interpretation suspect.

These effects are not mediated by an overt cytotoxicity, as ratios of viable cell counts/total cell counts remain similar during inhibition, but interferon-treated cells often plateau at a lower saturation density than do normal cells (Gresser *et al.*, 1970b, c; Knight, 1973). Collyn d'Hooghe *et al.* (1977) have reported that the intermitotic time, as followed by time-lapse photomicrography, was markedly and progressively increased, rather than arrested. Sokawa *et al.* (1977) recently reported that interferon suppressed the transition of 3T3 cells from a quiescent to a growing state.

Interferons also inhibit the growth of normal and tumor cells *in vivo*. The latter of these effects will be discussed later (Section XIII). Interferon or its inducers can inhibit the multiplication of allogeneic and syngeneic bone marrow cells in irradiated mice (Cerottini *et al.*, 1973), and an inhibitor of hemopoietic colony-forming cells was reported in serum of mice, rabbits and hamsters inoculated with poly rI·poly rC (McNeill and Fleming, 1971; Fleming, McNeill, and Killen, 1972; McNeill, Fleming, and McCance, 1972). Interferon inducers (poly rI·poly rC, Newcastle disease virus and statolon) were reported to inhibit the mitotic response of liver cells to partial hepatectomy (Jahiel *et al.*, 1971) and subsequently interferon itself was found to inhibit the regeneration of mouse liver after partial hepatectomy (Frayssinet *et al.*, 1973).

Early experiments with repeated inoculations of interferon preparations into newborn mice (32,000 to 64,000 units/day), showed no effect on growth or development (Gresser and Bourali, 1970b). However, when more potent preparations (800,000 units) were injected daily into newborn mice for about one week, mice died with extensive liver damage (Gresser *et al.*, 1975). If interferon treatment were stopped between days 6 and 9, liver damage appeared reversible and mice appeared to recover; however, in the next months several of these mice died from progressive glomerulonephritis (Gresser *et al.*, 1976a). Interestingly, inoculation of newborn mice with lymphocytic choriomeningitis virus results in decreased weight gain, liver necrosis and death; when antiserum to mouse interferon was inoculated into such mice, virus levels increased more than 100-fold but the disease manifestations were inhibited (Riviere *et al.*, 1977), suggesting that chronic virus-induced interferon leads to weight loss, liver disease and mortality. Also, Bekesi *et al.* (1976) have reported that repeated injections of high doses of interferon into AKR mice caused early death with marked signs of "wasting" (organ atrophy, small spleens and athymic), whereas lower doses prolonged survival.

3. *Metabolic Alterations in Interferon-Treated Cells*

The actual mechanism of the suppression of cell-multiplication is far from being approachable. As the antiviral mechanism seems to be related to a network of mechanisms to restrict translation, it could be envisioned that similar restrains might be imposed on at least certain classes of cellular messenger RNAs. In fact, in view of the wide range of sensitivities to insensitivities of viruses and their messenger RNAs to interferon-induced translation restrictions, it would be somewhat surprising to find that all classes of cellular messenger RNAs would be evenly sensitive or resistant to this restriction. Several early studies reported that crude interferon preparation exerted little, if any, effect on the metabolism of noninfected cells (Joklik and Merigan, 1966; Levy and Merigan, 1966; Merigan, Winget, and Dixon, 1965; Wagner, 1963b; Cocito, DeMaeyer, and Desomer, 1962b; Sonnabend, 1964; Friedman and Sonnabend, 1970), but again, these slight effects were readily related to impurities (Baron, Merigan, and McKerlie, 1966; Cocito, Schonne, and Desomer, 1965; Levy, Snellbaker, and Baron, 1963). Johnson, Lerner, and Lancz (1968) reported that mouse interferon preparations at about 800 units/ml inhibited amino acid incorporation in mouse L cells by 50%, but did not so effect heterologous cells. Falcoff and her associates (1975) reported that treatment of L cells with 1000 units/ml of mouse interferon consistently inhibited endogenous protein synthesis by 20 to 40%; this effect was obtained with several batches of interferon, some purified to 10^7 units/mg of protein. Inhibition paralleled kinetics of viral resistance.

Brouty–Boye *et al.* (1973a, b) reported that interferon in L1210 cells sensitive to interferon exerted a signicant decrease in synthesis of total cellular RNA and protein, but had no such effect in L1210 cells resistant to interferon action. O'Shaughnessy, Lee, and Rozee (1972) reported that interferon treatment of synchronized L cells delayed their thymidine uptake, and Fuse and Kuwata

(1976) found that DNA and protein synthesis are reduced in human RSa cells treated with interferon. Synchronized RSa cell cultures seemed to respond to interferon in the late G 1 phase by stopping DNA synthesis but did not do so when interferon was added in G 1/S boundary phase. However, Kariniemi (1977) reported that human leukocyte interferon did not depress DNA synthesis in human psoriatic epidermal cell cultures.

Tovey, Brouty–Boye, and Gresser (1975) and Brouty–Boye and Tovey (1977) have reported studies using the chemostat culture system developed in their laboratory (Tovey and Brouty–Boye, 1976). Mouse interferon preparations inhibited the multiplication of L1210 cells cultivated in steady-state conditions, and a rapid inhibition of incorporation of thymidine was detectable within two hours after interferon was added. Interferon did not inhibit the incorporation of amino acids or uridine. These investigators also reported that interferon reduced the uptake of thymidine but not of deoxyadenosine or deoxy-D-glucose (Brouty–Boye and Tovey, 1977), suggesting that interferon induced a specific alteration of cellular membranes. It seems worth noting that the interferon used in these studies was added continuously to maintain a rather high final concentration (24,000 mouse interferon reference units/ml).

