Current Topics in Microbiology and Immunology

102

Editors

M. Cooper, Birmingham/Alabama · P.H. Hofschneider, Martinsried · H. Koprowski, Philadelphia · F. Melchers, Basel
R. Rott, Gießen · H.G. Schweiger, Ladenburg/Heidelberg
P.K. Vogt, Los Angeles · R. Zinkernagel, Zürich

With 40 Figures

Springer-Verlag
Berlin Heidelberg New York 1983

ISBN-13: 978-3-642-68908-6 e-ISBN-13: 978-3-642-68906-2
DOI: 10.1007/ 978-3-642-68906-2

Softcover reprint of the hardcover 1st edition 1983
Library of Congress Catalog Card Number 15-12910

Typesetting: Fotosatz Service Weihrauch, Würzburg.

2121/3321-543210

Table of Contents

Indexed in Current Contents

Biosynthesis of the Collagen-like C1q Molecule and its Receptor Functions for Fc and Polyanionic Molecules on Macrophages

MICHAEL LOOS*

* Institute for Medical Microbiology, Johannes Gutenberg University, D-6500 Mainz, Federal Republic of West-Germany

Abbreviations. ATEe, *N*-acetyl-L-tyrosine ethyl ester; Con A, concanavalin A; CRP, C-reactive protein; $C\bar{1}$ INH, $C\bar{1}$ inhibitor; DEAE, diethylaminoethyl; DFP, diisopropyl fluorophosphate; DNP, dinitrophenyl; DNP-HSA, dinitrophenylated human serum albumin; DS, dextran sulfate; EDTA, ethylenediaminetetraacetate; E-TNP, trinitrophenylated erythrocyte; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GPS, guinea pig serum; HSA, human serum albumin; Ig, immunoglobulin; LPS, bacterial-derived lipopolysaccharide; M 199, medium 199; NaCl, sodium chloride; NPGB, *p*-nitrophenyl-*p'* -guanidinobenzoate; PA, polyanion; PBS, phosphate-buffered saline; PS, ant venom-derived polysaccharide; RB 200, lissamine rhodamine B; Sp54, pentosan polysulfo ester; PVS, polyvinyl sulfate; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SRBC, sheep red blood cell; TAMe, *N*-tosyl-L-arginine methyl ester; TNP, trinitrophenyl.

Nomenclature. The nomenclature used for the proteins of the classical complement pathway conforms with that agreed upon by the World Health Organization in 1968 (Bull WHO 39:935): sheep erythrocytes (E) sensitized with rabbit antibody (A) containing complement (C) components $EAC\bar{1}$, EAC4, $EAC\bar{1}4$. A component in the activated state is designated with a bar over the number, e.g., $C\bar{1}$; the activated forms of two subcomponents of native C1, C1r and C1s, are written with a bar over the letter, $C1\bar{r}$ and $C1\bar{s}$. The nomenclature of the alternative activating pathway of complement was drafted by a subcommittee of the IUIS WHO Nomenclature Committee in 1981 (J Immunol 127:1261).

"Alexine is nothing but a leucocytic product"
Elie Metchnikoff, 1905

1 Introduction

At the beginning of the nineteenth century, knowledge of immunity was limited to a few practical methods based on empirical observations, e.g., the observation by Jenner in 1798 that inoculation with cowpox material induced an immunity to smallpox. The discoveries by Louis Pasteur and Robert Koch that microorganisms caused fermentations and were responsible for a number of infectious diseases, greatly advanced the concepts of susceptibility and immunity in a limited number of diseases. In the late nineteenth century, the complement system was discovered by *Fodor* (1887), *Nuttall* (1888), and *Buchner* (1889a, b) through studying the bactericidal action of blood serum. It was recognized that killing of bacteria in fresh serum required at least two different substances: a heat-stable (30 min, 56 °C) factor, today known to be the antibody specific for the particular microorganism; and a heat-labile factor, which was normally present in each serum. This factor was at first termed "alexine" (*Buchner* 1889a, b) and later designated "complement" (*Pfeiffer* and *Issaeff* 1894; *Bordet* 1896). On 18 May 1889, Buchner made the following

statement at a lecture in Munich: "Das Vorhandensein bakterienfeindlicher Wirkungen durch flüssige Bestandtheile der Körpersäfte lässt die überall nachweisbare Thätigkeit der Phagozyten als weniger ausschlaggebend erkennen" (The presence of bactericidal action in body fluids reveals the overall detectable activity of phagocytes as less decisive). This statement by the chief advocate of the humoral theory of resistance to microbial infections was directed against the cellular theory of immunity proposed by Metchnikoff. In his answer to Buchner's critique, *Metchnikoff* (1889; English translation 1905) came to the conclusion, based on his own work, that "the postulates of this theory are often not in accord with the real facts," and that the bactericidal effect of body fluids has nothing in common with the phenomenon of immunity ("la propriété bactéricide des humeurs ne correspond nullement aux phénomènes de l'immunité"). However, "we expressed the opinion that a portion at least of the bactericidal power might come from substances that had escaped from the leucocytes during the preparation of the defibrinated blood and of the blood serum." Therefore, "alexine is nothing but a leucocytic product."

Today, almost a century later, it is well established that Metchnikoff's macrophages are one of the prominent cell types for serum C production. However, the individual C components are not merely liberated into the fluid phase, as was assumed by Metchnikoff. The living macrophages synthesize and secrete most of the C components (*Thorbecke* et al. 1965; *Colten* 1976; *Brade* and *Bentley* 1980; *Bentley* et al. 1981). Analysis and characterization of the C system in biological fluids were made possible by dramatic advances in protein chemistry and the development of suitable tissue culture techniques for analyzing protein synthesis by cells.

It has now been established that the C system is composed of 11 proteins of the "classical" pathway: C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8 and C9. There are three more proteins of the "alternative" pathway: B, D, and P. Finally, there are four control proteins: $C\bar{1}$ INH for the classical pathway, I (C3b inactivator or C3b INA) and H (β_1H or C3b INA accelerator) for the alternative pathway, and the anaphylatoxin inactivator. These 18 distinct serum proteins have been highly purified and physicochemically characterized, and for some of them at least, the amino acid composition and sequences are partially completed (for details, see *Müller-Eberhard* 1975; *Forthergill* and *Anderson* 1978; *Müller-Eberhard* and *Schreiber* 1980; *Porter* and *Reid* 1979). The third component, C3, which is probably the most important C component in relation to host defense, can be activated by two different pathways: the classical pathway with C1, C4, and C2, and the alternative pathway via a large cleavage product of C3, namely C3b, together with B, D, and P.

The purpose of this review is to summarize our present knowledge of the biosynthesis of C1 and its subcomponents C1q, C1r, and C1s by macrophages. From this discussion it will become evident that the individual subcomponents of macromolecular C1 are synthesized independently of each other. First, a functional description of C1 and its subcomponents will be presented together with their physicochemical characteristics. Second, focusing on the biosynthesis of the collagen-like C1q molecule, the similarities between the biosynthesis of collagen and C1q will be documented. Third, it is the major goal of this discussion to demonstrate that C1q during the secretion phase, mediates receptor functions for the Fc portion of immunoglobulins as well as for polyanionic molecules. This action occurs before C1q is released from cells and prior to its incorporation into the macromolecular C1 complex.

2 Biochemistry of C1

After treatment with the chelating agent EDTA, the macromolecular complex of C1 dissociates into three distinct proteins, designated C1q, C1r, and C1s. These subcomponents can be separated by chromatography on DEAE cellulose or by ultracentrifugation (*Lepow* et al. 1963; *Sassano* et al. 1972). Reassociation of the three subcomponents to macromolecular C1 occurs when they are mixed together in the presence of Ca^{++}, but not Mg^{++}. This indicates that C1 is a Ca^{++}-dependent molecule (*Lepow* et al. 1963). It is now known that calcium is the ligand between the subcomponents C1r and C1s, and that it enables the C1r-C1s complex to combine with C1q to form functionally active C1 (*Ziccardi* 1981). If C1 is reassembled from purified C1q, C1r, and C1s and then sedimented in a sucrose gradient, it is a 15–16-S molecule with a molecular weight of 739 000–774 000 (*Ziccardi* and *Cooper* 1977; *Siegel* et al. 1981). However, under physiological conditions (ionic strength $\mu = 0.15$) the C1 activity in either human or guinea pig serum has a sedimentation rate of between 18 S and 19 S (*Naff* et al. 1964; *Colten* et al. 1968a; *Loos* et al. 1976b). The sedimentation rate of guinea pig C1 varies inversely with the ionic strength of the sucrose gradient: at an ionic strength of $\mu = 0.065$, the S value is about 18, whereas at an ionic strength of $\mu = 0.90$, the activity is found in the 4-S region. The hemolytic activity of C1 disappears with increasing ionic strength, but it can be restored by reducing the ionic strength (*Colten* et al. 1968b). The sedimentation rates of serum C1 and C1 reassembled from purified subcomponents may vary because the compositions of the complexes differ – from 1 C1q: 2C1r:2C1s to 1C1q: 1C1r:1C1s respectively (*Gigli* et al. 1976; Borsos et al. 1980).

Macromolecular C1 is a euglobulin which can be precipitated from normal serum at an ionic strength of $\mu = 0.02$ or lower and pH 5.2 (*Pillemer* et al. 1941, 1943), or at low ionic strength ($\mu = 0.02$) but pH 7.5 (*Tamura* and *Nelson* 1968). Activated macromolecular C1 can be purified from normal human serum by affinity chromatography on Sepharose-bound IgG (*Assimeh* et al. 1974; *Bing* 1971, 1980; *Kolb* et al. 1979). Native (not activated) C1 can also be purified by this method when the purification steps are carried out in one of two inhibitors, DFP (*Gigli* et al. 1976) or NPGB (*Medicus* and *Chapuis* 1980).

2.1 Subcomponent C1q, a Collagen-like Molecule

In 1961, *Müller-Eberhard* and *Kunkel* and *Taranta* et al. independently reported a heat-labile humoral factor that was capable of precipitating soluble gamma globulin aggregates in the absence of divalent cations such as calcium and magnesium. Based on its sedimentation rate and its differences from the four C components of the human system that were known at that time (C1, C2, C3, C4), it was termed "11-S component." Two years later, when *Lepow* et al. (1963) resolved the first component of human C into three distinct subcomponents, C1q, C1r, and C1s, by chromatography on DEAE cellulose in the presence of EDTA, it was shown that C1q was functionally and immunochemically identical to the 11-S component.

The observation that C1q was insoluble at low ionic strength was useful for preparing the starting euglobulin fraction (*Calcott* and *Müller-Eberhard* 1972; *Reid* et al. 1972) and for isolating highly purified C1q by repeated precipitation and solubilization steps in the presence of EDTA (*Yonemasu* and *Stroud* 1971; *Volanakis* and *Stroud* 1972). Direct

binding of C1q to IgG coupled to Sepharose and its subsequent elution at high salt concentrations is an effective and rapid purification procedure (*Kolb* et al. 1979; *Arlaud* et al. 1979; *Reid* 1981). C1q has a molecular weight of 410 000, a sedimentation rate of 11 S, it migrates in an electric field as a gamma globulin, and its serum concentration ranges between 150 and 180 µg/ml (*Yonemasu* and *Stroud* 1971; *Calcott* and *Müller-Eberhard* 1972; *Porter* and *Reid* 1979; *Reid* 1982).

C1q, the recognition unit of trimolecular C1, is able to distinguish between the different classes of immunoglobulins and their subclasses by direct binding to the Fc portion of IgG and IgM, but not to IgA, IgD, and IgE (*Ishizaka* et al. 1966; *Isliker* et al. 1967; *Kehoe* and *Fougereau* 1969; *Augener* et al. 1971; *Plaut* et al. 1972; *Hurst* et al. 1974, 1975; *Bubb* and *Conradie* 1976; *Winkelhake* 1978). Studies of the interaction between human IgM from a patient with Waldenström's macroglobulinemia and human C1q suggested a valence of five for C1q (*McKenzie* et al. 1971). *Müller-Eberhard* (1969) described C1q as a multivalent molecule with five to six valences for IgG.

Electron-microscopic studies with human C1q revealed a unique structure, consisting of six peripheral subunits connected by fibrillar strands to a central core and resembling a "pot of flowers", as seen in Fig. 1 (*Shelton* et al. 1972; *Knobel* et al. 1975; *Strang* et al. 1982). The peripheral globular structures of the molecule are sensitive to proteolytic enzymes and represent the Fc binding portions of the molecule. The central portion, where the fibrillar strands terminate, represents the C1r and C1s binding site(s) of the molecule (*Knobel* et al. 1975; *Reid* and *Porter* 1976). From the lateral view of the C1q molecule (Fig. 1), the central subunit appears to be subdivided longitudinally into two parts.

It has been reported that an inhibitor exists in serum that forms a complex with C1q, leading to a reduction of its functional hemolytic activity. The inhibitor is retained by

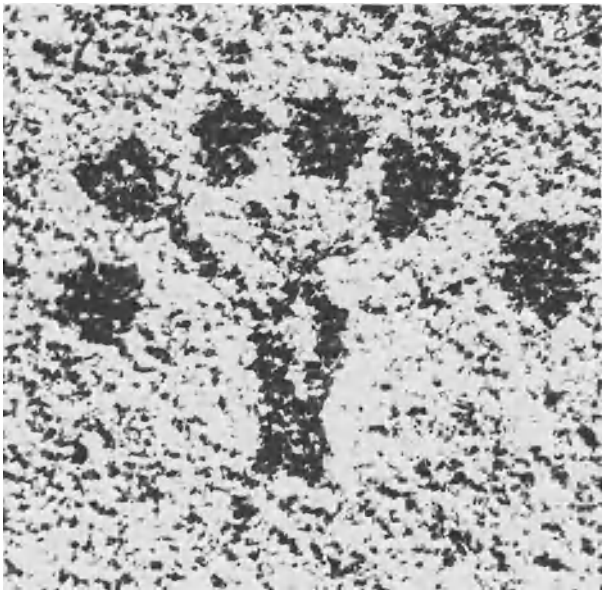

Fig. 1. Electron photomicrograph (negative) of human C1q. The six globular portions and the strands connecting these portions with a central piece are clearly visible. (*Knobel* et al. 1975)

chromatography on Con A-linked sepharose (*Conradie* et al. 1975) and has been purified from serum and identified as a chondroitin 4-sulfate proteoglycan (*Silvestri* et al. 1981). *Ghebrehiwet* (1981) isolated a C1q inhibitor from serum that shared some properties with a lymphocyte membrane-derived inhibitor. It was assumed that this inhibitor represented a cell surface C1q receptor that was shed into serum (*Sobel* and *Bokisch* 1975; *Tenner* and *Cooper* 1980, 1981).

Amino acid analysis revealed that C1q is an unusual serum protein due to its content of hydroxylysine and hydroxyproline and its high concentration of glycine residues (*Calcott* and *Müller-Eberhard* 1972; *Volanakis* and *Stroud* 1972; *Yonemasu* and *Stroud* 1971, *Yonemasu* et al. 1971, 1972). These amino acids are unusual because they are found predominantly in collagen and in basement membrane proteins.

Structural analysis showed that the C1q molecule could be dissociated into three smaller subunits upon reduction and alkylation (*Volanakis* and *Stroud* 1972; *Yonemasu* et al. 1972). The individual polypeptides of C1q are designated the A, B, and C chains. It was proposed, based on electron-microscopic pictures of C1q, that disulfide bonds link one A and one B chain together, and two C chains together (*Reid* 1974; *Porter* and *Reid* 1979). It was assumed that the two strands composed of A-s-s-B are linked by the C chains (C-s-s-C), resulting in a doublet, (A-s-s-B): (C-s-s-C): (A-s-s-B). One C1q molecule seems to be composed of six A–B dimers and three C-C dimers that together form three doublets. The mode of linkage between the doublets remains unclear (*Reid* and *Porter* 1976).

Amino acid analysis confirmed a collagen-like structure: glycine was in every third position and hydroxyproline and hydroxylysine were often in the Y position of the repeating X-Y-glycine amino acid sequence (*Reid* 1974; *Reid* 1977; *Reid* et al. 1977). Since all collagen molecules with three peptide chains form a triple helical structure, such a form is also postulated for each of the strands which are composed of the A, B, and C chains (*Reid* and *Porter* 1976).

2.2 Subcomponent C1r, Proenzyme of a Serine Esterase

The subcomponent C1r consists of two identical, noncovalently-linked 83 000–93 000-dalton polypeptide chains (*Ziccardi* and *Cooper* 1976a; *Sim* 1981). In the linked form, they have a molecular weight of approximately 168 000–188 000 and a sedimentation rate of 7.5 S. The electrophoretic mobility of purified C1r is similar to β-globulin, and its serum concentration is about 50 μg/ml (*DeBracco* and *Stroud* 1971; *Valet* and *Cooper* 1974a).

The hypothesis by *Naff* and *Ratnoff* (1968), that C1r is an enzyme capable of activating C1s from its precursor form, has been confirmed in several laboratories. The C1r exists in the native macromolecular complex of C1 as a proenzyme. It can be isolated in the zymogen form only when the purification is done at low temperature (0 °C), or when protease inhibitors such as DFP or NPGB are used during the entire purification procedure (*Valet* and *Cooper* 1974a; *Gigli* et al. 1976; *Chapius* et al. 1977; *Ziccardi* and *Cooper* 1976a, b). Precursor C1r is a single-chain molecule which, during activation, undergoes a cleavage into two chains: chain "a" with a molecular weight of about 56 000 and chain "b" with a molecular weight of about 27 000. The chains are linked by disulfide bridges (*Takahashi* et al. 1975a; *Gigli* et al. 1976; *Sim* et al. 1977). The enzymatic site of C1r is located in the smaller chain "b", shown by the uptake of radiolabeled DFP (*Takahashi* et al. 1975a; *Ziccardi* and *Cooper* 1976b; *Assimeh* et al. 1978).

The natural substrate of activated C1r is the zymogen C1s, which is then converted to the esterase $C1\bar{s}$ (*Naff* and *Ratnoff* 1968; *DeBracco* and *Stroud* 1971; *Valet* and *Cooper* 1974a; *Ziccardi* and *Cooper* 1976b). $C1\bar{r}$, a serine esterase, has been reported to cleave synthetic substrates such as amino acid methyl esters and amino acid *p*-nitrophenyl esters (*Naff* and *Ratnoff* 1968; *Andrews* and *Baillie* 1979). However, these observations remain controversial (*Sim* et al. 1977).

C1r and the other two components C1q and C1s form a macromolecular complex which by crossed immunoelectrophoresis can be demonstrated to be in the gamma region (*Laurell* and *Mårtensson* 1974; *Laurell* et al. 1979; *Bartholomew* and *Esser* 1980). There is some evidence that C1r requires only C1q and calcium to bind to sensitized erythrocytes, but C1r by itself does not bind to these cells, as shown by the uptake of [^{125}I]-C1r (*Ziccardi* and *Cooper* 1976a). Furthermore, in the presence of calcium, C1r and C1s form a C1r-C1s complex, which by crossed immunoelectrophoresis was demonstrated to be in the β region (*Nagasawa* et al. 1974; *Laurell* and *Martensson* 1974; *Laurell* et al. 1978). The esterolytic activity of C1r can be inhibited not only by the protease inhibitors DFP and NPGB, but also by a naturally occurring glycoprotein, termed the C1 esterase inhibitor. The latter is of biological importance because it modulates the activity of $C\bar{1}$ (*Naff* and *Ratnoff* 1968; *Ratnoff* et al. 1969; *Ziccardi* and *Cooper* 1976a; *Andrews* and *Baillie* 1979). Even in the presence of calcium ions, the activity of isolated $C1\bar{r}$ is inhibited (*Valet* and *Cooper* 1974a; *Ziccardi* and *Cooper* 1976a).

2.3 Subcomponent C1s, Proenzyme of a Serine Esterase

The subcomponent C1s, dissolved in dissociating solvents, has an apparent molecular weight of 83 000–110 000 (*Nagaki* and *Stroud* 1969; *Valet* and *Cooper* 1974b; *Sim* et al. 1977; *Arlaud* et al. 1977b). The molecule exists in two forms: the native or precursor form, C1s, and the activated form C1 esterase, or $C1\bar{s}$. Although a comparison of the amino acid composition of both precursor and activated forms was inconclusive, the conversion of the native form to $C1\bar{s}$ resulted in a faster anodal migration on immunoelectrophoresis; C1s migrates as a β-globulin and $C1\bar{s}$ as an α-globulin (*Sakai* and *Stroud* 1973; *Valet* and *Cooper* 1974b; *Laurell* et al. 1976; *Sim* 1981).

The purification of the proenzyme C1s is particularly successful when protease inhibitors such as L-lysine, DFP, or NPGB are included in the buffers. The zymogen C1s is a single-chain molecule which, like C1r, is cleaved upon activation into two chains (*Sakai* and *Stroud* 1973, 1974; *Valet* and *Cooper* 1974b; *Gigli* et al. 1976; *Sim* et al. 1977; *Arlaud* et al. 1977a). Chain "a" has a molecular weight of about 56 000 and chain "b" of about 27 000; the chains are linked by disulfide bonds (*Sakai* and *Stroud* 1973; *Takahashi* et al. 1975b; *Arlaud* et al. 1977a, b; *Sim* et al. 1977). The enzymatic site of $C1\bar{s}$ is located in the smaller chain "b", as shown by the incorporation of radiolabeled DFP (*Sakai* and *Stroud* 1973; *Arlaud* et al. 1977a). The active center of human $C1\bar{s}$ consists of an anionic binding site in conjunction with a hydrophobic binding site. This was shown by using a variety of guanidine, amidine, and aromatic compounds of low molecular weight (*Bing* 1969). The natural substrates of $C1\bar{s}$ in serum are C4 and C2, the next two components in the C cascade of the classical pathway (*Haines* and *Lepow* 1964b; *Gigli* and *Austen* 1969; *Budzko* and *Müller-Eberhard* 1970; *Patrick* et al. 1970). Cleavage of C4 and C2 on the cell surface by $C1\bar{s}$ leads to the formation of the classical pathway C3 convertase, $C\overline{4b, 2a}$ (*Borsos* et al.

1961; *Müller-Eberhard* and *Lepow* 1965; *Müller-Eberhard* et al. 1967). Under certain conditions, this cleavage also occurs in the fluid phase. The C1s can be converted directly to $C1\bar{s}$ by proteases such as plasmin and trypsin (*Valet* and *Cooper* 1974b). During continuous incubation of C1s with plasmin, trypsin, or a mixture of lysosomal enzymes isolated from human peripheral leukocytes, it was found that C1s was progressively degraded, resulting in an increase in smaller fragments and a progressive loss of esterase activity (*Taubmann* and *Lepow* 1971). From these experiments it is clear that the activation of C1s to $C1\bar{s}$ and the subsequent breakdown of $C1\bar{s}$ into smaller fragments by proteolytic enzymes depends not only on the type of enzyme and its concentration, but also on the time of incubation. Furthermore, the cleavage of C1s is not necessarily synonymous with its activation.

$C1\bar{s}$, in addition to hydrolyzing its natural plasma substrates C4 and C2, also hydrolyzes a variety of synthetic substrates containing arginine or tyrosine esters, such as ATEe and TAMe (*Haines* and *Lepow* 1964a; *Bing* 1971). The esterase activity of $C1\bar{s}$ or active $C\bar{1}$ is inhibited by the naturally occurring $C\bar{1}$ INH (*Haines* and *Lepow* 1964b; *Lepow* et al. 1965; *Bing* 1969, 1971). DFP, first described by Becker (1956) as inhibiting the esterolytic action of $C\bar{1}$, inhibits $C1\bar{s}$ as well, indicating that $C1\bar{s}$ is a serine esterase. There are other phosphonate esters which are potent inhibitors of C1 esterase activity, and the length of the phosphonate ester influences its inhibitory potency (*Becker* 1956; *Becker* and *Austen* 1964; *Haines* and *Lepow* 1964b; *Becker* 1967; *Bing* 1969; *Sakai* and *Stroud* 1974).

The cleavage of C4 and C2 by isolated $C1\bar{s}$ is also inhibited by various PAs, such as DS, Liquoid (polyanetholesulfonate; Roche), and Sp54. In contrast to the experiments on polyanion binding to C1q, there were no differences, on a weight basis, among the polyanions tested, in binding to $C1\bar{s}$, this indicates that the mechanism of action of PA on $C1\bar{s}$ is different from that on C1q. Thus the PAs do not appear to bind to $C1\bar{s}$ and do not affect the enzymatic site, but they may interfere with the C4 and C2 binding site(s) (*Loos* et al. 1976a; *Raepple* et al. 1976).

There are a number of similarities between the two subcomponents C1r and C1s: both are serine esterases which are inhibited by $C\bar{1}$ INH, DFP, and NPGB, and both precursor forms are single-chain molecules which are cleaved upon activation into larger "a" and smaller "b" chains linked by disulfide bonds. The similarities of precursor chains and chain fragments were confirmed by showing a high frequency of identity in their amino acid composition, especially of the esterolytic center (*Sim* et al. 1977; *Fothergill* and *Anderson* 1978; *Sim* 1981). However, the two subcomponents differ in their substrate specificities: $C1\bar{r}$ can activate only C1s, but not C4 and C2; $C1\bar{s}$ cannot activate C1r, but it does cleave C4 and C2. Furthermore, C1s does not bind to C1q in the absence of C1r, but [^{125}I]-C1r is taken up by EA provided that C1q is present (*Ziccardi* and *Cooper* 1976a, b).

3 Acceptor Molecules for C1 and C1q

3.1 Immunoglobulins: IgM and IgG

Both IgM and IgG have a binding site for the C1q subcomponent of macromolecular C1 (*Müller-Eberhard* and *Kunkel* 1961; *Ishizaka* et al. 1966; *Winkelhake* 1978), and various subclasses within these classes of immunoglobulins are potent activators of the classical

C pathway. The other classes of immunoglobulins, including IgA, IgD, and IgE, do not activate the classical C pathway. The IgM molecule has a sedimentation rate of 19 S, a molecular weight of about 900 000, and is a pentamer of five 7-S subunits, each of which is able to bind C1 (*Augener* et al. 1971). The acceptor site for C1 is localized within the C_H4 domain of the Fc portion of the IgM molecule (*Plaut* et al. 1972; *Sledge* and *Bing* 1973; *Hurst* et al. 1974; *Bubb* and *Conradie* 1976). The binding capacity of a single IgM molecule is increased after it combines with a corresponding specific antigen, suggesting that a conformational change has occurred within the C1 binding site of the Fc portion (*Hyslop* et al. 1970). One cell-bound IgM molecule seems to be sufficient to fix one C1 molecule (*Borsos* and *Rapp* 1965a, b). Each C1 molecule capable of interacting with cell-bound antigen-antibody complexes is able to initiate the C sequence, leading to lysis of the respective cell (*Colten* et al. 1967). These data support Mayer's one-hit theory of immune hemolysis; namely, that one discrete site on a cell surface is sufficient for completion of the C sequence and lysis of the cell (*Mayer* 1961). The C1q binding site of IgG is also located in the Fc portion of the molecule (*Isliker* et al. 1967; *Kehoe* and *Fougereau* 1969; *Augener* et al. 1971). The human IgG subclasses have different binding affinities for C1q and C1: IgG3 is most efficient, followed by IgG1 and IgG2. IgG4 does not bind C1 or C1q (*Augener* et al. 1971), and in this respect is similar to Fab or $F(ab')_2$ fragments. For IgG, the C1 binding site was shown to be located within the C_H2 domain of the heavy chain (*Kehoe* and *Fougereau* 1969; *Arlaud* et al. 1976; *Lee* and *Painter* 1980, *Burton* et al. 1980; *Lukas* et al. 1981; *Painter* et al. 1982). The binding of C1 or C1q to monomeric IgG is very weak, even at low ionic strength. Aggregation of IgG, binding of the antibody to the antigen, and linkage of the immunoglobulin to Sepharose all amplify the C1q/C1 binding capacity (*Müller-Eberhard* and *Kunkel* 1961; *Ishizaka* et al. 1966; *Agnello* et al. 1970; *Augener* et al. 1971; *Bing* 1971; *Hughes-Jones* and *Gardner* 1978; *Lin* and *Fletcher* 1978). The IgG molecule is, however, less efficient than IgM in initiating C fixation. A single molecule of anti-Forssman antigen IgM on the surface of an SRBC is capable of binding one C1 molecule and initiating the C cascade. In contrast, to achieve a single C1 binding site, it has been calculated that more than 1000 molecules of IgG are needed (*Humphrey* and *Dourmashkin* 1965). However, at least two IgG molecules in close proximity ("doublet") on the cell surface can fix a C1 molecule (*Borsos* and *Rapp* 1965a, b; *Colten* et al. 1967), and C1 fixation by a single molecule of IgG antibody on the surface of a sheep erythrocyte has also been reported. Immunochemical analysis of this latter IgG hemolysin revealed that it differed from other IgG hemolysins and may represent a unique subclass of rabbit immunoglobulin. Thus, not only the human IgG subclasses differ in their C1 binding affinities, but other examples of this variability exist in nature (*Frank* and *Gaither* 1970).

Recently, it was shown by *Golan* et al. (1981) that different populations of antisheep erythrocyte IgG exist on the surface of sensitized SRBCs (EA). All these populations were able to bind C1q. However, because of the distribution and arrangement of IgG on the cell surface, it was thought that one part of bound C1q was unable to interact with C1r and C1s, another part did bind C1r and C1s but did not activate them, and a smaller part of bound C1q not only bound, but also activated C1r and C1s. From these observations it was concluded that in addition to the binding of C1q to IgG, a certain configuration of C1q is essential for the interaction with C1r and C1s. This configuration is triggered by IgG. This interpretation was supported by the recent work of *Circolo* and *Borsos* (1982) showing that the angle of the Fab arms of the antigen-bound IgG molecule is important to obtain maximal C fixing and activating properties. From these findings it appears that

the density of the epitopes is important for the Fc region of IgG and subsequently for the conformational changes of C1q.

3.2 Polyanionic Factors and Other Acceptors for C1 and C1q

In addition to the immunoglobulins, a number of other substances interact directly with C1 and C1q (*Loos* 1982b). A variety of PAs from complexes with C1q due to their negative charges reacting with cationic C1q, the most basic protein in serum, with an isoelectric point at pH 9.3 (*Heinz, Golan* and *Loos,* manuscript in preparation). A direct interaction with C1q was also shown for polynucleotides such as polyinosinic acid (*Yachnin* et al. 1964), DNA (*Agnello* et al. 1970; *Peltier* et al. 1978), carrageenan (*Borsos* et al. 1965), heparin, DS, PVS, Liquoid, and chondroitin sulfate (*Rent* et al. 1975; *Raepple* et al. 1976; *McKay* et al. 1981; *Almeda* et al., to be published). Although almost all of these substances bind C1q and C1, only a few of them cause activation of C1 after the binding step. Treatment of serum with DS, PVS, or Liquoid resulted in a dose-dependent activation of C1 via the classical C pathway, as well as of C3 via the alternative pathway (*Loos* and *Bitter-Suermann* 1976). This dual function of PAs as activators of both C pathways was also demonstrated using sulfated Sephadex particles as insoluble PAs (*Burger* et al. 1977). Thus, an important observation is that a substance may bind to C1q, but not necessarily cause activation of macromolecular C1.

The interaction of certain PAs (such as heparin) with protamine, a polycation, leads under certain conditions to activation of serum C1. Thus the reaction is independent of antibody (*Rent* et al. 1975; *Fiedel* et al. 1976).

Certain LPS preparations bind directly to isolated C1 via C1q (*Müller-Eberhard* et al. 1970; *Loos* et al. 1972c; *Loos* et al. 1974). The C1q-LPS interaction appears to be influenced by the core and the O-specific sugar portion of LPS because the lipid A region, which is identical in all LPSs, has a strong C1q binding capacity (*Loos* et al. 1974). The lipid A region was shown not only to bind C1 but also to activate the classical C pathway (*Cooper* and *Morrison* 1978). This antibody-independent interaction of LPS with the classical pathway is probably an important defense mechanism against the invading pathogen (*Loos* and *Brunner* 1979). It was also demonstrated that intact bacteria such as *Escherichia coli, Klebsiella pneumonia,* and *Mycoplasma pneumoniae* bind directly to C1 via C1q (*Bredt* et al. 1977; *Loos* et al. 1978). Furthermore, it was shown for *Salmonella minnesota* that the antibody-independent bactericidal activity of normal human and guinea pig serum was dependent upon C1q, C4, C2, and Ca^{++} (*Clas* and *Loos* 1980, 1981, 1982). Similar results were published later with the *E. coli* strain J5 (*Betz* and *Isliker* 1981; *Betz* et al. 1982). The experiments of *Clas* and *Loos* (1980, 1981, 1982) also revealed that the core-deficient R mutants of *Salmonella minnesota* were more susceptible than the S form. E-TNPs were described as a model for an antibody-independent activation of the classical C pathway (*Loos* and *Thesen* 1978). These experiments were based on the observations that DNP-HSA precipitated C1q and that C1 binding capacity was proportional to the DNP substitution rate (*Loos* and *König* 1977). With E-TNPs, the cell intermediates E-TNPC1, E-TNPC14, and E-TNPC142 were formed; after the addition of the remaining C components, including C9, the cells were lysed (*Loos* and *Thesen* 1978; *Thesen* et al. 1978). The envelopes of some RNA viruses, including Moloney leukemia virus and vesicular stomatitis virus, also have receptors for C1. This promotes the lysis of

these tumor viruses even in the absence of a specific antibody, as a result of activation of the classical C pathway (*Cooper* et al. 1976; *Bartholomew* et al. 1978).