4. Restraints Imposed by Interferon

Interferon treated cells are slowed from rapid proliferation and are also resistant to being stimulated to proliferate. Lindahl–Magnusson, Leary, and Gresser (1972) showed that interferon preparations inhibited DNA-synthesis induced in mouse splenic lymphocytes by phytohemagglutinin or allogeneic lymphocytes. Blomgren, Strander, and Cantell (1974) reported that human leukocyte interferon (at 10 units/ml) suppressed the proliferative response of human lymphocytes to the mitogens phytohemagglutinin, concanavalin A and PPD and to the mixed lymphocyte reactions. Interferon was also able to suppress cells *in vivo.* Pacheco *et al.* (1976) reported that spleen cells from mice inoculated with 10^5 units of interferon 18 hours before excision were resistant to stimulation of thymidine incorporation when exposed to lectin, phytohemagglutinin or concanavalin A, or to lipopolysaccharide. Cupples and Tan (1977) found that cells from Down's syndrome patients were inhibited in DNA synthesis to a greater degree than normal cells by interferon treatment prior to antigenic or mitogenic stimulation.

It has also been reported that induction of specific cellular products can be inhibited in interferon-treated cells. Beck *et al.* (1974) reported that mouse interferon, which had antiviral activity in rat cells, and rat interferon could significantly inhibit the ability of rat HTC cells to produce tyrosine amino-transferase (TAT) when exposed to dexamethasone. Vassef *et al.* (1974) also reported partial depression of TAT induction in interferon-treated rat HTC cells, but only with 200 to 700 times higher concentrations than those required to inhibit reovirus or vesicular stomatitis virus replication; however, some viruses can also be that much less sensitive to interferon than these viruses, so the argument for these results revealing a selectivity of interferon action for viral rather than cellular functions is not valid. Illinger *et al.* (1976) have reported

that pretreatment of rat glial cells with rat interferon prior to induction with hydrocortisone hemisuccinate inhibits production of glycerol 3-phosphate dehydrogenase. Chicken interferon was reported to suppress the induction of glutamine synthetase in chick embryonic neural retina by hydrocortisone (Matsuno, Shirasawa, and Kohno, 1976); levels of lactate dehydrogenase, and acetylcholine esterase, were unaffected.

Recently, Smith and his associates (1976) reported, also, that interferon treatment prevented the proliferative response induced in normal erythroid precursor cells by erythropoietin.

These data all indicate a general restraint induced in cells by interferon which manifests itself as an anti-proliferative mechanism and limits several cellular syntheses. The recent reports that interferons can stimulate release of prostaglandin E (Yaron *et al.*, 1977; Karmazyn *et al.*, 1977) which is able to regulate cell replication, indicate the further complexity of evaluating the mechanism of interferon actions. Cortisol and indomethacin, which inhibit prostaglandin E production, reportedly prevented the inhibitory effect of interferon on synovial fibroblast growth (Yaron *et al.*, 1977).

F. Toxicity Enhancement

Several studies have found direct correlations between the ability of polyribonucleotides to induce interferons and their toxicity (Niblack and McCreary, 1971; Declercq, Stewart II, and Desomer, 1972; Declercq and Stewart II, 1972; Stewart II and Declercq, 1974); in fact, there has been no convincing data reporting separability of these properties of double-stranded RNAs, though several such claims have been made (O'Malley *et al.*, 1975a, b; Tso *et al.*, 1976). Thus, the findings that interferon-treated (primed) cells could be induced to produce interferon by concentrations of poly rI·poly rC that were insufficient to stimulate interferon synthesis in unprimed cells (Rosztoczy, 1971; Stewart II *et al.*, 1972) suggested two possibilities: either interferon-treated cells can be induced by less toxic levels of double-stranded RNA inducers, or the inducer double-stranded RNA is more toxic for interferon-treated cells than it is for normal cells. In fact, Stewart II *et al.* (1972) found that the latter was case: interferon-treated cells were markedly more sensitive than normal cells to toxicity of the double-stranded RNA, poly rI·poly rC. Normal L cells, human skin fibroblasts and mouse embryo cell cultures remained healthy after exposure to poly rI·poly rC, but interferon-treated cells lysed between 3 and 6 hours after exposure to this material (Fig. 20).

This enhanced sensitivity to toxicity was also found with several natural and synthetic double-stranded RNAs (Stewart II, Declercq, and Desomer, 1973), and correlated with the relative abilities of these polynucleotides to induce interferon and with required polynucleotide size for induction of both effects (Stewart II and Declercq, 1974). Toxicity enhancement was not seen with single-stranded RNAs, DNA, tilorone, endotoxin, COAM, cycloheximide, actinomycin D, DEAE-dextran, rattlesnake venom, diphtheria toxin or cholera toxin, and it was thus suggested that the enhancement of toxicity of interferon treated cells was specific for double-stranded RNAs.