The C-reactive protein, an acute phase serum protein, binds directly to C1q and C1; the complex behaves remarkably similarly to C1q and C1 bound to immunoglobulins and causes activation of the classical C pathway (*Volanakis* and *Kaplan* 1974; *Siegel* et al. 1976; *Claus* et al. 1977).

Fibronectin, a glycoprotein with a molecular weight of 400 000, is associated with many different cell types and is also present in plasma and other body fluids. It has been shown to react with collagen and collagen-like molecules such as gelatin or C1q (*Menzel* et al. 1981a; *Isliker* et al. 1981, 1982; *Pearlstein* et al. 1982; *Bing* et al., to be published). There is some evidence that the binding of fibronectin is mediated by the collagen-like region of the A chain of C1q, whereas the globular regions of the C1q molecule are not involved. Fibronectin, which is thought to stimulate endocytosis and promote the clearance of particulate material from the circulation, may also function in the clearance of C1q-coated material such as immune complexes (*Bing* et al., to be published).

A polysaccharide (PS) that was isolated and characterized from the venom of the tropical arboreal ant *Pseudomyrmex sp.* causes the activation of human C1, resulting in the consumption o the serum substrates C4 and C2. PS had no effect on the human $C\bar{1}$ INH, thus eliminating the possibility that C1 was activated because its natural inhibitor was removed. There is considerable evidence that the action of the PS is directly on C1q. The site on C1q is available to the PS when purified C1q is bound to cells (EAC1q), or when C1q is combined with the other subcomponents C1r and C1s in the fluid phase. When macromolecular $C\bar{1}$ is cell-bound, such as on EA$C\bar{1}$, the PS does not bind to $C\bar{1}$, presumably because bound C1q is not accessible. In addition, fluid phase C1q devoid of C1r and C1s does not bind the PS. It was shown that the globular heads of C1q which bind the molecule to the Fc region of IgG are probably not the binding site of the PS. The site of binding and possibly the subsequent activation of macromolecular C1 are thought to be generated on the collagenous portion of C1q. Thus, the PS differentiates two forms of C1q: bound and fluid-phase (*Schultz* et al. 1979, 1980).

The interaction between platelets and C1q, as well as the role of C1q in platelet aggregation, has been reported earlier. It was shown that the collagen-like region of C1q, especially that of the A chain, has receptors on platelets. Thus, C1q inhibited the collagen-induced aggregation of platelets (*Suba* and *Csákó* 1976; *Wautier* et al. 1977, 1980). *Sobel* and *Bokisch* (1975) showed that human peripheral lymphocytes are endowed with a specific, trypsin-sensitive C1q receptor. *Tenner* and *Cooper* (1980, 1981) also found that monocytes as well as B-lymphocytes (Ig-negative, C3b receptor positive) bound C1q. This cell surface C1q receptor may function as a serum C1q inhibitor after it is shed into biological fluids (*Ghebrehiwet* 1981).

4 Activation of C1

Activation of C1 is defined as the conversion of native precursor C1 to $C\bar{1}$. Native C1 is not able to act on the substrates C4 and C2, but $C\bar{1}$ has this capacity, leading to the formation of $C\overline{4b, 2a}$, the classical pathway C3 convertase (*Loos* 1982a). The activation of precursor C1 by antigen-antibody complexes is dependent on time and temperature (*Borsos* et al.

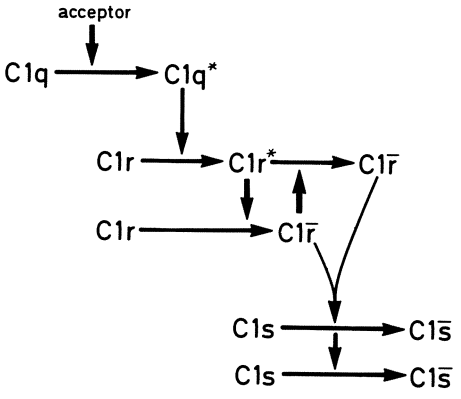

Fig. 2. Internal activation of the trimolecular complex C1 to C1̄ by different acceptors, e.g., the Fc portion of IgG or IgM antibodies (*Fc**), PAs, PS, or lipid A, the common portion of the LPS derived from gram-negative bacteria. *, conformational changes; - serine esterases

1964). Experimental evidence exists indicating that different steps are involved in the internal activation of C1 to C1̄ (Fig. 2) (*Loos* et al. 1972a, 1973; *Loos* 1982a):

1. Binding of C1 to antigen-antibody complexes via C1q, the recognition unit of C1. This step is temperature- and time-independent and is inhibited by PAs such as DS, PVS, carrageenan, Liquoid, and heparin, as well as by LPS and its lipid A portion.
2. Conversion of bound C1q from an Sp54-insensitive to an Sp54-sensitive form, which is designated C1q*. This step is dependent on temperature and represents a conformational change within the C1q molecule. Experiments designed to investigate the interaction of PS with fluid-phase and cell-bound C1q led to the same conclusion, namely that conformational changes are induced within the C1q molecule upon binding to immune complexes (*Schultz* et al. 1980). This postulation, drawn from functional studies, has been confirmed immunochemically by the detection of neoantigens within the C1q molecule following its binding to immune complexes. The neoantigens were detected by different monoclonal anti-C1q antibodies (*Golan* et al. 1982). It was confirmed that binding of C1 to the Fc portion of antibody in antigen-antibody complexes caused a reversible alteration of C1q, designated C1q*.
3. It has been assumed that the conformational change within the C1q molecule influences the conformation of C1r. This leads to the formation of a reversible transition state of C1r, designated C1r* as proposed by *Dodds* et al. (1978). In this single-stranded molecule the enzymatically active site of C1r becomes exposed. C1r* now cleaves the second C1r molecule present in the C1 complex to irreversible form a double-stranded C1r̄.
4. In the next step, C1r̄ cleaves the C1s dimer enzymatically to form the active C1̄ molecule.

The internal activation of C1 is therefore composed of two different activation steps: the first involves two conformational changes leading to the formation of C1q* and C1r*, followed by two proteolytic cleavage events leading to the generation of C1r̄ and C1s̄. Thus the C1q molecule, the trigger of the classical C cascade, serves as a transmitter between C1 activators, e.g., immune complexes, PAs (*Loos* and *Bitter-Suermann* 1976), LPS (*Loos* et al. 1974; *Cooper* and *Morrison* 1978), and a venom polysaccharide (*Schultz* et al. 1980). This transmission then alters the zymogens C1r and C1s. However, C1 bound to

molecules that are not able to initiate internal activation can be recognized by external activation with proteases such as trypsin, chymotrypsin, plasmin, thrombin, pronase, and elastase (*Loos* et al. 1973; *Loos* 1982a; *Heinz* and *Loos,* manuscript in preparation).

5 Biosynthesis of C1 and its Subcomponents

In the early 1960s, investigations aimed at determining the site(s) of synthesis of individual C components were stimulated when immunochemical techniques and advanced methods for protein chemistry were introduced to C research. Currently, two approaches for demonstrating serum protein synthesis in vitro have been developed: (a) the demonstration of a net increase of the specific protein in an in vitro system, (b) the incorporation of radioactive amino acids into the protein followed by autoradiographic demonstration of the labeled protein in an immunoelectrophoretic pattern. With the latter method, C3 was the first C protein to be studied. It was found to be synthesized in mouse spleen tissue (Hochwald et al. 1961), in human lymph nodes, bone marrow, and ileum, as well as in monkey spleen, lymph nodes, and bone marrow (*Asofsky* and *Thorbecke* 1961). In order to ascribe the synthesis of β1C (C3) in different tissues to a particular cell type, it was assumed that Kupffer cells and other tissue macrophages were the source of C3. Furthermore, it was assumed to be unlikely that peripheral blood cells, lymphocytes, plasma cells, or eosinophils were responsible for C3 synthesis (*Thorbecke* et al. 1965). In addition to the method of incorporating ^{14}C-labeled amino acids into the newly synthesized protein, *Siboo* and *Vas* (1965) performed hemolytic assays to demonstrate similar properties of the secreted protein to individual C components, including C2, C3, and C4. However, with this methodology these authors could not distinguish between release of preformed C components and synthesis.

Meanwhile, the ability to analyze and characterize the C system increased dramatically, and suitable tissue culture techniques were developed for the study of protein synthesis in well-characterized cells. Thus, in order to establish that a given cell or tissue is a site of synthesis for an individual C protein, the following "minimum criteria" have been outlined by *Colten* (1976):

1. The tissues and cells must be well characterized and, whenever possible, must be available in pure culture or in highly enriched populations.
2. Tissue culture media and culture conditions must be chosen for optimal maintenance of the cells and for stability of the complement components.
3. The baseline levels of preformed complement must be reduced, without damage to the cell.
4. Production of biologically active protein should be detected using one or more quantitative measures of complement activity. Optimally, the kinetics of synthesis and secretion should be monitored.
5. Incubation of the cells in medium containing radiolabeled amino acids should result in incorporation of label into immunochemically identifiable complement protein. It must be established that the radiolabeled protein has the functional and physicochemical characteristics of the complement component. The demonstration of labeled peptides by mapping of proteolytic digests of the newly formed protein indicates uniform incorporation of radiolabel.

6. The appearance of functional protein and the incorporation of radiolabel must be reversibly inhibited by established inhibitors of protein synthesis, or by incubating the cells at low temperature. The effect of inhibitors on total protein synthesis should also be assessed.
7. Results of studies using, e.g., fluorescent antibody, organ transplant, or extirpation, should be consistent with results of in vitro studies.

It is especially important to use several methods (points 4, 5 and 6 above) for establishing de novo synthesis in vitro.

5.1 Biosynthesis of C1 by Cells of the Intestinal Tract

Colten et al. (1966) first presented evidence that C1 is synthesized in the tissue of the small intestine. De novo synthesis was demonstrated by incorporation of ^{14}C-labeled amino acids, by reversible inhibition of synthesis by actinomycin D and puromycin (suggesting that the increase of C1 activity was not due to the release of preformed C1), and by hemolytic assay in which the synthesized, radiolabeled protein behaved like hemolytically active C1. By adapting Jerne's hemolytic plaque assay for detecting the synthesis of C1 in individual cells, these authors found that only the columnar epithelial cells of the small intestine produced hemolytic plaques. Again, the production of hemolytic plaques was prevented in the presence of puromycin (*Colten* et al. 1968c). Whereas in guinea pigs the small intestine was found to be the only tissue capable of in vitro synthesis of C1, human C1 was synthesized in both the large intestine and the ileum. Synthesis of human C1 was not found in the jejunum, stomach, liver, kidney, lung, spleen, lymph nodes, or thymus (*Colten* et al. 1968d). Using the same plaque assay system, *Moore* and *Vas* (1968) also were unable to demonstrate the production of hemolytically active C1 by bone marrow and spleen cells.

Investigating the appearance of C1 in the sera of sheep embryos, it was found that C1 was detectable from the 39th day of gestation. Since the placenta is an effective barrier to the transmission of maternal proteins, it was concluded that synthesis of C1 by the foetus began before the 39th day of gestation (*Colten* et al. 1968e). *Day* et al. (1970) also found, in studying the ontogenetic development of C1q synthesis in the piglet, that the intestine was the first site of synthesis. Portions of the fore-, mid-, and hindgut were found to be the first tissues synthesizing C1q protein. However, synthesis of C1q subsequently occurred in lymph nodes and spleen and still later in lungs and liver. Incorporation of labeled amino acids into C1q was prevented by chloramphenicol, indicating the de novo synthesis in these organs. Since nonintestinal tissues, particularly those rich in peripheral lymphoid cells, also produced C1q, the authors concluded that C1q is produced by cells derived from the mesenchyme rather than by epithelial cells. The method introduced by *Thorbecke* et al. (1965) was used to detect C1q synthesis, since no hemolytic activity was detectable in any embryonic tissue studied. The lack of detection of hemolytic C1 activity could be explained by components of the tissue culture medium, especially the 10% FCS, which is now known to strongly inhibit $C\bar{1}$ activity.

5.2 Biosynthesis of C1 and its Subcomponents by Epithelial Cells

Based on the observations of *Colten* et al. (1968c) that columnar epithelial cells are the site of C synthesis, *Bing* et al. (1975) used longer-term cultures to follow the synthesis of C1

and its subcomponents in primary cultures of human colon and urogenital tract tumor cells. They found that cells derived from the urogenital tract and transitional cell carcinoma, as well as from colon mucosa and a colonic carcinoma, were all capable of C1 synthesis. All tissue examined from the urogenital tract had in common the transitional cell epithelium, the cell type in which transitional tumors originate. *Morris* et al. (1978) compared the capacity of human fetal intestine columnar epithelium and bladder transitional epithelium to synthesize C1 and its subcomponents with that of skin and lung fibroblasts and monocytes. They found that epithelial cells synthesized and secreted 400–3700 times more hemolytically active C1 than fibroblasts or monocytes. Immunochemical and physicochemical analyses provided evidence that C1q from columnar epithelial cell cultures and C1r from each of the cell types tested had comparable subunit structures to serum C1q and C1r. C1s produced by epithelial cells and monocytes was found comparable to serum C1s, whereas C1s from fibroblast medium consisted of subunits with larger molecular sizes. C1q isolated from bladder transitional epithelial cells also consisted of subunits with higher molecular weights than that of serum C1q. From these experiments it was concluded that one cell type can synthesize and secrete C1q, C1r, and C1s. However, it is not known whether one individual cell can synthesize all three subcomponents or whether the individual C1 subcomponents are synthesized independently.

Secretion of an esterase from HeLa cells (derived from a cervical carcinoma), immunochemically and enzymatically identical with C1s of human serum, was reported by von *Zeipel* et al. (1973); however no evidence was provided for de novo synthesis. Examination of at least two other HeLa lines has failed to reveal synthesis of C1q (*Stecher* and *Thorbecke* 1967a), C1s, or intact C1 (*Colten* 1972a).

5.3 Biosynthesis of C1 and its Subcomponents by Fibroblasts

Since the subcomponent C1q is a collagen-like molecule, investigations were undertaken to determine whether it is synthesized by collagen-producing cells, namely fibroblasts. *Al-Adnani* and *McGee* (1976) showed by immunoperoxidase and immunoprecipitation procedures that material similar to the subcomponent C1q is synthesized by human fibroblast cell lines in vitro. However, these authors were unable to demonstrate the origin of C1q in vivo by the immunoperoxidase procedure using various rat organs, including fetal and neonatal organs. C1q was detected only in fibroblasts surrounding a silica granuloma which had been produced in the subcutaneous tissue of adult Wistar rats. From these data the conclusion was drawn that C1q is also produced in vivo, at least in pathological conditions where fibroblasts in a silica granuloma are functioning at a much higher level. However, no evidence for in vivo synthesis was provided, nor was it excluded that intracellular peroxidase activity, which also may increase in fibroblasts upon silica treatment, mediated staining.

Bing et al. (1975) found no evidence for synthesis of macromolecular C1 in one fibroblast cell line. In contrast, *Reid* and *Solomon* (1977), testing nine human fibroblast cell lines, provided evidence that all nine lines synthesized and secreted a hemolytic agent with activity comparable to that of serum C1. However, examination of the individual C1 subcomponents revealed that the fibroblasts were synthesizing low amounts of C1q compared with the C1r and C1s synthesized and secreted by these cells. From this it

was concluded that the rate of synthesis and secretion of C1r and C1s may be independent of the rate of synthesis and secretion of subcomponent C1q. It was demonstrated further that fibroblast-derived C1q has a higher apparent molecular weight than serum C1q. Analogous to the synthesis of collagen, which is present in a higher molecular weight form before secretion (termed procollagen), it was considered that C1q may also be produced by fibroblasts in a precursor form. However, these authors did not consider the possibility that some collagen types (I, II, and III) can bind C1r and C1s (*Valet* et al. 1978; *Menzel* et al. 1981b) and may mimic C1q function, although they are less potent on a weight basis than C1q (*Csákó* et al. 1981). Based on a rate of synthesis in vitro of approximately 10^4 effective C1 molecules per day (the cell number was not given), it was assumed that if this rate of synthesis and secretion occurred in vivo, fibroblasts may be the major source of hemolytic C1 activity in plasma. This conclusion was made in spite of the fact that fibroblast C1q was shown to differ markedly in at least two respects from serum C1q: it reacted very weakly with antiserum to serum C1q, and its apparent molecular weight, judged by SDS PAGE, was approximately 626 000 compared to 410 000 for serum C1q (*Reid* and *Solomon* 1977). Recently, a genetic defect of the C1q subcomponent associated with an immune complex nephritis in a 4-year-old Asian boy was described (*Thompson* et al. 1980). This defect was shown to be the consequence of an abnormal C1q gene that produced an antigenically deficient, nonfunctional molecule, and was expressed as a codominant allele. Skin fibroblasts of this patient were shown in vitro to secrete hemolytically active C1q, similar to that of C1q from fibroblasts of a normal individual (*Skok* et al. 1981). Similar results were obtained from in vitro cultured skin fibroblasts of a 5-year-old Turkish girl (A. K.) who had a selective complete deficiency of serum C1q (*Berkel* et al. 1981; *M. Loos, R. Burger,* and *A.I. Berkel*, unpublished data). Confirming the observations by *Reid* and *Solomon* (1977), it was also found that these fibroblast culture supernatant fluids contained higher concentrations of C1r and C1s than C1q-like activity. *Skok* et al. (1981) concluded from their findings that "a cell type other than fibroblasts was responsible for the synthesis of serum C1q, and that distinct genes exist for the fibroblast C1q and serum C1q. The products of these two genes would be two antigenically and physicochemically different C1q molecules." However, as pointed out by *Al-Adnani* and *McGee* (1976) it must be considered whether these in vitro findings have anything in common with what occurs in vivo.

5.4 Biosynthesis of C1 and its Subcomponents by Macrophages

Stecher et al. (1967b) reported that human and monkey macrophages, isolated from peritoneal exudates or lungs, produced not only β1C (C3) and β1E (C4), but also the 11-S globulin identified as C1q. This was shown immunochemically using the incorporation of radiolabeled amino acids into the protein, followed by autoradiography of immunoelectrophoretic patterns. Although this method is both specific and sensitive, these authors noted a limitation of this procedure: it demonstrates "neither net synthesis nor biological activity of a protein." However, with this approach it was ruled out that peripheral blood leukocytes synthesized C1q. These results were confirmed by *Lai A Fat* and *van Furth* (1975). In further experiments studying the medium requirements for serum protein production by rat and mouse macrophages in vitro, *Stecher* and *Thorbecke* (1967a) demonstrated that macrophages of both species showed much higher levels of serum

protein synthesis in a medium containing 20% instead of 1% FCS. Furthermore, these authors made the interesting observation that hydrocortisone is an effective substitute for FCS, at least for rat macrophages. *Moore* and *Vas* (1968) were able to demonstrate plaque formation produced by peritoneal exudate cells from guinea pigs with EA and R1, a reagent containing all C components except C1. De novo synthesis of C1 by macrophages could not be proven with this method. However, *Day* et al. (1970), using 10% FCS in the culture medium, failed to find hemolytic C1, although they provided immunochemical evidence that nonintestinal tissues, particularly those rich in peripheral lymphoid cells, produced C1q. It was also proven immunochemically that macrophages synthesized C1q. *Colten* and *Wyatt* (1972) and *Wyatt* (1974) were unable to detect the biosynthesis of hemolytically active C1q or C1 in guinea pig peritoneal exudate cells.

5.4.1 Culture Conditions and Methods

In all the experiments that failed to show de novo synthesis of hemolytically active C1q or C1 by peritoneal exudate cells, FCS was present in the culture medium. However, *Colten* (1972b) described, in a study of the biosynthesis of the $C\bar{1}$ inhibitor ($C\bar{1}$ INH), that "M 199 FCS was found to be an unsuitable tissue culture medium for these studies since preliminary experiments showed that this medium interfered with the biologic assay of $C\bar{1}$ INH." Studying the biosynthesis of C1q and C1 by peritoneal macrophages, *Colten* and *Wyatt* (1972) decreased the amount of FCS in culture medium from 10% to 1%, but they were still unable to detect hemolytically active C1q or C1. From this, the question arose of whether the proper culture conditions were used for peritoneal exudate cells in order to detect hemolytically active C1q or C1. Therefore before studying protein biosynthesis by peritoneal macrophages, we were cognizant of the "minimum criteria" by *Colten* (1976) mentioned above and tested culture media for optimal maintenance and stability of the complement components.

In our preliminary experiments, cultures of guinea pig peritoneal macrophages with and without heat-inactivated (2 h, 56 °C), 10% FCS or 10% autologous GPS were incubated in parallel for 72 h at 37 °C. The FCS was heated for 2 h at 56 °C to possibly avoid the inhibitory effect of FCS heat-inactivated as normally done for tissue culture media

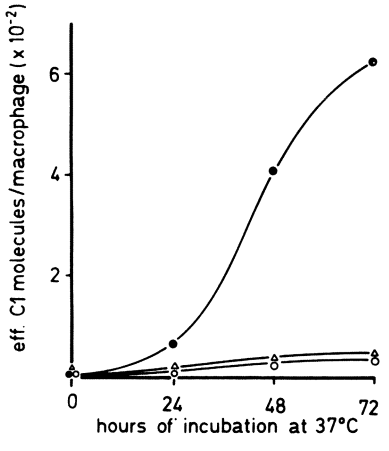

Fig. 3. Effect of heat-inactivated (56 °C, 120 min) FCS or autologous GPS on the detection of $C\bar{1}$ activity in culture supernatants of guinea pig peritoneal macrophages. Serum-free M 199 (●—●); M 199 containing 10% FCS (○—○); M 199 containing 10% autologous GPS (△—△)

(30 min, 56 °C). C1 activity was detected in the supernatants only from serum-free cultured monolayers after a latent period of 24–48 h (Fig. 3). In culture fluids containing 10% heat-inactivated FCS or GPS, however, C1 activity was rarely detected. In some of the experiments, performed with different batches of FCS, a small number of effective C1 molecules were detected by the hemolytic assay, but they never represented more than 30%–40% of the activity found in serum-free culture controls (*Müller* et al. 1978a). In contrast to the detection of the whole C1 molecule, C1q was found after 1 and 4 h of incubation. Its detection was not affected by FCS. Production of C1q was sometimes even higher in cultures containing FCS or GPS (*Müller* et al. 1978a). For further characterization of the inhibitory effect of FCS on C1, different concentrations of heat-inactivated FCS were added to supernatants from macrophage monolayers that had been cultured without serum for 72 h. After incubation at 0 °C and 37 °C for 30 min, tests for C1 were performed. Fig. 4 shows that the activity of C1 decreased depending not only on the temperature, but also on the concentration of FCS. In this experiment we also found a slight decrease in C1q activity with 10% FCS at 37 °C, probably due to the interference of traces of FCS on the hemolytic assay of C1q. The inhibitory effect of FCS or GPS on C1 in supernatants was similar to the mode of interaction of $C\bar{1}$ INH with purified $C\bar{1}$. In earlier experiments, we showed that purified $C\bar{1}$ INH from guinea pig serum, in contrast to human $C\bar{1}$ INH (*Levy* and *Lepow* 1959; *Gigli* et al. 1968), was resistant to incubation at 56 °C and was not destroyed unless heated to 60 °C for 30 min (*Loos* et al. 1971, 1972b). Based on these data, we considered the inhibitory affect of 56 °C-inactivated GPS and FCS to be due to $C\bar{1}$ INH that remained active. Therefore, we assumed that the C1 molecules found in macrophage supernatants were mostly present in the activated form, $C\bar{1}$, since only this form is inhibited by the $C\bar{1}$ INH. Based on this observation, M 199 with the addition of streptomycin and penicillin (100 units/ml) but without serum was used for

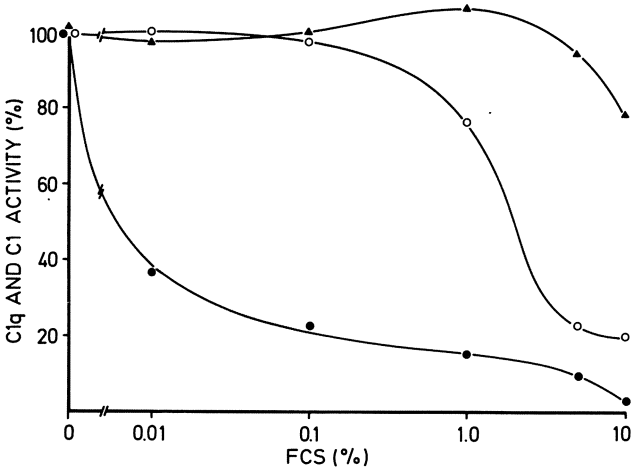

Fig. 4. Effect of heat-inactivated FCS (56 °C, 120 min) on C1q and $C\bar{1}$ activity in culture supernatants. FCS was added at different concentrations to supernatants removed from guinea pig macrophage monolayers, previously cultured serum free for 72 h. Hemolytically active C1 molecules were determined after incubation at 37 °C (●—●) and 0 °C (○—○) for 30 min; and C1q molecules after incubation at 37 °C (▲—▲) for 30 min. 100% = 4.9×10^8 effective C1 molecules/ml or 11.5×10^9 effective C1q molecules/ml respectively. (*Müller* et al. 1978a)

biosynthesis studies of C1 or C1q in human, guinea pig, and mouse peritoneal exudate cells.

5.4.2 Independent Synthesis of the C1 Subcomponents and Antigenic Similarities between Macrophage C1q and Serum C1q

5.4.2.1 Guinea Pigs

Male and female guinea pigs weighing 400–500 g were injected i.p. with 30 ml 2% starch gel in isotonic NaCl. Four days later, peritoneal exudate cells were harvested by washing the peritoneal cavity with 120 ml cooled (2°–4 °C) M 199, modified (Flow Laboratories Ltd., Bonn, W. Germany) without anticoagulant. The cells were collected by centrifugation (350 *g*) and resuspended in M 199 at a concentration of 1.25×10^6/ml. Aliquots (2 ml) of cell suspension were distributed into plastic Petri dishes (35-mm diameter, Greiner and Söhne, Nürtingen, W. Germany). After incubation in a humidified atmosphere containing 5% CO_2 for 90 min at 37 °C to allow attachment of adherent cells, the dishes were washed vigorously four times with PBS, pH 7.3. The macrophages were then cultured in M 199 (2 ml/dish), in the absence of serum, with addition of streptomycin and penicillin (100 units/ml).

Morphology. At the beginning of each experiment, monolayers consisted of 90%–96% of cells that had the morphological characteristics of macrophages and were able to form rosettes with and phagocytose IgG or C3-coated SRBC. At time zero and at different intervals thereafter supernatants from six replicate cultures were pooled, centrifuged to remove any cell fragments, and stored at –70 °C. The monolayer cultures were washed twice in PBS, three of them were stained with trypan blue, and the adherent viable cells counted; the remaining three cultures were taken for DNA determination. At time zero, nearly all cells were viable. After 24 h, about 98% of the cells could be identified as macrophages, and most of them were well spread. The cells became increasingly round after 48–72 h in culture. After 96 h only a few macrophages were well spread and 90% of the adherent macrophages were viable.

DNA content and the number of viable cells per dish were examined over the time course of incubation (see Table 1). From these data, it was calculated that 1 µg DNA

Table 1. Macrophage viability and DNA content during serum-free incubation of monolayer cultures[a]

Incubation (h)	DNA content (µg)	Viable cells/dish ($\times 10^{-5}$)
0	16.5 ± 1.6	14.1 ± 0.1
24	14.9 ± 1.8	11.8 ± 1.4
48	14.2 ± 1.7	9.4 ± 1.1
72	9.4 ± 2.3	6.7 ± 1.5
96	6.9 ± 2.5	4.9 ± 1.9

[a] Macrophages were cultured on a glutaraldehyd cross-linked BSA-covered surface in plastic dishes (35 mm diameter) without changing of medium. The values are means ± S.D. from five experiments. (*Müller* et al. 1978a)

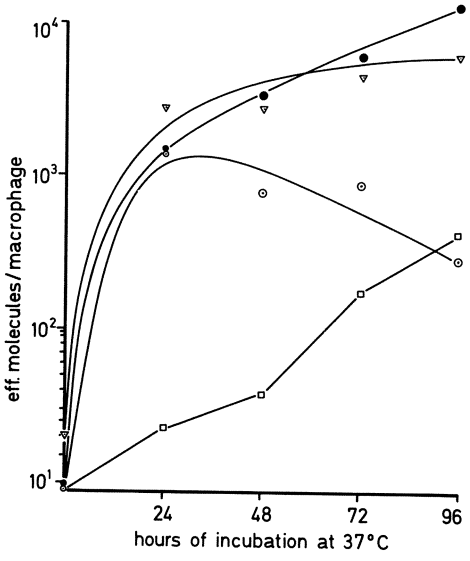

Fig. 5. Kinetics of C1 (□—□), C1q (●—●), C4 (⊙—⊙), and C2 (▽—▽) production by serum-free cultured guinea pig peritoneal macrophages. (*Müller* et al. 1978a)

correlated 0.75×10^5 cells. The number of effective molecules of the different C components, found at various times in the supernatants, was always related to the number of cells counted at time zero.

In the following experiments with C1, C1q, C4, and C2, the effective molecules were determined in the supernatant from serum-free-cultured macrophage monolayers according to *Rapp* and *Borsos* (1970). The components C4 and C2 were previously known to be synthesized by primate and guinea pig macrophages (*Stecher* et al. 1967c; *Littleton* et al. 1970; *Wyatt* et al. 1972). Synthesis of C2 by human monocytes was reported by *Einstein* et al. (1976) and *Littman* and *Ruddy* (1977). As shown in Fig. 5, C1q, C4, and C2 activities were detected after 24 h. Compared with C1q, C4, and C2, relatively few effective C1 molecules were found during the 96-h incubation period. C1q production differed from that of whole C1, both in rate and in quantity, indicating an independent synthesis of C1 subcomponents. It was calculated that the macrophages synthesized and secreted 9.3×10^3 hemolytically effective C1q molecules/cell/h and 3.1×10^2 effective C1 molecules/cell/h. In this experiment, the ratio of C1q to C1 was 30:1.

The discovery of selective complete C1r and C1q deficiencies confirms the interpretation that the individual subcomponents of C1 are synthesized independently (*Stroud* et al. 1970; *Pickering* et al. 1970; *Day* et al. 1972; *Berkel* et al. 1979; Loos et al. 1980b).

In testing the hemolytic activity of C1r and C1s, it was found that C1s activity was slightly increased over whole C1 activity, whereas C1r activity corresponded to C1 activity. Since all three subcomponents are necessary for measuring C1 activity, C1r seems to be the limiting component in the reassembly of macrophage-derived C1. The findings of *Reid* and *Solomon* (1977), which were confirmed in our laboratory, showed that fibroblasts produce higher amounts of C1r and C1s than C1q. In our experiments, the ratio of C1q to C1 in macrophage supernatants varied from 15:1 to 30:1. Macrophages harvested from untreated guinea pigs produced only 50%–60% of the C1q and C1 compared with starch gel pretreated animals, indicating that the rate of synthesis and release of C1 sub-

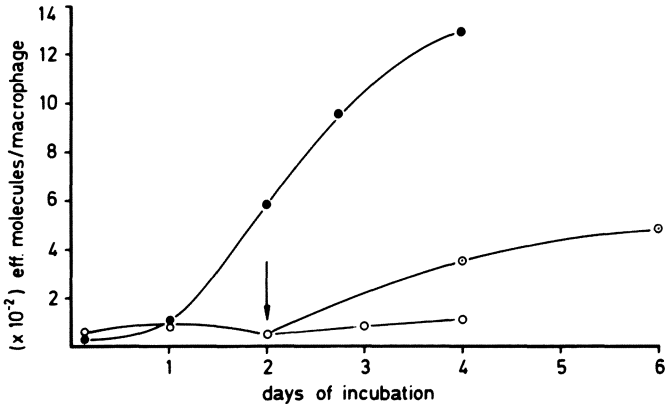

Fig. 6. Effect of cycloheximide on the production of C1 by guinea pig macrophages. Monolayers were incubated in serum-free medium without cycloheximide (●—●) or with 0.5 µg/ml cycloheximide (○—○). At the time indicated by the *arrow*, the monolayers were washed and a portion of the cycloheximide-cultured cells was then incubated in medium without cycloheximide (⊙—⊙)

components may depend on the state of activation of the macrophages. Further evidence on this subject, using mouse macrophages, will be discussed in a later section. However, the ratio of C1q to C1 in peritoneal macrophages of noninduced animals was similar to that found in monolayers of starch gel-induced ("elicited") macrophages.