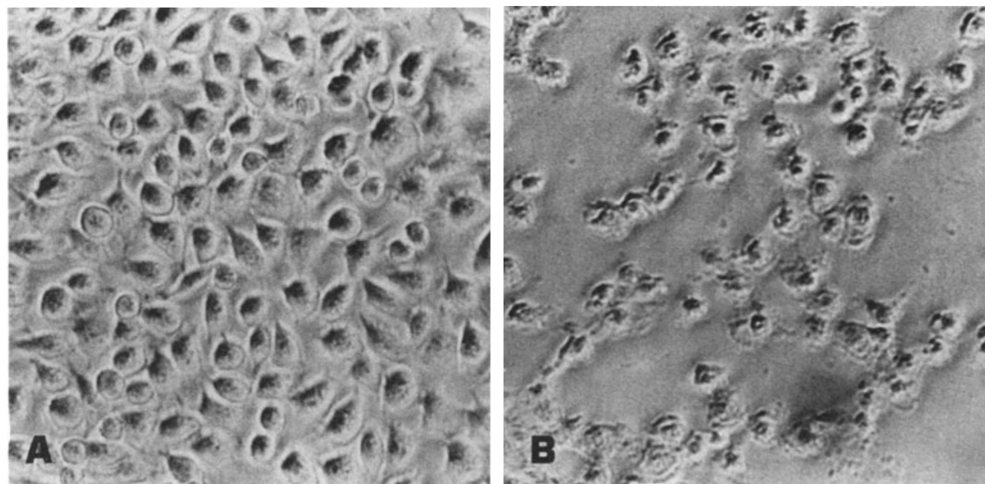

Fig. 20. Double-stranded RNA toxicity-enhancing activity of interferon. Cultures of L929 cells were incubated in growth medium *(A)* or growth medium containing 10 units of interferon/ml *(B)*. Both cultures were then exposed to 25 μg of poly rI·poly rC and photographed about 3 hours later. (Experiment courtesy of Sohan Gupta)

This interpretation of double-stranded RNA specificity of toxicity-enhancement was further supported by the observations that vaccinia virus infection caused an early disintegration of interferon-treated cells (Joklik and Merigan, 1966; Horak, Jungwirth, and Bodo, 1971; Jungwirth *et al.*, 1972a, b; Bodo *et al.*, 1972; Stewart II *et al.*, 1972). Joklik and Merigan (1966) first observed that interferon-treated L cells began to be converted to "ghosts" about 3 hours after infection with vaccinia virus and by 6 hours after infection practically all the cells had been lysed, while infected cells not previously exposed to interferon were all intact. Horak, Jungwirth, and Bodo (1971) found that a viral gene product must be synthesized for the lysis of vaccinia virus-infected interferon-treated cells, which in their L cells occurred between 4 and 8 hours after infection. Stewart II, Declercq, and Desomer (1973) also found that vaccinia virus was not lytic for interferon-treated cells if cells were treated with cycloheximide or actinomycin D when infected (however, these inhibitors did not prevent poly rI·poly rC from lysing interferon-treated cells). These data suggest that a viral gene product, not the input virus itself, is responsible for the lysis of interferon-treated cells. As doublestranded RNA was reported in vaccinia virus-infected cells (Colby and Duesberg, 1969), it is tempting to speculate that this type of toxicity enhancement is specific for double-stranded RNA.

This effect emphasizes the myriad mechanisms involved in the antiviral network of interferon-treated cells. As illustrated in Fig. 17, viruses which are able to avoid the early inhibitory actions of interferon-treated cells and which form sufficient double-stranded replicative structures can activate the kinase $\rightarrow$ phosphorylated initiation factor block and the LMWIT $\rightarrow$ endonuclease effect and then may push the cell to suicide, taking the premature "embryonic" virus with it.

Enhanced lysis of interferon-treated cells has also been reported with L cells infected with vesicular stomatitis virus (Katz, Lee, and Rozee, 1974) and with human conjunctival cells infected with influenza virus (Green and Mowshowitz, 1978); in the latter case it was also reported that a viral synthetic process in cells was required to produce lysis. Other have observed enhanced cytopathic effects in cells repeatedly exposed to poly rI·poly rC (Billiau, Van den Berghe, and Desomer, 1972; Martelly and Jullien, 1974), possibly owing to induced interferon priming cells for enhanced toxicity of the double-stranded RNA.

The amount of interferon required to induce this effect is similar to that required for measuring resistance to viruses, and the amount of destruction of the interferon-treated cells is dependent on both the amount of double-stranded RNA added to the cells and the concentration of interferon applied (Stewart II, Declercq, and Desomer, 1972a; Lackovic and Borecky, 1976); this relationship was used to develop a sensitive non-antiviral interferon assay for this effect. Toxicity enhancement with double-stranded RNA can be demonstrated in several lines of L cells; it can be demonstrated in mouse embryo cell cultures (Stewart II *et al.*, 1972; J. Desmyter, personal communication, 1972). It is seen in mouse leukemia L1210S cells which are interferon sensitive, but not in the L1210R cells resistant to (and not binding) interferon (Werrene and Rousseau, 1976a; Gresser and Stewart II, unpublished data). I have observed a distinct toxicity-enhancement in 3 of 10 different human fibroblast cultures, a slight effect in 4 of 10 cultures and no detectable effect in 3 of these cultures. Nor have I seen this effect in rabbit kidney cell cultures (unpublished data). In view of the variable abilities of human fibroblasts to express this action, it would perhaps not be surprising that Declercq, Edy, and Cassiman (1975) were unable to correlate toxicity-enhancement to numbers of chromosome 21 in human fibroblast. In view of my experience with this effect, what was surprising with the study of Declercq *et al.*, however, was their phenomenal luck in finding 5 out of 5 human fibroblasts that not only expressed the double-stranded RNA toxicity-enhancement but all expressed it very distinctly and to the same extent.

Several hours of treatment with interferon are required for cells to develop toxicity-enhancement, during which time protein synthesis is required (Stewart II, unpublished data). This suggests that while perhaps priming can develop through a direct membrane alteration effect, toxicity-enhancement like antiviral activity, requires cellular protein synthesis to develop, in contrast to the direct membrane alteration mechanism proposed by Declercq and Desomer (1975).

Double-stranded RNA toxicity-enhancement appears to lead to cell lysis within about three hours after addition of the double-stranded RNA; no damage is visible before that time and lysis is complete in those cells effected by no later than about 6 hours. Just prior to lysis it was possible, by use of cytochemical staining techniques, to detect intracellular injury to mitochondria, as evidenced by marked loss of succinic dehydrogenase, lysosomal injury, as suggested by diffuse acid phosphatase activity and nuclear swelling concurrent with lowered protein synthesis (Stewart II *et al.*, 1975b). By using electron

microscopy it was similarly revealed that mitochondrial and nuclear damage precede cell disruption (Heremans *et al.*, 1976). As the mitochondrial distortions seen in these studies are common to numerous cellular toxicities, these studies only tell us the obvious, that the cells have been damaged somehow. Preliminary attempts to isolate various cell membranes from normal and interferon-treated cells and to mix these directly with double-stranded RNA and detect membrane disruptions by altered sedimentation patterns proved technically difficult (Stewart II, unpublished data) but should be interesting to attempt by improved membrane isolation methods. It would be tempting to relate the toxicity-enhancement directly to the shut-off of protein synthesis, as seen in cell-free protein synthetic systems derived from interferon-treated cells whose translating functions are arrested by addition of double-stranded RNA. However, in interferon-treated cells it is possible to completely arrest cellular RNA and/or protein synthetic processes by addition of various metabolic inhibitors, and in such cultures no lytic activity is detectable, even after 24 hours (Stewart II, unpublished data).

Interferon-treated L cells were also reported to be more sensitive to arginine starvation (Lee and Rozee, 1975) and to the toxicity of DEAE-dextran, protamine sulfate and methylated albumin (Katz, Lee, and Rozee, 1974); however, others did not find enhancement of sensitivity to DEAE-dextran (Stewart II, Declercq, and Desomer, 1973; M. Tovey, personal communication, 1972). However, Stewart II *et al.* (1975b) did observe an increased toxicity of concanavalin A in interferon-treated cells, but this toxicity was distinct from the lysis seen with vaccinia virus and double-stranded RNA; it was rather an increased vacuolation of the cytoplasm. It seems likely this increased toxicity of the lectin for interferon-treated cells relates to the increased binding reported with concanavalin A in interferon-treated cells (Huet *et al.*, 1974).

It could be expected that toxicity enhancement plays a role *in vivo* as well as in cell cultures. The presence of interferon in virus infected patients might preclude the utility of double-stranded RNAs in treatment of such diseases. Stewart II *et al.* (1972) cautioned that safety assessments based on toxicity levels of poly rI·poly rC in normal individuals might not accurately reflect the therapeutic index of this inducer in virus-infected patients who might have infection-induced interferon levels. In fact, Declercq, Stewart II, and Desomer (1973a) reported that virus-infected mice were markedly more susceptible to the toxicity of double-stranded RNAs than were normal uninfected mice. Cantell, Pyhala, and Strander (1974) also observed that the combined administration of interferon and virus was dangerous to rabbits: none of more than 150 rabbits injected with a few million units of human leukocyte interferon died; likewise, none of several rabbits injected with Newcastle disease virus died; however, a significant number of the rabbits injected with both virus and interferon died, and most of the survivors were toxified to some extent. In a perhaps related effect, Huang and Landay (1969) reported that interferon inducers poly rI·poly rC and Newcastle disease virus enhanced the lethal effect of endotoxins in mice.

On the assumption that the antitumor activity of double-stranded RNA is related at least in part to its direct toxicity for tumor cells, and on the basis

that a significant amount of intramuscularly inoculated interferon binds locally (Stewart II, unpublished data; Harmon and Janis, 1975), Stewart II *et al.* (1975c) attempted to take advantage of the toxicity-enhancing activity of interferon to attack Moloney sarcoma virus-induced tumors in mice. Mice developing autochthonous tumors were given injections of interferon or poly rI·poly rC, or interferon followed by poly rI·poly rC, all injections directly into the palpatable tumors. Whereas treatments with interferon alone were ineffective in prolonging life, and poly rI·poly rC alone was only marginally effective (Declercq and Stewart II, 1974b), sequential local treatment with interferon and poly rI·poly rC was, in this preliminary trial, markedly more effective in both prolonging life and inducing tumor regressions. Clearly this apparent *in vivo* application of double-stranded RNA toxicity-enhancement merits further investigations.

The toxicity-enhancing factor in interferon preparations has been shown to possess all the attributes of interferons (Section VII. A. 7.); it purified with interferon through more than 1000-fold (Stewart II *et al.*, 1972) and 1 million-fold (Stewart II *et al.*, 1973) increases in the specific activity. It was found to parallel the activity profiles of mouse and human interferons in SDS gels as seen in Fig. 13 and 14 (Stewart II, 1975b), and recently we have found identical ratios of antiviral/toxicity-enhancing activity in mouse interferon purified to 10^9 units/mg of protein (Stewart II, Lin, and Wiranowska-Stewart, 1978) and in mouse interferoid (paralleling the profile in Fig. 15) (Stewart II *et al.*, 1978).

Thus, toxicity enhancement, priming, blocking and cell-multiplication-inhibition are all cellular alterations induced by interferons. The subsequent activities described in this section are not so well characterized in terms of the proof that interferon is the factor inducing them, but present evidence at least strongly implicates interferons as the active component. Studies with pure interferons should soon clarify this involvement.

G. Enhanced Synthetic Activities

In addition to enhancing the ability of cells to produce interferon (priming), interferon can apparently also assist cells to make other products.

Yaron *et al.* (1976) reported that either poly rI·poly rC or human interferon could stimulate production of hyaluronic acid by rheumatoid and non-rheumatoid human synovial fibroblasts, and suggested a relationship between interferon production and pathology of accumulated joint fluid during virus infections. Yaron *et al.* (1977) also found that either poly rI·poly rC or human interferon (100 units/ml) could stimulate prostaglandin E production by these synovial cells or by foreskin fibroblasts. As prostaglandin E is a mediator of inflammatory processes, it is tempting to speculate that the "interferon fever" reported in skin wheals following inoculation with human fibroblast interferon (Desomer *et al.*, 1977) and the febrile response often observed with systemic administration of human leukocyte interferon (Section XIV) may be related to this activity.