To prove de novo synthesis, macrophage monolayers were incubated in M 199 containing cycloheximide (0.5 µg/ml) for 48 h. After washing the cultures twice with PBS, they were divided into two portions. The first was incubated again with cycloheximide (0.5 µg/ml) and the second in M 199 without inhibitor for another 48 h. Controls were

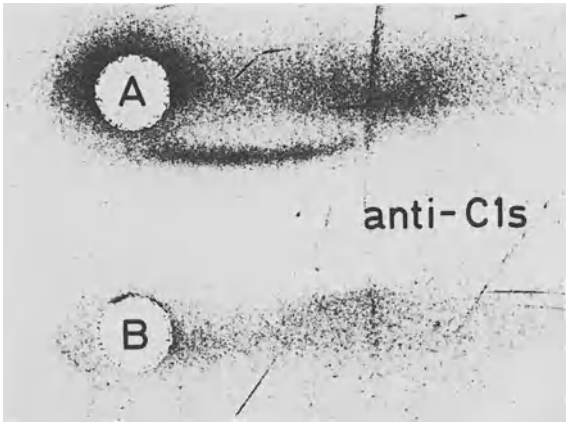

Fig. 7. Autoradiograph of immunoelectrophoretic patterns demonstrating incorporation of ^{14}C-labeled amino acids into C1s. Wells were filled with dialyzed, concentrated supernatants obtained from guinea pig macrophage monolayers incubated in the presence of radiolabeled amino acids (2.5 µCi/ml) for 72 h without (*A*) or with (*B*) cycloheximide (0.5 µg/ml). The immunelectrophoretic patterns were developed with rabbit anti-guinea pig C1s. GPS was used as carrier. The anode is to the *right*. (*Müller* et al. 1978a)

washed and incubated in M 199 only. In the presence of cycloheximide, production of C1 and C1q was inhibited by 99% and 87% respectively (Fig. 6). After removal of cycloheximide, production was partially restored (43% and 61%, respectively). The viability of monolayers with cycloheximide was about 20% less than in controls. When puromycin (1 µg/ml) was used, instead of cycloheximide, similar results were obtained. In controls, it was demonstrated that neither cycloheximide nor puromycin had any effect on the stability of C1 or C1q activity.

To prove further the de novo synthesis of C1s in vitro, a modification of the method of *Hochwald* et al. (1961) was used (see *Müller* et al. 1978a, for experimental details) for incorporation of ^{14}C-labeled amino acids into the newly synthesized protein. Fig. 7 shows that radiolabeled amino acids were incorporated into a protein immunochemically identical to C1s. No incorporation was detected when 0.5 µg/ml cycloheximide was added to the culture medium. In control experiments, nonspecific binding of radioactive amino acids to unlabeled C1s or to carrier protein was excluded.

Since C1q is a collagen-like molecule consisting of relatively high amounts of hydroxyproline and hydroxylysine, de novo synthesis of C1q was assessed by incorporation of ^{14}C-labeled proline (*Loos, Reske, Golan,* and *Heinz,* manuscript in preparation). After incubating guinea pig peritoneal macrophages for 4 h with [^{14}C]-proline, a Nonidet P40 (NP40) extract of the cells was then incubated with monospecific guinea pig antiserum to C1q (*Hitschold* et al. 1981). The immunoprecipitate was analyzed on long, calibrated SDS polyacrylamide gradient gels (5%–20%) in the absence and presence of reducing agents. Under nonreducing conditions, the major portion of labeled protein did

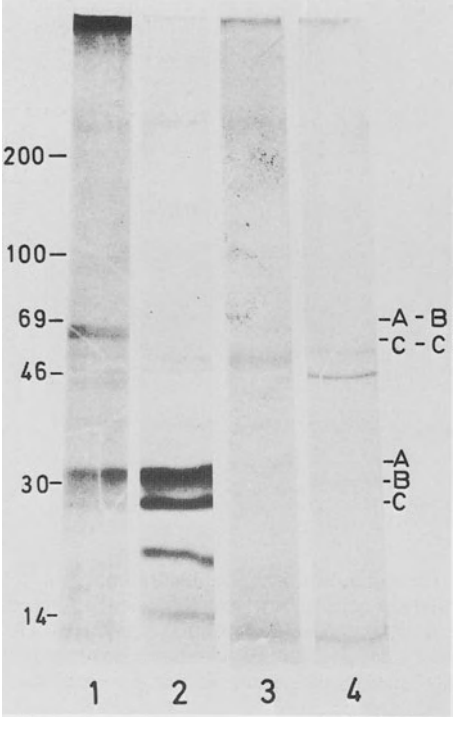

Fig. 8. Comparison of autoradiographs of unreduced (*1*) and reduced (*2*) [^{14}C]proline-labeled proteins from guinea pig peritoneal macrophages. Starch gel-induced guinea pig peritoneal macrophages were labeled for 4 h at 37 °C. After washing, the cells were treated with Nonidet P40 (NP40). A solution of cold carrier C1q (10 µg C1q/ml), prepared according to Hitschold et al. (1981), was added. The cell extract was mixed with excess heat-inactivated rabbit anti-guinea pig C1q and incubated at 4 °C overnight. The immunoprecipitate was washed and electrophoresed on SDS PAGE in the absence (*1*) and presence (*2*) of dithiothreitol. Normal GPS treated in the same way was used as control (*lane 3,* nonreduced; *lane 4,* reduced). Standards were run under reducing conditions. A-B, C-C, and A, B, and C correspond to what are thought to be the dimers and monomers, respectively, of GPS C1q. The gel was exposed to an X-ray film for 2 weeks at -70 °C

not enter the gel (Fig. 8). Two faint bands were detected with molecular weights of 65 000 and 63 000, and three faint bands were detected with molecular weights of 34 000, 32 000, and 25 000. The higher-molecular weight bands may represent the dimers of A-B (65 000) and C-C (63 000) chains of C1q. The lower-molecular-weight bands may represent monomers of A- (34 000), B- (32 000) and C- (25 000) chains of C1q. Individual polypeptide chains became more prominent when the immunoprecipitates were analyzed under reducing conditions. From an experiment done in duplicate, mean values of 31 300 for chain A, 28 700 for chain B, and 24 500 for chain C were obtained. These values are almost identical to those found for guinea pig serum C1q (*Hitschold* et al. 1981): A-chain 30 200, B-chain 28 200 and C-chain 24 000. In addition to the above mentioned polypeptides, additional prominent bands with incorporated radiolabeled proline can also be seen: one band corresponded to molecular weight 19 500 and another to 16 000. These proteins might be linked by disulfide bridges to the A-, B-, and/or C-chain of C1q since they are not detectable under nonreducing conditions. The linkage of A-, B-, and C-chains to additional proteins may explain why the higher-molecular-weight C1q protein of macrophages does not enter the gel under nonreducing conditions. Since only 4 h were used for the incorporation of ^{14}C-labeled proline, after disruption of the cells, the immunoprecipitated material may represent intracellular and membrane-associated C1q molecules that have not been secreted through the membrane. This high-molecular-weight protein may therefore represent a precursor form of macrophage-derived C1q, "pro-C1q". Furthermore, the faint bands found at 63 000 and 65 000 may originate from a secreted C1q molecule that dissociates under nonreducing conditions into the A-B and C-C dimers. This would indicate that, similar to collagen biosynthesis, secretion of C1q through the membrane and its subsequent release into the supernatant is accompanied by the cleavage and release of smaller peptides. Furthermore, the secreted A-B and C-C dimers are dissociated even further under nonreducing conditions into individual A-, B-, and C-chains. This observation is similar to that found for guinea pig serum C1q (*Hitschold* et al. 1981) and raises the question of whether A- and B- and both C-chains are linked by disulfide bridges, as proposed for human C1q by *Reid* and *Porter* (1976).

Due to the striking similarities of the subunits of macrophage-derived C1q and serum C1q, *Rabs* et al. (1981) isolated C1q from guinea pig macrophage supernatants and compared it to purified GPS C1q. C1q from macrophage supernatants was purified using ammonium sulfate precipitation, IgG-Sepharose affinity chromatography, and gel filtra-

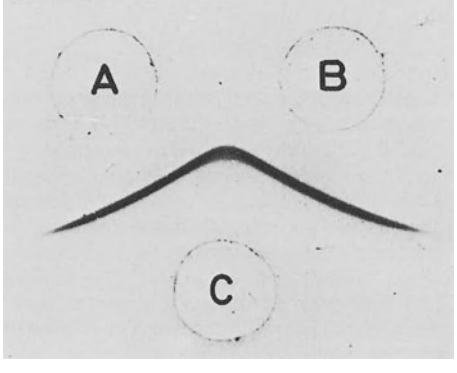

Fig. 9. Immunodiffusion of purified guinea pig peritoneal macrophage-derived C1q (*A*) and purified GPS-derived C1q (*B*) against a monospecific anti-guinea pig serum C1q antiserum (*C*) showing antigenic identity between both C1q molecules

tion on AcA 34 ultrogel. The purified molecule had a sedimentation rate of 11.7 S, which is comparable to 11.3 S observed for serum C1q (*Hitschold* et al. to be published), and an electrophoretic mobility in the gamma region. With rabbit anti-GPS C1q, the immunodiffusion analysis revealed antigenic identity between macrophage-derived and serum C1q (Fig. 9). This is the first evidence that macrophages are the site of synthesis of serum C1q.

5.4.2.2 Human

Monolayers of human peritoneal macrophages were prepared as follows: Ascitic fluid was obtained from a 60-year-old female patient suffering from severe cardiac insufficiency and from a 65-year-old patient with liver cirrhosis and portal hypertension. The fluid was withdrawn into cooled siliconized and sterilized glass bottles. Cells were collected by centrifugation, resuspended in M 199 at a concentration of 1.5×10^6 nucleated cells/ml, and then treated as previously described for guinea pig macrophages. Since the ascitic fluid contained a lower percentage of macrophages than the peritoneal exudate from stimulated guinea pigs, a second portion 1 ml of cell suspension, which had been kept at 8 °C, was added to the dishes. After incubation for 60 min, the procedure was repeated. The cultures were then incubated for 20 h and subsequently washed three times with PBS. Confluent monolayers were obtained consisting of about 95% macrophages. Monolayers were cultured for 72–96 h without changing the medium. The morphology of the peritoneal cells was observed after staining with May-Grünewald and Giemsa solution (Merck, Darmstadt, Germany), and viability was assessed using trypan blue stain.

Macrophage monolayers from human ascites fluid produced hemolytically active C1 molecules at nearly the same rate as guinea pig macrophages. Also, the ratio of human C1q/C1 molecules (12:1) after 72 h was similar, and the rate of synthesis of C1 was also reduced when the human macrophages were cultured in the presence of 0.5 µg/ml of cycloheximide (Fig. 10).

Double-staining experiments were performed with fluorescein-labeled antibodies to determine if C1q and C1s were found in the same or different cells. The IgG fraction of an antiserum to C1q, labeled with FITC, and the IgG fraction of an antiserum to C1s, labeled with RB 200, were used for this study (*Loos* et al. 1981). Fig. 11 shows the detection of C1s

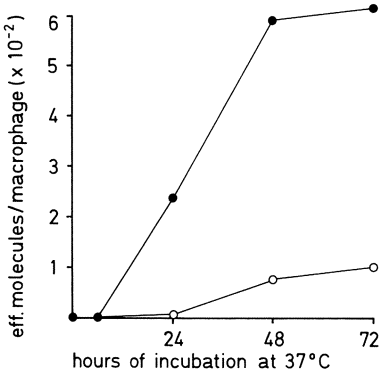

Fig. 10. Kinetics of C1 production by human peritoneal macrophages incubated in the absence (●—●) or presence (○—○) of cycloheximide (0.5 µg/ml). At time zero human macrophage monolayers contained about 1.3×10^6 cells/dish. The cells were obtained from human ascitic fluid by centrifugation. Morphologically and functionally, the cells were macrophages: they formed rosettes and phagocytosed EIgG and EAC1-3b. The cells were cultured for 72–96 h in M 199 containing 100 units penicillin and streptomycin/ml in the absence of serum. (*Müller* et al. 1978a)

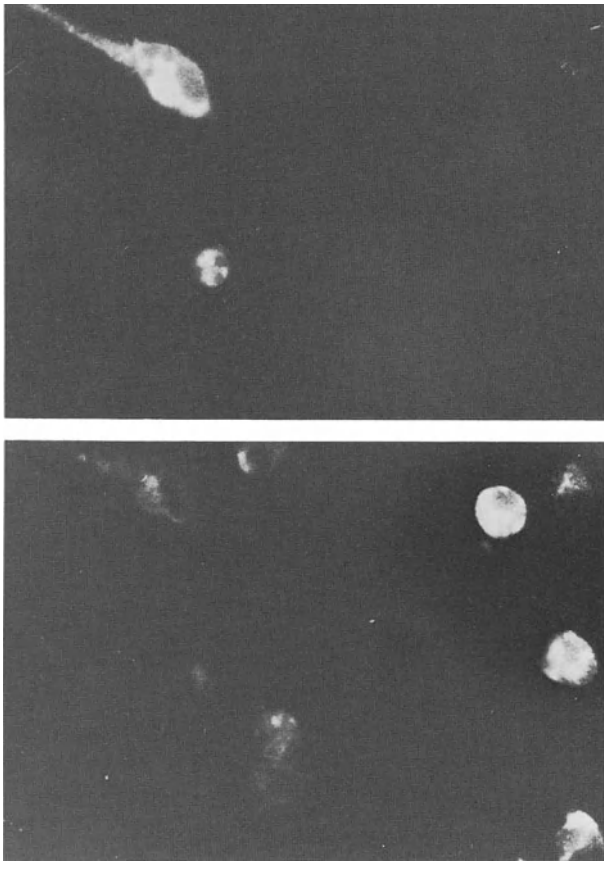

Fig. 11. Detection of C1q and C1s on human peritoneal macrophages by direct immunofluorescent double staining. *Upper panel*: Rhodamine-labeled anti-C1s, green excitation H 546 nm; *lower panel*: staining of the same section with FITC-conjugated anti-C1q, blue excitation 450–490 nm. Planopochramat 40 × 11.0 with oil immersion. Ektachrome 400. × 228

(upper panel) and C1q (lower panel) in the same field of cells. More cells stained for C1q than C1s, but in some cells both C1q and C1s were detected. Similar results were obtained with guinea pig macrophages (*Loos* et al. 1981). During the course of cell culture, the number of cells detected with RB 200 anti-C1s increased. It was also of interest that the number of cells detected with anti-C1q did not change, even after repeated washings, whereas the number of cells detected with anti-C1s was reduced to zero by washing.

The double-staining procedure was also used to detect C1q and C1s in individual cells of the mucosa of the gut, a possible site of synthesis. The cells were stained with RB 200-labeled anti-C1s and FITC-labeled anti-C1q. However, the interpretation of the staining pattern obtained on biopsy material taken during rectoscopic examination is no longer valid (*Loos* et al. 1981). It was discovered that the staining pattern changed from biopsy specimen to specimen. "Sometimes no cells stained for C1q or C1s." Since at that time no information was available concerning the medical status of the patients from whom the

biopsy material was taken, the staining patterns were reinvestigated with pathologically defined biopsy specimens. In this study, three groups of patients were selected: (1) patients with inflammatory colitis, (2) patients with ulcerative colitis, and (3) patients with Crohn's disease. Biopsy material from healthy individuals served as controls. The specimens were stained for C1q, C4, C3, IgA, IgG, and IgM. The results showed that in specimens from healthy individuals, single cells stained only with FITC-conjugated anti-IgA. Material from patients with Crohn's disease showed a reduced staining compared with the controls. However, the specimens from patients with inflammatory colitis and ulcerative colitis stained with all of the antisera tested. Based on these observations, this fluorescent method is now used as an adjuvant for differentiating ulcerative colitis and Crohn's disease (*Schneider, Loos, Störkel,* and *Gross,* manuscript in preparation).

The staining pattern observed with human peritoneal macrophages was confirmed using the immunoperoxidase method. In control experiments, staining due to endogenous peroxidase or unspecific binding of peroxidase-labeled anti-rabbit IgG was excluded (*Loos* et al. 1981).

5.4.2.3 Mouse

Peritoneal macrophages were obtained from approximately 8-week-old unstimulated NMRI mice by peritoneal lavage with 5 ml serum-free M 199. Monolayers of the peri-

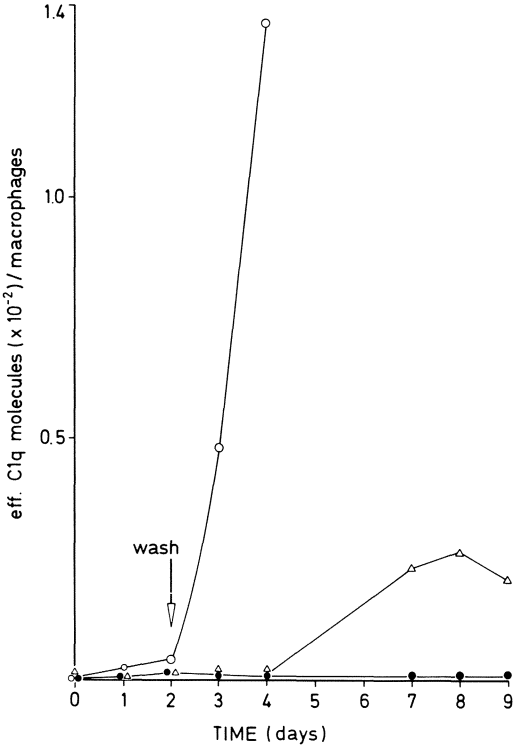

Fig. 12. Kinetics of C1q production by mouse peritoneal macrophages. Monolayers were incubated in serum-free medium without cycloheximide (○—○) or with addition of 0.5 µg cycloheximide/ml (●—●). At the time indicated by the *arrow,* cells were washed and part of the monolayer cultured with cycloheximide was incubated in medium without inhibitor (△—△)

toneal exudate cells were established in culture as described above for guinea pig peritoneal macrophages.

Monolayers of mouse peritoneal macrophages produced effective C1q molecules after a lag period of 48–72 h (Fig. 12). The mouse C1q activity was determined by reassociation experiments to macromolecular C1, using human C1r, C1s, and Ca^{++}, as discussed above. The detection of macromolecular C1 activity indicated that mouse C1q can interact with the human subcomponents C1r and C1s and is similar in this respect to guinea pig C1q, as described above. This confirms earlier observations by *Sassano* et al. (1972) that hybrids can be formed with C1 components derived from different species. However, determinations of the rate of synthesis were not undertaken since, in assays using C1 subcomponents from different species, the hemolytic activities of the individual subcomponents are, for the most part, underestimated (*M. Loos,* unpublished observations). The production of C1q was inhibited when the culture medium contained 0.5 µg/ml cycloheximide. After removal of the cycloheximide, production of C1q was partially restored. Again, as in untreated controls, after removal of the inhibitor, there was a lag phase of 48–72 h before C1q activity in the supernatants could be detected.

It has been documented that marked differences exist in biological activities between resident, elicited, and activated macrophages (*Karnovsky* and *Lazdins* 1978). It was reported above that during the study of C1 biosynthesis by guinea pig macrophages, resident macrophages harvested from untreated guinea pigs produced only 50%–60% of the C1q and C1 compared with macrophages harvested from starch gel-pretreated animals (elicited). It was therefore of interest to test the effects of different substances which stimulate peritoneal macrophages in vivo on the synthesis and secretion of C1q in vitro.

Eight-week-old NMRI mice were injected i.p. with a 1-ml aliquot of one of five substances described in the literature as stimulating lymphocyte activity: HSA, 0.5% dissol-

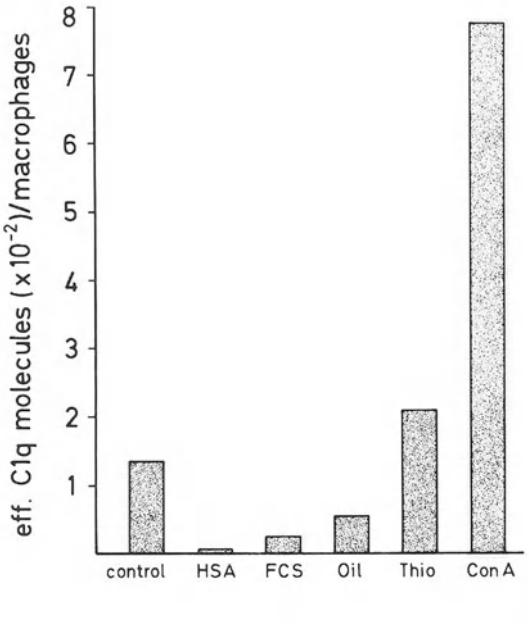

Fig. 13. Effect of in vivo stimulation on the synthesis and secretion of C1q in vitro by mouse peritoneal macrophages. Eight-week-old NMRI mice were injected i.p. with 1 ml of HSA (0.5% in physiological NaCl solution), thioglycolate, unheated FCS, incomplete Freund's adjuvant (oil), or Con A (40 µg/ml). Four days later, the cells were collected by i.p. lavage and adjusted after washing to 1×10^6 cells/ml. After 4 days culture at 37 °C, the cell supernatants were collected and the C1q activity was determined

ved in physiological NaCl; unheated FCS; incomplete Freund's adjuvant; thioglycolate broth; or Con A, 40 µg/ml. Physiological NaCl was used as a control. For each substance, three animals were used. Four days after injection, peritoneal exudate cells were collected, adjusted to 1×10^6 cells/ml, and transferred to petri dishes. After the cells had adhered to the surface of the plates (2 h, 37 °C), they were washed and incubated for 4 days in M 199 without serum. Supernatants were then collected and tested for C1q activity. Fig. 13 shows that the cells stimulated in vivo with Con A showed a fivefold increase in C1q activity compared with the control. In supernatants from cells pretreated in vivo with thioglycolate, only a slight increase of C1q activity was found, and cells pretreated in vivo with HSA, FCS, or adjuvant showed a reduced number of hemolytically active C1q molecules. Similar results were obtained when the experiments were repeated. It was concluded that in vivo stimulation of peritoneal macrophages with Con A, and to a minor extent with thioglycolate, leads to an increase of C1q release in vitro, probably due to an increased rate of synthesis. However, whether these substances have a direct effect on macrophages in vivo or whether the macrophages were activated as a result of lymphocyte-mediated stimulation is not certain at this time.

5.4.3 Similarities between the Biosynthesis of C1q and that of Collagen

The amino acid analysis of C1q revealed a high content of hydroxyproline (5%), hydroxylysine (2.1%), and glycine (17%). It was concluded that C1q is a collagen-like molecule (*Calcott* and *Müller-Eberhard* 1972; *Reid* et al. 1972). This interpretation was confirmed by the amino acid sequences found in the three different polypeptide chains. Each chain was shown to have a collagen-like region that was characterized by repeating X-Y-glycine triplets, often with hydroxylysine or hydroxyproline as Y, constituting about 40% of the molecule (*Reid* et al. 1972; *Reid* and *Porter* 1976). The occurrence of hydroxyproline, as found in the collagen molecule, has been shown in only a few proteins of vertebrates: elastin, C1q, and the tail structure of acetylcholinesterase (*Foster* 1982; *Rossenberry* et al. 1982).

The three polypeptide chains of the collagen molecule, each coiled into a left-handed helix, are twisted around each other into a right-handed triple-helical conformation. This conformation depends on the presence of hydroxyproline to stabilize the triple helix. Collagen polypeptides that lack hydroxylation fold into a triple-helical conformation at 25 °C or lower, but the helix formed is not stable at body temperature. These findings were made in studies on collagen biosynthesis (reviewed by *Prockop* et al. 1976; *Fessler* and *Fessler* 1978; *Prockop* et al. 1979; *Miller* and *Gay* 1982; *Kivirikko* and *Myllylä* 1982; *Prockop* and *Tuderman* 1982). Collagen synthesis consists of different post-translational steps, one of which is the hydroxylation of proline and lysine by proline 4- and proline 3- as well as lysyl-hydroxylases. Both types of enzymes are dependent on the same cofactors, namely ferrous ions, molecular oxygen, and α-ketoglutarate. Incubation of collagen-producing cells with 2,2'-dipyridyl, a chelator of ferrous ions (*Fessler* and *Fessler* 1974; *Harwood* et al. 1976), or with 3,4-dehydro-DL-proline, a proline analogue which is incorporated into the collagen chains but cannot be hydroxylated (*Kerwar* and *Felix* 1976; *Nolan* et al. 1978), prevents the folding of the individual chains into triple-helical conformation. This conformation is a prerequisite for secretion of the collagen molecule at 37 °C. Other studies with such inhibitors of post-translational hydroxylation have revealed that hydroxylation takes place before the interchain disulfide bonds are formed. The

synthesized procollagen is converted, after secretion, by at least two different proteases, into collagen molecules which spontaneously assemble into fibrils. The latter cannot be distinguished microscopically from the mature fibrils found in tissues.

Based on the biosynthesis studies of collagen, it was of interest to test whether the collagen-like C1q molecule underwent similar post-translational modifications. For these studies, guinea pig macrophage monolayers were incubated for 72 h in M 199 containing 10^{-4} *M* 2,2′-dipyridyl. The number of adherent viable cells was as high as in the controls (incubated in M 199), and the spreading of the macrophages was actually increased. Samples removed at different times were investigated for hemolytically active C1q. As a control to rule out inhibition of protein synthesis or nonspecific blocking of secretion by 2,2′-dipyridyl, the number of C4 and C2 molecules in the hemolytic assays was also determined. Fig. 14 shows that in the supernatants of macrophages cultured with 2,2′-dipyridyl, strikingly lower amounts of hemolytically active C1q were detected, whereas the number of C4 and C2 molecules was not significantly different from the control values during the time course of incubation. In subsequent experiments, the effect of 2,2′-dipyridyl was fully reversible when the inhibitor was removed by washing.

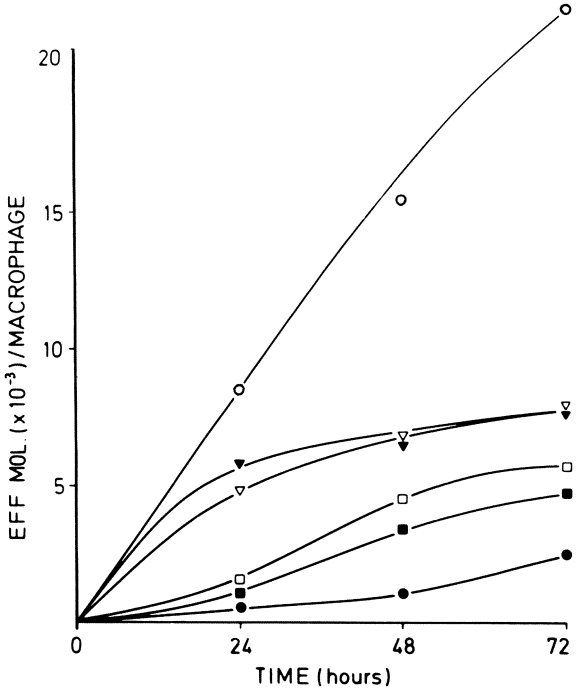

Fig. 14. Effect of 2,2′-dipyridyl on the secretion of C1q, C4, and C2 by peritoneal macrophages. Guinea pig peritoneal exudate cells were harvested by washing the peritoneal cavity with 120 ml cooled M 199, after 4 days of in vivo stimulation of the animals with 30 ml 2% starch gel. The cells were distributed into petri dishes and cultured for 72 h in serum-free M 199 (*open symbols*) or in M 199 containing 10^{-4} *M* 2,2′-dipyridyl (*closed symbols*). From 90% to 96% of the cells had the characteristics of macrophages. After the indicated times of incubation, the supernatants were tested for C1q (○—○; ●—●) C2-(▽—▽; ▼—▼), and C4-(□—□; ■—■) activity in hemolytic assays. The results were correlated to the number of macrophages per dish at time zero

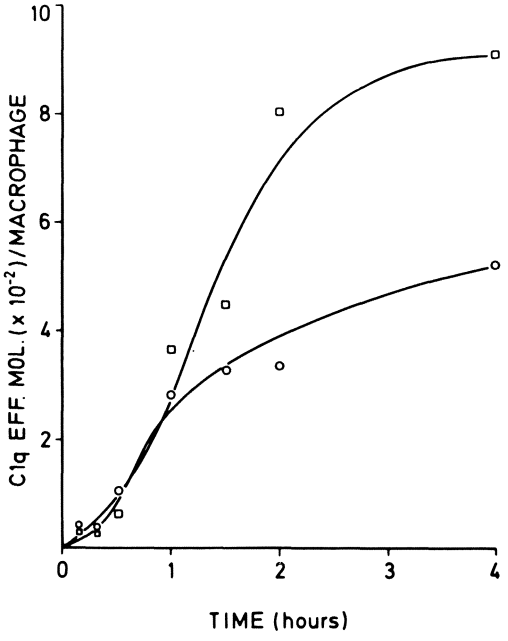

Fig. 15. Release of hemolytically active C1q molecules after preincubation of macrophage monolayers in M 199 for 24 h with (□—□) or without (○—○) 10^{-4} *M* 2,2'-dipyridyl. (*Müller* et al. 1978b)

During the first 6 h after removing the inhibitor, the increase of C1q activity exceeded the control values (*Müller* et al. 1978b). This observation was repeated. Monolayers were preincubated with 2,2'-dipyridyl for 24 h. After washing of the cells and adding fresh M 199 without the inhibitor, samples were removed at short intervals and analyzed for C1q activity. Fig. 15 demonstrates that after a short lag phase of 60 min, a higher rate of C1q secretion occurred from the cells preincubated with the inhibitor. After incubation for 2 h, the rate of C1q secretion returned to that of the control value. As mentioned above, hydroxyproline is crucial for triple-helical conformation under physiological conditions, and unhydroxylated procollagen molecules are not secreted. Thus, from the data in Fig. 15, it is possible that "pro-C1q" molecules, containing only peptides with random coil formation, might not be secreted, but rather accumulated intracellularly. This interpretation is supported by the finding that after removing the inhibitor, a marked increase in release of C1q over the control was measured. This could be due to rapid hydroxylation and secretion of the accumulated pro-C1q molecules. Therefore, 2,2'-dipyridyl has no effect on the synthesis of the C1q chains, but rather on the post-translational hydroxylation step. To investigate these interpretations further, the effect of temperature on the secretion of C1q in the presence or absence of 2,2'-dipyridyl or 3,4-dehydro-DL-proline was tested. Separate monolayers of guinea pig peritoneal macrophages were incubated for 48 h at 37 °C and 22 °C in M 199 in the presence or absence of the two inhibitors. During the time of incubation, samples were removed and assayed for hemolytic C1q activity. Fig. 16 shows that both inhibitors prevented secretion of C1q at 37 °C, whereas at 22 °C no significant differences were found compared to the controls. These experiments proved that the post-translational steps of hydroxylation and secretion of C1q were similar to those described for collagen synthesis. It was concluded that the collagen-like C1q molecule, similar to procollagen, must undergo a series of post-translational steps as

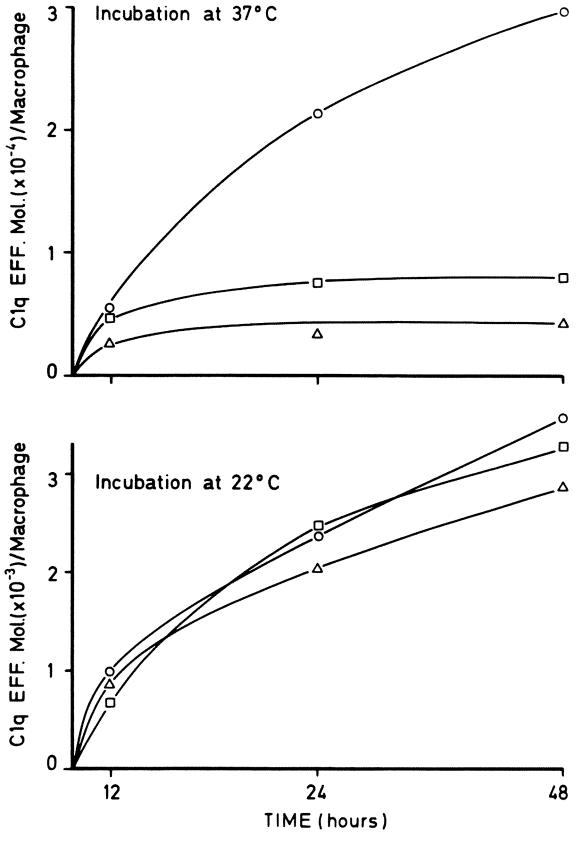

Fig. 16. Effect of 10^{-4} *M* 2,2′-dipyridyl (△—△) and 10^{-3} *M* 3,4-dehydro-DL-proline (□—□) on the secretion of hemolytically active C1q molecules by guinea pig peritoneal macrophages at different temperatures. M 199 without inhibitor was used as the control (○—○)

a prerequisite for secretion. Furthermore, based on the data obtained at 22 °C, it can be concluded that only at 37 °C hydroxylation is necessary in order to form a triple-helical C1q molecule for secretion. Hydroxylation and the subsequently occurring glycosylation of the hydroxylysyl residues do not influence the hemolytic activity of C1q. However, this interpretation does not rule out the importance of glycosylation of amino acids other than hydroxylysine for the functional activity of C1q.