Interferon can also enhance the amount of certain induced cell products.

Nebert and Friedman (1973) reported that mouse interferon pretreatment (100 units/ml) enhanced the amount of aryl hydrocarbon hydroxylase induced in cultures with benzanthracene. And Ida *et al.* (1977) found that either virus, poly rI·poly rC or human leukocyte interferon enhanced the ability of human leukocytes to release histamine when exposed to ragweed antigen E or anti-IgE. These authors suggest that interferon may, therefore, play a role in precipitating or potentiating attacks of bronchial asthma during viral infections. In this regard it is interesting that Skurkovich and Eremkina (1975) have suggested the role of interferon in allergy and have proposed use of anti-interferon immunoglobulins in the treatment of certain autoimmune diseases.

H. Surface Alterations Induced by Interferons

Interferon treatment has been repeatedly reported to increase the resistance of cultured cells to diphtheria toxin (Yabrov, 1966, 1967; Moehring, Moehring, and Stinebring, 1971; Boquet, 1975; Marchenko *et al.*, 1976b). However, this effect could not be detected in some laboratories (Stewart II, Declercq, and Desomer, 1973), possibly because it is very slight. As interferon and cholera toxin can apparently interfere with receptor-interactions of one another (Kohn *et al.*, 1976) it seems likely that interferon interferes with binding of the diphtheria toxin to cells, though Yabrov (1966, 1967, 1975, 1976) has interpreted that interferon acts by stabilizing cells against perturbations of protein syntheses.

Knight and Korant (1977) have provided direct evidence for a surface alteration of cells by interferon, though not a direct one resulting from binding (or priming), as it required several hours and protein synthesis for such an effect to develop. The interferon-treated cells then migrated in an electric field with a greater net negative charge than normal cells.

Huet *et al.* (1974) also reported that mouse interferon treatment induced an increase in binding of ^{63}Ni-labeled concanavalin A to interferon-sensitive L1210 cells but did not do so in interferon-resistant L1210 cells. The increased binding was slight at 24 hours but was greatest after 48 hours.

A number of workers have found that interferon treatment can increase the expression of surface antigens on cells. Lindahl, Leary, and Gresser (1973) demonstrated that mouse interferon preparations enhanced the expression of surface antigens of leukemia L1210 cells. Interferon treatment increased the cell-antibody-absorbing capacity of interferon-sensitive cells but not of interferon-resistant cells. About 100 units/ml for 24 hours enhanced the expression of histocompatibility antigens of thymocytes from mice of various ages, splenic lymphocytes from young mice and did not affect the expression of theta antigens (Lindahl, Leary, and Gresser, 1974). Killander *et al.* (1976) demonstrated further that interferon treatment of L1210 cells enhanced the expression of histocompatibility antigens similarly in all stages of the cell cycle, suggesting that the enhancement was not due to a concentration of cells in a particular phase of the cycle. Skurkovich *et al.* (1976b) demonstrated that interferon-treated L1210 cells exerted an enhanced cell-mediated immune response in mice. Lindahl *et al.* (1976a, b) also found that injection of interferon into young or ma-

ture mice enhanced the histocompatibility displays on the surfaces of their thymocytes and splenic lymphocytes.

The availability of restricted antisera against specific determinants of the H-2 complex allowed Vignaux and Gresser (1977) to determine that interferon-treated mouse splenic lymphocytes displayed increased amounts of H-2 K and H-2 D antigens (4- to 8-fold greater than normal), but did not express enhanced Ia antigens. In these studies cells were treated with high concentration of interferon of about 3000 units of interferon/ml (about 12,000 reference units), and in one experiment Ia antigen was increased slightly by treatment with 10^5 units/ml. This study again emphasized the dose-effect of interferon actions not being an "all-or-none phenomenon". These authors also found an enhanced $H\text{-}2^k$, $H\text{-}2K^k$ and $H\text{-}2D^k$ on splenic lymphocytes and thymocytes of mice injected with interferon (dosage: "mice injected i. p. four times with an interferon preparation titering 1×10^{-6}"; volume administered not specified). Lonai and Steinman (1977) reported that mouse interferon (4000 units/ml)[8] increased the H-2 antigens exposed on mouse T cells but did not enhance Ia antigen expression, and suggested that interferon influences suppressor or killer cells. In this regard it is interesting that interferon is released by immunocytes in response to antigens or target cells (Svet–Moldavsky *et al.*, 1974), again relating the induction-action ends of the interferon system.

I. Enhanced Immunolysis

Svet–Moldavsky and Chernyakovskaya (1967) demonstrated that in the presence of crude mouse interferon preparations normal mouse lymphocytes acquired a killer activity on target mouse L cell, though it did not enhance the sticking of the lymphocytes to the L cells. Syngeneic lymphoid cells in interferon preparations also acquired the ability to exert an antitumor effect against sarcomas in mice inoculated with benzanthracene (Chernyakhovskaya and Slavina, 1972). Skurkovich and Aleksandrovskaya (1973) also reported stimulation of cytotoxic activity of lymphocytes of mice towards target cells following administration of crude interferon preparations *in vivo*. Svet–Moldavsky and his colleagues (1974) also demonstrated that lymphoid cells removed from mice at the peak of interferon production, which had been induced by injection of allogeneic cells, exhibited a strong cytotoxic effect on non-specific target L cells. This interesting study emphasized that interferon may play a role as a mediator molecule for initiations of various immune reactions.