Based on the striking similarities between collagen biosynthesis and the synthesis of the collagen-like C1q molecule, the following steps for C1q synthesis by macrophages are proposed (Fig. 17):

1. Biosynthesis of the individual A-, B-, and C-chains of C1q on ribosomes. This step is reversibly inhibited by inhibitors of protein synthesis, such as cycloheximide and puromycin.
2. Hydroxylation of proline and lysine residues by prolyl- and lysyl-hydroxylases. This step is prevented by the removal of ferrous ions with 2,2′-dipyridyl, or by incorporation of the proline analogue 3,4-dehydro-DL-proline, which does not undergo hydroxylation.
3. Assembly of interchain disulfide bonds. Based on data for collagen biosynthesis, this

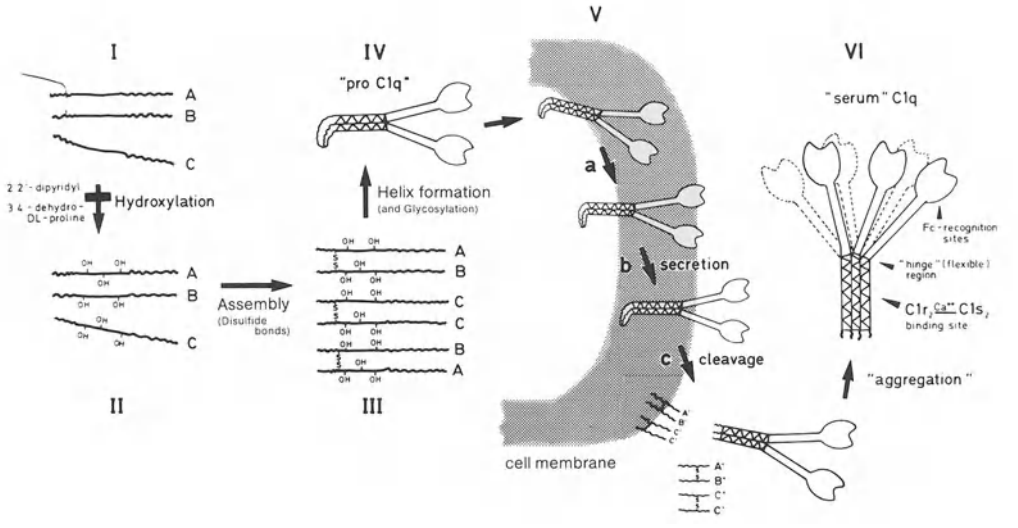

Fig. 17. Post-translational steps involved in the biosynthesis of C1q

step occurs even when hydroxylation is prevented. Also supporting this interpretation are the data obtained for C1q secretion at 22 °C in the presence of the inhibitors mentioned above.

4. Formation of pro-C1q. This involves folding of the collagen-like portions into a triple-helical conformation, a prerequisite for C1q secretion. Folding does not take place at 37 °C when hydroxylation is inhibited, and nonhydroxylated, non-triple-helical C1q molecules accumulate in the cell. Since there is no evidence for covalent linkage of the three C1q dimers, it is assumed that only the dimers, and no higher order structures, are formed intracellularly.
5. Secretion of C1q through the cell membrane. During secretion, C1q is detected with FITC-conjugated anti-C1q on the membrane of macrophages. The collagen-like portion of the C1q molecule may stabilize the globular portion within the membrane, similar to membrane-associated acetylcholinesterase (*Bon* et al. 1978; *Bon* and *Massoulie* 1978). The observed lag in C1q secretion may be due to this stabilization step.
6. Reassociation of the three C1q dimers to fluid-phase C1q. This molecule is now able to associate with C1r, C1s, and Ca^{++} to form macromolecular C1.

Since incorporation of ^{14}C-labeled proline provided evidence for a precursor C1q present in macrophages, the existence of pro-C1q during the intracellular steps 1 to 5 is postulated. During or shortly after secretion cleavage of pro-C1q occurs followed by an aggregation of C1q in the fluid phase, but not to the same extent as collagen. This statement is made because less than half of each C1q chain (approximately 80 residues) shows the collagen-like sequence, followed by approximately 110 residues of the globular sequence up to the C terminus (*Reid* and *Porter* 1976; *Porter* and *Reid* 1979).

6 Endogenous C1q as an Fc Receptor on Macrophage Membranes

6.1 Similarities between C1q and the Fc Receptor

C1q is the Fc-recognizing subcomponent of the first component of C (*Müller-Eberhard* and *Kunkel* 1961). However, the individual classes and subclasses of immunoglobulins in different species have different binding affinities for C1q and C1, and this is summarized in Table 2 for human, guinea pig, and mouse immunoglobulins. For human, IgM and IgG3 are the most efficient in binding C1 or C1q, followed by IgGI, and to a lesser extent, IgG2. IgG4 is similar to Fab or $F(ab')_2$ fragments, IgA, IgE, and IgD, in that it is not recognized by C1q. For the guinea pig, C1 and C1q bind to IgM, although some IgM molecules were found that did not bind C1 (*Linscott* and *Hansen* 1969). Also, IgG2 but not IgG1 was capable of binding C1q (*Sandberg* et al. 1971). In the mouse, IgG2a and IgM have been reported to bind C1 via C1q, but IgG1, IgA, and IgE do not (reviewed by *Spiegelberg* 1974).

Macrophages have been reported to express Fc receptors for different subclasses of Ig on their surface (reviewed by *Dickler* 1976; *Unkeless* et al. 1981). Comparing the heterogeneity of immunoglobulins with their binding behavior, it appears that those which bind to C1q are recognized by the same receptor population, e.g., human IgG1 and IgG3 both have strong binding capacities for C1q and both compete equally for the macrophage IgG1 receptor. On the other hand, mouse IgG2a binds to C1q but does not compete with the binding of mouse IgG1 (not recognized by C1q) to the Fc receptor. Similarly, guinea pig IgG1 does not interfere with binding of guinea pig IgG2 to macrophages.

Based on the striking ability of C1q and the Fc receptors to distinguish different Ig subclasses, and the fact that C1q is synthesized by macrophages, the question was asked whether endogenous C1q may serve, during the secretion phase, as a Fc-recognizing molecule in the macrophage membrane. In kinetic studies on the appearance of C1q activity in mouse peritoneal macrophage supernatants, it was found that C1q activity occurred in the fluid phase 48–72 h after initial culture in vitro. The Fc receptor activity of mouse macrophages was therefore examined and compared to C1q activity in the supernatants during the first hours of in vitro culture. The IgG fraction of a rabbit antiserum to SRBCs was used to coat SRBCs (EIgG) in order to detect Fc receptor activity. This anti-

Table 2. C1q binding capacity of immunoglobulin subclasses from different species[a]

Human	C1q binding	Guinea pig[b]	C1q binding	Mouse	C1q binding
IgG1	+	IgG2	+	IgG2a	+
IgG2	(+)	-		IgG2b	
IgG3	++	IgG1	-	IgG1	-
IgG4	-	-			
IgA	-	IgA	-	IgA	-
IgM	+	IgM[c]	+/−	IgM	+
IgE	-	IgE	-	IgE	-
IgD	-				

[a] Reviewed by *Spiegelberg* (1974); [b] *Sandberg* et al. (1971); [c] *Linscott* and *Hansen* (1969)

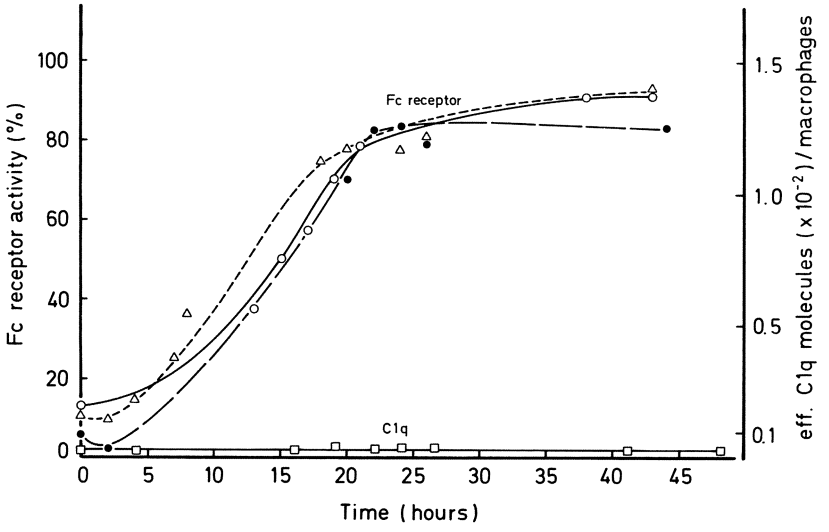

Fig. 18. Kinetics of appearance of Fc receptor activity for EIgG in macrophage monolayers after 2 h adherence at 37 °C. Three different experiments; ○—○, △---△, ●—●. C1q hemolytic activity was determined in the supernatants of the same macrophages monolayers (□--□)

body is also recognized by C1q. The kinetics in Fig. 18 represent three different experiments performed with peritoneal macrophages of 8-week-old NMRI mice. The individual experiments were performed at different times using new mice and newly prepared reagents. After a 2 h period of adherence and washing (time 0), the Fc receptor activity for EIgG was reduced to 10% (Fig. 18). The Fc receptor activity increased as a function of time and reached a plateau after 30–40 h incubation. During this time, no C1q activity was detected in the supernatants; findings similar to this were shown in earlier experiments (see Fig. 12). This suggested that during the procedures for preparing macrophage monolayers in vitro, most of the Fc receptor activity was removed from the cells but was restored within 40 h. After this time, C1q activity appeared in the supernatants. Thus it was concluded that after restoration of C1q in the membrane, its release occurs due to further incorporation of endogenous C1q into the membrane. This interpretation is supported by the observation of *Molenaar* et al. (1977) and *Kay* and *Douglas* (1981) that Fc receptor material from monocytes is released during the time of incubation into the supernatants. *Kay* and *Douglas* (1981) have suggested the use of monocyte culture supernatants as a source of monocyte receptor material. Such a procedure has been used for the purification of C3b material from culture supernatants (*Landen* and *Dierich* 1979). Furthermore, the delay observed in the secretion of C1q is similar to that for basement membrane collagen (*Grant* et al. 1972) and for the protein component of Ig (*Parkhouse* and *Melchers* 1971; *Melchers* 1971). In addition, preincubation of EIgG with various quantities of guinea pig peritoneal macrophage supernatants reduced the ability of the EIgG to form rosettes with macrophages in a dose-dependent fashion. The inhibition factor was shown to bind to EIgG. The addition of C1q-depleted serum to EIgG pretreated with macrophage supernatants caused complete lysis of these cells, indicating that macrophage-derived C1q was bound to EIgG (*Rabs* et al. 1981).

6.2 Inhibition of Expression of Fc Receptor Activity by Inhibitors of C1q Synthesis and by Anti-C1q F(ab')$_2$

The secretion of C1q is inhibited by 2,2'-dipyridyl and 3,4-dehydro-DL-proline by preventing hydroxylation of proline and lysine residues incorporated into the individual C1q chains (see Figs. 14–16). To test whether these inhibitors also influence the Fc receptor activity on macrophages, guinea pig peritoneal macrophages were incubated in M 199, and in M 199 without proline and lysine, containing 10^{-3} *M* 3,4-dehydro-DL-proline or 10^{-4} *M* 2,2'-dipyridyl, for 18–24 h at 37 °C. The cells were then washed in M 199 and tested for Fc receptor activity using EIgG. In order to make the Fc receptor assay more sensitive, the number of Fc sites on the SRBC was reduced (*Loos* et al. 1980a; *Loos,* 1982c). As demonstrated in Fig. 19, the inhibitor-treated macrophages expressed lower Fc receptor activity compared to the controls. The effects of both the inhibitors on the Fc receptor activity were more prominent when instead of optimally sensitized SRBC (anti-SRBC, 1:200), a lower concentration of antibody was used. From these experiments it was concluded that the Fc receptor, if not identical with C1q, is at least a hydroxyproline- and/or hydroxylysine-containing collagen-like molecule that depends for its expression on intracellular hydroxylation of proline and/or lysine residues. Antigenic identity between the postulated hydroxyproline- and hydroxylysine-containing Fc receptor and the hydroxyproline- and hydroxylysine-containing C1q molecule are supported by the selective, dose-dependent reduction of Fc receptor activity after incubation of peritoneal macrophages from NMRI mice and guinea pigs with anti-C1q F(ab')$_2$ (Fig. 20). In additio-

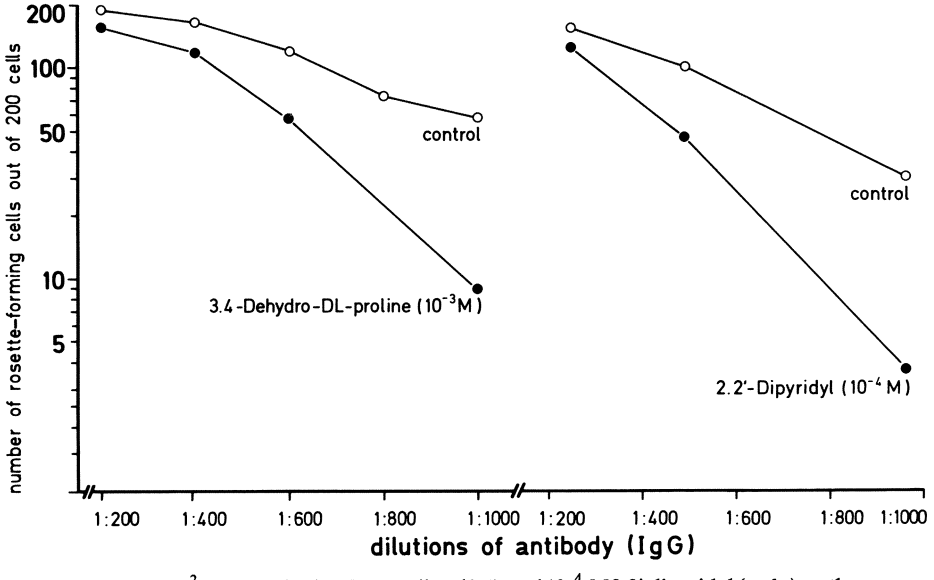

Fig. 19. Effect of 10^{-3} *M* 3,4-dehydro-DL-proline (*left*) and 10^{-4} *M* 2,2'-dipyridyl (*right*) on the expression of Fc receptor activity on guinea pig peritoneal macrophages. The macrophages were incubated with (●—●) or without (○—○) inhibitors for 24 h at 37 °C; the cells were then washed and rosette formation was tested using SRBCs sensitized with different amounts of anti-SRBC. (*Loos* et al. 1980a)

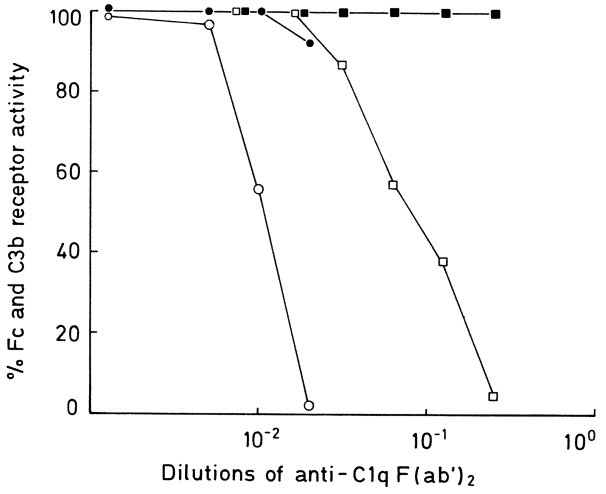

Fig. 20. Effect of anti-C1q F(ab')$_2$ on the Fc and C3b receptor activity of guinea pig (□, ■) and mouse (○, ●) peritoneal macrophage monolayers incubated in M 199 with different concentrations of anti-C1q-F(ab')$_2$ or in M 199 alone (control). After washing, the cells were assayed for Fc receptor activity with EIgG (*open symbols*) and for C3b receptor activity (*closed symbols*) with EIgMC1-C3b. The results were calculated as the number of rosettes (three or more E per macrophage) out of a total of 200 macrophages counted

nal controls, preincubation of the macrophages with anti-IgG F(ab')$_2$ did not influence Fc receptor activity. Anti-C1q F(ab')$_2$ however, had no effect on the C3b receptor activity of the macrophages. It is known that fibronectin binds to collagen as well as to C1q (*Menzel* et al. 1981a; *Isliker* et al. 1981, 1982). Since fibronectin is produced by macrophages (*Tsukamoto* et al. 1981), in additional experiments the possibility of fluid-phase C1q being bound by macrophages via fibronectin was excluded. These data indicate that immunochemically, as well as functionally, there is no difference between the subcomponent C1q and the Fc receptor activity for EIgG. Therefore it is assumed that C1q produced by macrophages represents the membrane-associated Fc receptor activity for those Ig that are also recognized by C1q in the fluid phase.