Lindahl, Leary, and Gresser (1972) extended these findings by demonstrating that in the interferon-mediated sensitization of sensitized lymphocytes for leukemia L1210 cells, the enhanced specific cytotoxicity resulted from an effect of interferon on lymphocytes rather than from an effect on the target cells. She and her coworkers also provided evidence that interferon was the active agent in the preparations. In contrast to previous reports, however, these workers

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[8] This report does not indicate interferon *reference* units, and as this preparation was provided by Gresser, who reports his units to equal 4 mouse reference units (Vignaux and Gresser, 1977), this dose may mean 16,000 reference units/ml. This again emphasizes the need for stating titers in reference units.

did not detect cytotoxicity with interferon-treated or normal lymphocytes of mice not specifically sensitized to target cells. Slavina and Svet–Moldavsky (1973) reported rather that interferon induced the sensitivity of L cells to the cytotoxic effects of lymphocytes. Thus, in the nonspecific system the alteration seems to be in the target cell, while in the specific system the alteration appears to be in the effector cells.

Recently, Heron, Berg, and Cantell (1976) found that the killer activity of human lymphocytes was enhanced by treatment with human leukocyte interferon. In related studies, Skurkovich *et al.* (1976a) reported that treatment of human lymphocytes with interferon enhanced their cytotoxicity toward cells of lymphoblastoid lines. These authors (Skurkovich *et al.*, 1976b; Skurkovich, Klinova and Eremkina, 1976) also reported that L1210 cells incubated with interferon preparations were more sensitive to the action of cytotoxic antibodies.

J. Enhancement of Phagocytosis

Huang and his associates (1971) demonstrated that mouse peritoneal macrophages exposed to interferon preparations (50 units/ml) exhibited significantly (200 to 300%) enhanced phagocytic activity for colloidal carbon particles. These investigators demonstrated this effect with a variety of different mouse interferon preparations and showed that the enhancing effect of such preparations was neutralized by antiserum prepared against partially purified mouse interferon (Donahoe and Huang, 1973). It was also demonstrated that interferon preparations or an inducer, Newcastle disease virus, injected into mice enhanced phagocytic activity against the carbon particles injected intraperitoneally (Donahoe and Huang, 1976). Enhancement began after several hours and lasted for at least 60 hours.

Imanishi *et al.* (1975) also reported that human leukocyte interferon preparations enhanced the phagocytic activity of human peripheral monocytes for latex particles. At 4000 interferon units/ml the interferon preparations increased both the number of phagocytic cells and the degree of phagocytosis by individual cells. However, in this study no preincubation of cells with interferon was required for phagocytosis-enhancement to develop: latex and interferon added simultaneously gave the same enhancement.

It was suggested that interferon and its inducers could exert an antibacterial and anti-protozoal effect by stimulating phagocytosis (Donahoe and Huang, 1973). In fact, some studies have demonstrated that interferon inducers enhanced reticuloendothelial clearance of bacteria (Remington and Merigan, 1970) and protozoa (Herman and Baron, 1970). On the other hand, Kazar, Krautwurst, and Gordon (1971) did not find an increased uptake of *Chlamydia trachomatis* by interferon-treated mouse peritoneal cells. However, Gresser and Bourali (1970a) observed that macrophages of mice treated with interferon and then injected with RC19 leukemia cells exhibited greater phagocytosis of tumor cells than controls.

It could also be imagined that enhanced pinocytosis of virions could be induced in cells by interferons (perhaps not only in specialized motile phagocytic elements) and that this enhancing effect on uptake could obscure inhibitory ef-

fects on binding and penetration. Further studies on both these phenomena seem appropriate.

K. Macrophage "Activation"

Several interferon inducers administered to mice enhanced the spreading of peritoneal macrophages when these were then plated on glass (Rabinovitch *et al.*, 1977), and it was suggested that this "activation" of macrophages resulted from interferon action. Khesin *et al.* (1973) reported that by studies of comparative morphology, cytochemistry and biochemistry of peritoneal exudate macrophage of mice it was determined that macrophages from mice treated with interferon preparations were "activated". Schultz, Papamatheakis, and Chirigos (1977) reported that a number of interferon inducers, pyran copolymer, poly rI·poly rC, and dextran sulfate when injected into mice rendered their peritoneal macrophages cytotoxic for leukemia cells, and demonstrated that partially purified mouse interferon (2×10^7 units/mg of protein) at 1000 units/ml rendered macrophages cytotoxic for tumor cells. Interferon-treated macrophages showed enhanced spreading on plastic and had prominent cytoplasmic granulations; cytotoxicity appeared to result from direct activity as it was not transferred as a soluble factor, and did not result from enhanced phagocytosis. The effect was induced by as little as 100 units/ml, was maximal at 1000 units/ml. Human interferon was about 1 percent as effective, requiring at least 10^4 units/ml to "activate" the mouse macrophages.

These findings further implicate the interferon system as the determinants in yet another mechanism of "immune"-mediated antitumor activity.

L. Delayed-Type Hypersensitivity-Inhibition

The effect of interferon and interferon inducers in mice on the delayed-type hypersensitivity (DTH) response to picryl chloride or sheep red blood cells (SRBCs) was first investigated by DeMaeyer, DeMaeyer–Guignard, and Vandeputte (1975). Mice inoculated with Newcastle disease virus, Sendai virus, or statolon eight hours prior to challenge by picryl chloride painting of shaved ears showed decreased ear swelling at 24 hours. As a control for this study they used both If-1^l and If-1^h mice; the latter group, being better interferon producers with Newcastle disease virus (Section V. D. 1.), also showed better DTH inhibition with this inducer. Also, four doses of about 10^6 units of mouse interferon administered on the day before or the day of challenge also inhibited the reaction. Similarly, interferon inhibited foot-pad swelling in sensitized mice inoculated with SRBCs. The interferon used in these studies was purified to about 10^8 units/mg of protein.

These investigators (DeMaeyer–Guignard, Cachard, and DeMaeyer, 1975) also showed that the afferent arc of the DTH response (the primary sensitization) as well as the efferent arc, was inhibited by interferon. Mice treated with interferon 1 or 2 days before sensitization had decreased response to SRBCs. Similar results were obtained with both afferent and efferent arcs of the DTH response using a viral (Newcastle disease virus) immunogen (DeMaeyer, 1976).

Thus interferon can inhibit sensitization and expression of the DTH reactions to a hapten, a T-cell-dependent antigen or a virus.

These studies point up a paradox of interferon activities: interferon, an antiviral agent important in recovery from virus diseases and tumors, can inhibit cell-mediated immune reactions which are important in elimination of certain virus diseases and tumor cell destruction. It is possible that this effect of interferon explains the enhanced tumor growth reported in certain systems with interferon inducers and interferon (Gazdar, 1972; Gazdar *et al.*, 1972a, b, 1973).

It is also possible (as will be described below) that at different doses, and under different schedules, interferon might decrease immune response in one case, and increase it in another. Such effects on the cell-mediated immunity, DTH reaction, likely explain the following observation on graft survivals.

M. Graft-vs-Host Reactions: Effects of Interferon on Transplantation

The effects of interferon on graft survivals are quite conflicting. It was first reported by Chernyakhovskaya and Slavina (1972) that injection of an interferon preparation under allogeneic skin graft in mice accelerated the processes of necrosis. Similarly, Skurkovich *et al.* (1973a, b) reported that daily injections of mice with crude mouse interferon preparations intraperitoneally after they were grafted with allogeneic skin accelerated the rejection process, in association with an enhanced cytotoxic activity of their lymphocytes for target cells. Repeated transplantations to mice which were treated with interferon during the first grafting accelerated rejection of these second-set grafts. These workers concluded that interferon given after transplantation enhanced cell-mediated rejections.

Cerottini *et al.* (1973) also found that interferon-inducers or interferon preparations exerted inhibitory effects on the multiplication of transplanted allogeneic spleen cells and syngeneic bone marrow cells in irradiated mice. However, this effect was more likely a result of the cell-multiplication-inhibitory action than an immunomodulation. OReilly *et al.* (1976) reported that interferon did not suppress marrow engraftment in two bone marrow transplant patients given daily doses of 10^6 units of crude human leukocyte interferon.

Hirsch *et al.* (1973) also reported that interferon could inhibit the proliferative response of the graft-vs-host reaction in mice and could inhibit the activation of leukemia virus concomitant with this reaction. Mice injected with about 10^5 units daily of mouse interferon starting a day before the injection of spleen cells showed suppression of the graft-vs-host-response.

Mobraaten, DeMaeyer, and DeMaeyer–Guignard (1973) found also that injection of mice with Newcastle disease virus or statolon sufficient to give serum interferon levels of about 10^5 units/ml on the day of or after grafting significantly prolonged allograft survival. If given prior to grafting, the inducer was ineffective. Tilorone, orally, also prolonged allograft survivals. In other studies tilorone has been reported to prolong both skin and heart allografts in rats (Wildstein, Stevens, and Hashim, 1976) and kidney transplants in dogs (Wildstein *et al.*, 1976)[9].

[9] The presence of interferon in these experiments was not determined as the authors con-

DeMaeyer, Mobraaten, and DeMaeyer–Guignard (1973) also found that injections of mouse interferon preparations, intraperitoneally, delayed allograft rejections across an H-2 barrier in mice. These workers also showed that allografts on the If-1^h mice injected with Newcastle disease virus survived longer than on the If-1^1 mice so induced (DeMaeyer, Mobraaten, and DeMaeyer–Guignard, 1973).

Hirsch *et al.* (1974) reported that interferon treatment of mice twice daily from 1 day before grafting until graft rejection increased transplant survival by 2 days with A/J skin on Balb/c mice, by 4 days with DBZ/2 skin on Balb/c, indicating that minor loci as well as the major H 2^d histocompatibility locus were involved in rejection. Hirsch, Black, and Proffitt (1977) also showed that about 10^5 units of crude mouse interferon daily reduced virus activation and the incidence of lymphomas following graft-vs-host reaction in mice and reduced the severity of the rejection reaction itself. These data suggest that interferon may prove useful in immune disorder, in viral infection, in neoplasia, and as an immunosuppressive with antiviral activity in organ transplant recipients, who are at high risk for both virus infection and neoplasia[10].

Imanishi *et al.* (1977) also reported that high doses of rabbit interferon, in eyedrops (2×10^6 units/ml; one drop/eye × twice daily) suppressed rejection of rabbit corneal xenograft. Interestingly, a low dose (2×10^3 units/ml) enhanced rejection. Thus this high dose-low dose response relationship proferred by the Japanese workers may reconcile the discrepancies between increased rejections seen by Eastern block workers and increased survivals reported by their Western counterparts. It seems that several more years of careful attention to timings, dosages and purities of interferon administrations will be necessary for sufficient data to accumulate to allow accurate analysis of these variables in regard to application for transplantation survival.

N. Effects of Interferon on Antibody Production

1. In vivo *Antibody Production*

Early experiments on the effects of interferon on antibody production in mice reported no effects (Anderson, 1965; Desomer, Billiau, and Declercq, 1967; Mazzur and Paucker, 1967; Mazzur, Ellsworth, and Paucker, 1967). The demonstration of an effect of interferon on antibody production was first reported by Braun and Levy (1972) who found that intraperitoneal injection of mouse interferon with SRBCs either enhanced or inhibited the plaque-forming-cell response measured 2 days later. Low doses (250 to 1500 units) enhanced antibody producers whereas high doses (5000 to 10,000 units) inhibited the production of plaques. Chester, Paucker, and Merigan (1973) reported that intravenous injection of about 10^5 units of interferon into mice two days prior to the antigen significantly suppressed their antibody response to SRBC. When

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tended that "interferon assay is not well established in the canine species"; such a situation did not, however, deter Depoux (1965), or Aurelian and Roizman (1965); see Table 1.

[10] In view of the growing hopes for this substance and in light of its cell-multiplication-inhibitory activity, it seems surprising that no one has yet proposed using it to treat giantism!

poly rI·poly rC was used to induce interferon, however, a slight increase in antibody-producing cells was observed after SRBC challenge (Merigan, Chester, and Paucker, 1975). Brodeur and Merigan (1974) showed that both the primary and secondary hemolysin and agglutinin antibody responses were inhibited in mice injected intravenously with about 10^5 units of interferon 2 days before primary immunization with SRBC, and both IgM and IgG antibody synthesis was depressed, as revealed by sensitivity and resistance to mercaptoethanol.

Brodeur and Merigan (1975) also demonstrated that about 10^5 units of interferon inhibited the primary antibody response of mice to a thymus-independent antigen, *Salmonella typhimurium* lypopolysaccharide, and thus indicated a direct action of interferon on B lymphocytes.

Others have reported that intravenous injection of rabbits with about 10^6 units of rabbit interferon did not alter the ability of these animals to produce antibodies to SRBCs (Thorbecke, Friedman-Kien, and Vilček, 1974).

Ngan, Lee, and Kind (1976) reported that mouse interferon was able to strongly inhibit an heterologous adoptive cutaneous anaphylaxis (HACA) reaction. Sensitized mouse spleen cells which synthesized IgE caused HACA reaction in rat skin; when these cells were treated with 200 units of interferon/ml, the reaction was reduced about 50%; when treated with 600 units/ml, the reaction (i. e., IgE antibody production) was completely inhibited.

Virelizier, Virelizier, and Allison (1976) found that infection of mice with the coronavirus, murine hepatitis virus-3, modified their humoral response to SRBCs, and showed that the modification correlated with the circulating interferon levels: presence of interferon before antigen reduced antibody responses; its presence after antigen enhanced antibody response. These authors speculate that interferon induction may account for the many modifications of immune responses seen in man during virus diseases. Berencsi and Baladi (1977) reported that interferon induced in hamsters by Newcastle disease virus apparently suppressed both their primary and anamnestic responses to SRBCs.

2. In vitro *Antibody Production*

In the first report on effects of interferon on *in vitro* antibody response, Gisler, Lindahl, and Gresser (1974) showed that several thousand units (about 3000 "units"/ml; about 12,000 reference units/ml) were required to suppress the *in vitro* PFC response of Balb/c mouse spleen cells to SRBCs. When these workers treated spleen cells that were poor responders with lower doses of interferon (1000 to 2000 units/ml), antibody production was enhanced. These effects were seen when cells were treated with interferon 6 hours before antigen or up to 40 hours after it. Johnson, Smith, and Baron (1974, 1975) also found that the antibody response of mouse spleen cells to SRBCs was inhibited by interferon *in vitro,* by using C 57 Bl/6 mouse cells they were able to show a marked inhibition (90% or greater) with only 20 units of interferon and more than 90% inhibition with 40 units/ml. Reasons for this apparently large quantitative discrepancy are not clear. Booth *et al.* (1976) also found that the antibody response of mouse spleen cells to SRBC, inhibited by 80 units of interferon, was slightly enhanced by 0.8 unit/ml. Interferon treatment apparently

did not inhibit the growth of spleen cultures (Booth, Booth, and Marbrook, 1976).

The *in vitro* antibody response to T cell- and macrophage-independent antigen, *E. coli* lipopolysaccharide, was also found to be sensitive to interferon, at about 100 units/ml (Johnson, Bukovic, and Baron, 1975; Johnson and Baron, 1976a, b, 1977).

It has also been shown that poly rI·poly rC is able to inhibit primary antibody response to SRBCs and *E. coli* lipopolysaccharide in mouse spleen cells (Johnson, Bukovic, and Smith, 1975). Concanavalin A and Staphylococcal enterotoxin (T-cell mitogens) were also able to inhibit the PFC response to SRBC or lipopolysaccharide (Johnson and Baron, 1976b, c). Interestingly, the antiviral activity induced by double-stranded RNA and the inhibitory effect of double-stranded RNA on antibody synthesis were both inhibited by antibody prepared against mouse type I interferon, whereas the interferon and the immunosuppression induced in these cultures by concanavalin A or Staphylococcal enterotoxin were not neutralized by this antiserum.

It has recently been suggested that type I and type II interferons are functionally distinct, in that the latter, often being a product of immune recognition reactions, or at least produced by immunocompetent cells, would have a greater predilection for cells of the immune system. It has even been reported that type II interferons have significantly more antitumor activity than type I interferon (Salvin *et al.*, 1975b). In this regard I should again point to the fact emphasized in Fig. 5: these suppositions are premature until type II interferons are significantly purified, and will be difficult to accept until demonstrated with pure type II interferons.

The demonstrations that interferons are able to induce a number of alterations in cells other than selective inhibition of virus replication has opened the interferon system to explorations by many fields of research. The applications of interferon to immunomodulations is particularly exciting, and is a fertile new field for rampant speculation: I shall resist rushing in.

Thus far we have followed the interferon system from its start, the descriptions of the inducers, to the interaction of these with cells, through the processes leading to production of interferons. We have purified and characterized these proteins and have examined what they can do at the molecular level to inhibit viruses and have examined their other actions and possible actions at the cellular level. Now we shall examine reports of what they do in the intact animal.

The remainder of this book will deal with the uses of interferons against virus diseases and tumors in animals and in the clinic.