7 Endogenous C1q on Macrophages as a Receptor for Polyanionic Molecules

Natural and synthetic PAs have antiviral, antibacterial, and antitumor properties; they inhibit hormone action, inhibit or activate specific enzymes, and influence cell membranes, surface charges, and cell adhesion (reviewed by *Regelson* 1968a, b). Injection of DS 500 into mice caused loss of bactericidal resistance against *Listeria monocytogenes*. Mononuclear phagocytes in liver and spleen, and both circulating and fixed macrophages, showed uptake of electron-dense material in vivo. It was concluded that pronounced changes occurred in the mononuclear phagocytes after treatment with DS, and they were unable to express cellular resistance (*Hahn* 1974; *Hahn* and *Bierther* 1974). DS interacts with mouse macrophages in vitro to induce a dose- and time-dependent release of lysosomal enzymes. The selectivity of the release, which occurs without cell killing,

was shown morphologically and also by the lack of lactate dehydrogenase in the medium. The induction of enzyme release by various DS preparations correlated with the molecular weights and the degree of sulfation (*Schorlemmer* et al. 1977a, b; *Riches* and *Stanworth* 1981). The fusion of phagosomes and lysosomes in vitro was inhibited by DS, Liquoid, and suramin, whereas phagocytosis of yeast cells was enhanced by small doses of these PAs (*Bloksma* et al. 1980). The mechanism by which these polyanionic molecules modulate macrophage functions was not clear. However, it was assumed that PAs capable of activating the alternative C pathway may indirectly trigger macrophage activation by generation of the C3 split product C3b (*Schorlemmer* et al. 1977a; *Riches* and *Stanworth* 1981). PAs, however, have been described as activators of both the classical (C1, C4, C2) and the alternative pathways of C (*Hadding* et al. 1973; *Loos* and *Bitter-Suermann* 1976; *Burger* et al. 1977; *Loos* 1982b). The interaction of various PAs - including DS, Liquoid, and heparin - within the classical C pathway was due to direct binding to the subcomponent C1q (*Raepple* et al. 1976).

Since C1q is synthesized by macrophages and serves during secretion as an Fc-recognizing molecule in the membrane of macrophages, the question was asked whether the effects of PAs on macrophages may be due to their direct interaction with membrane-associated, endogenous C1q (*Loos* and *Schorlemmer* 1981, 1982).

Mouse (NMRI) peritoneal macrophages were cultured in the presence of different concentrations of DS 500 for 24 h at 37 °C. The supernatants were then tested for the lysosomal enzyme activity of *N*-acetyl-β-D-glucosaminidase, and the cells were tested for Fc receptor activity using EIgG. Figure 21 shows that lysosomal enzyme release increased as

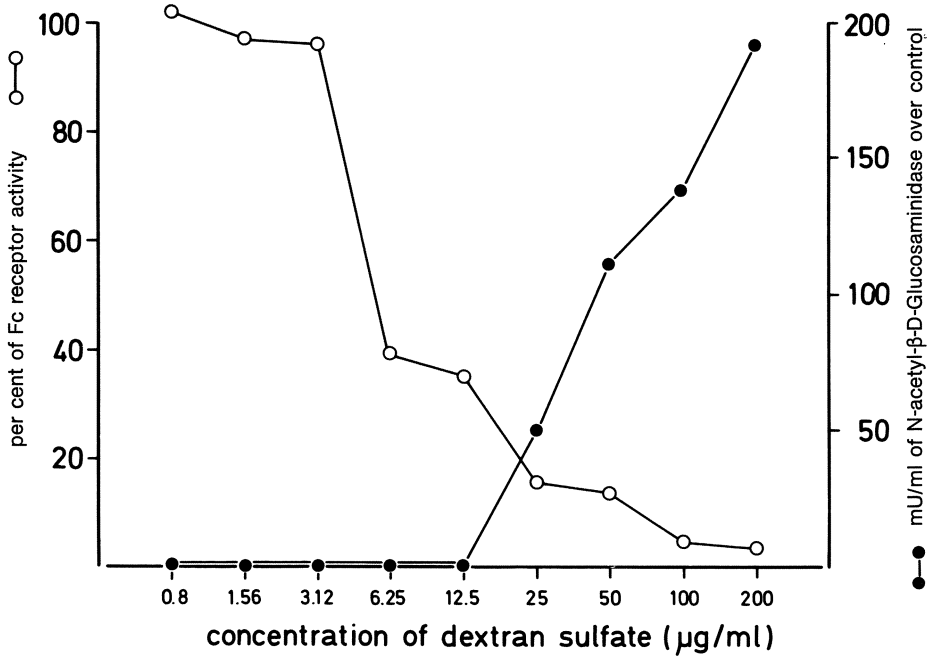

Fig. 21. Effect of DS on the secretion of lysosomal enzymes (e.g., *N*-acetyl-β-D-glucosaminidase, ●—●) and on the Fc receptor activity (○—○) of mouse peritoneal macrophages

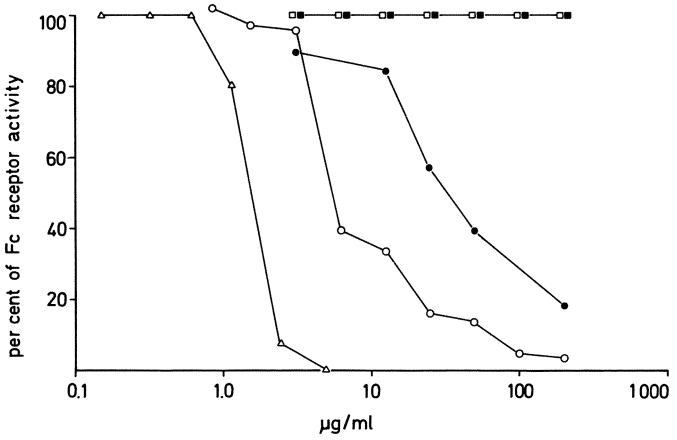

Fig. 22. Dose-dependent effect of different polyanionic substances (○—○, Dextran sulfate; □—□, Heparin; ■—■, Sp 54; ●—●, PVS; △—△, Liquoid) on the Fc receptor activity for EIgG of mouse (NMRI) peritoneal macrophages after 24 h at 37 °C

a function of DS concentration, as reported by *Schorlemmer* et al. (1977b). In contrast, the Fc receptor activity for EIgG decreased with increasing concentrations of DS. In additional experiments, pretreatment of DS with purified C1q abolished the capacity to induce lysosomal enzyme release. Incubation of DS with C2, C3, and C4 (also produced by macrophages) did not affect the DS-induced enzyme release.

To confirm the effect of DS on macrophage Fc receptor activity, dose-response curves were obtained using Liquoid, PVS, DS, heparin, and Sp54. Figure 22 shows that dose-dependent inhibition of the Fc receptor activity of mouse peritoneal macrophages (1.5×10^6/ml) was observed with Liquoid (50% inhibition; 1.5 µg/ml), DS (50% inhibition; 5.5 µg/ml), and PVS (50% inhibition; 33 µg/ml). No inhibition was observed with heparin and Sp54, both having relatively small molecular weights, approximately 17 000 and 2000 respectively. However, preincubation of macrophages with heparin before exposure to DS abolished the ability of DS to induce lysosomal enzyme release. Pretreatment of the heparin with C1q neutralized its effect on macrophages (unpublished data). From these findings, it appeared that a certain minimum molecular size was required to induce lysosomal enzyme release and/or to block Fc receptor activity. Similar results were described by *Schorlemmer* et al. (1977b).

The interaction of PAs with C1q is a temperature-independent, direct binding reaction (*Raepple* et al. 1976). In preliminary experiments, binding (e.g., Liquoid to macrophages) occurred within a few minutes. To test the effect of incubation time on the expression of Fc receptor activity, mouse peritoneal macrophages were incubated for 10 min, 60 min, or up to 18 h at 37 °C with Liquoid (5 µg/ 1.5×10^6 cells/ml). Fig. 23 shows that after 10 min, more than 40% of the Fc receptor activity was blocked. Even after removing the inhibitor by washing, inhibition up to 55% occurred for a further 2 h. However, all Fc receptor activity was restored after 4 h following removal of Liquoid. Similar findings were observed with an incubation period of 60 min. Fc receptor activity was inhibited 70% as before; it was further reduced after removal of Liquoid but started to increase after 4 h. After incubation of the cells for 18 h, all Fc receptor activity was in-

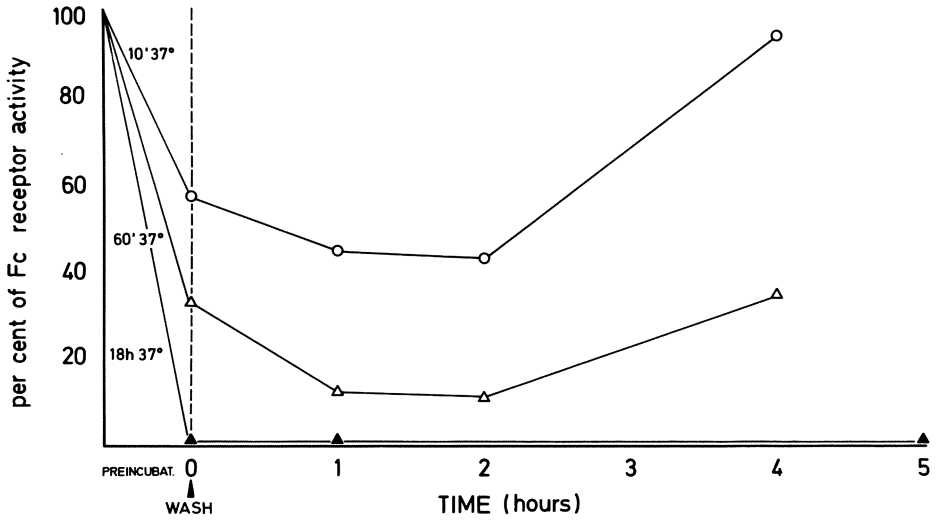

Fig. 23. Effect of different times of preincubation of mouse macrophages with Liquoid (1.5×10^6 cells/ml; 5 µg/ml) on the reappearance of Fc receptor activity after removal of the inhibitor by washing

hibited, but it reappeared after more than 30 h incubation at 37 °C. Therefore, even with a limited amount of Liquoid, inhibition of Fc receptor activity occurred within minutes. However, it is not known if Liquoid only adsorbs to the cells, or whether it is also taken up by the cells. The latter possibility may account for the further reduction of Fc receptor activity observed after removal of Liquoid by washing, and also for the lag phase before the reappearance of activity after 18 h incubation. Future experiments will attempt to answer these questions.

In earlier investigations (see Fig. 20), Fc receptor activity was inhibited by treatment of macrophages with anti-C1q $F(ab')_2$, indicating that endogenous C1q served as an Fc receptor for EIgG. Now there is evidence that the Fc receptor activity is blocked by different polyanionic molecules known to bind directly to fluid phase C1q. The assumption is that endogenous C1q is the receptor for these polyanionic molecules, leading to a loss of C1q-mediated Fc receptor activity and to an increase in lysosomal enzyme release. Therefore, it may be possible to prevent DS-induced enzyme release by pretreatment of cells with anti-C1q $F(ab')_2$. This hypothesis was tested in the next experiment. Mouse (NMRI) peritoneal macrophage monolayers were preincubated with an excess of anti-C1q $F(ab')_2$. Controls were preincubated with anti-C3 $F(ab')_2$ and anti-IgM $F(ab')_2$ for 1 h at 37 °C. After washing, the cells were exposed to 200 µg DS/ml for an additional 24 h at 37 °C to induce lysosomal enzyme release. The results (Fig. 24) indicate that preincubation of macrophages with anti-C1q $F(ab')_2$ abolished the subsequent DS-mediated release of *N*-acetyl-β-D-glucosaminidase. Preincubation of the cells with anti-C3 $F(ab')_2$ or anti-IgM $F(ab')_2$, however, did not prevent DS-induced enzyme release. The slight reduction of enzyme activity noted in the supernatants was also found when cells were incubated with albumin, indicating an unspecific protein binding effect. From this experiment it was concluded that polyanionic substances that induce lysosomal enzyme

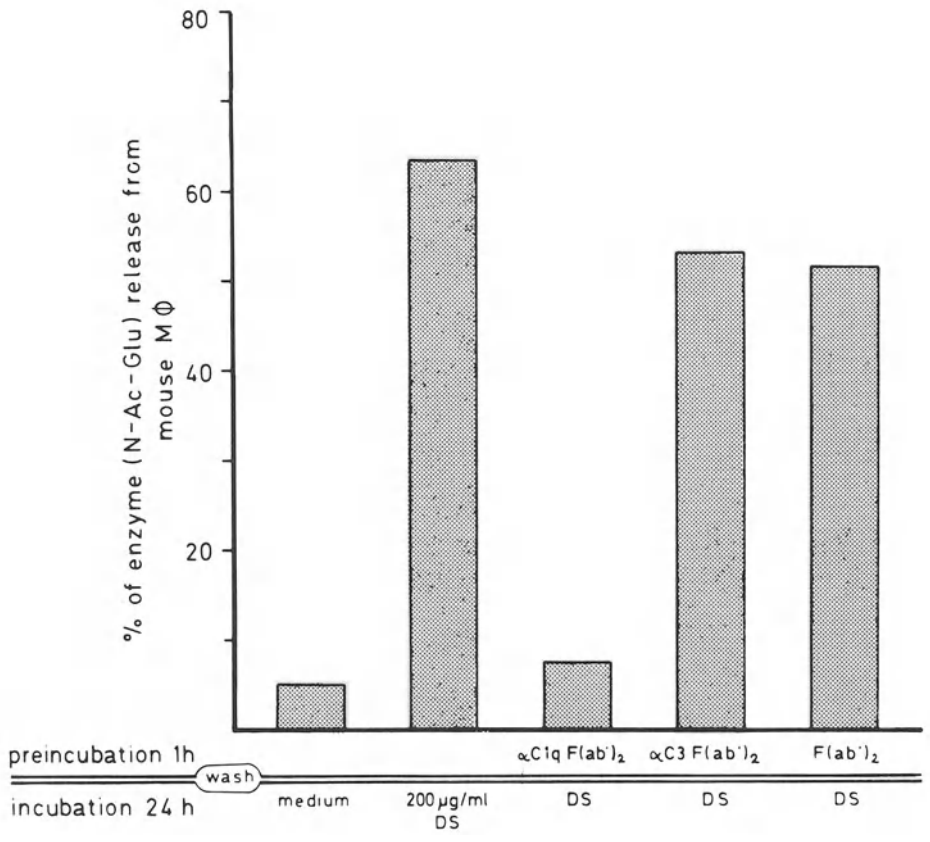

Fig. 24. Effect of preincubation (1 h, 37 °C) of mouse (NMRI) macrophages with different $F(ab')_2$ antibody preparations on the DS (200 µg/ml)-mediated release of *N*-acetyl-β-D-glucosaminidase

release and reduce Fc receptor activity are binding to membrane-incorporated, endogenous C1q.

7.1 Modulation of Fc Receptor Activity by Polyanions and Protamine

Polyanionic molecules inhibit the Fc receptor activity for EIgG on macrophages (see above). Macrophages, however, also possess receptors for C3b. Therefore it was of interest of determine if binding of polyanionic molecules to the C1q-mediated Fc receptor influences the expression of the C3b receptor activity on macrophage membranes. Mouse peritoneal macrophage monolayers were incubated for 30 min at 37 °C in M 199 on M 199 containing either Liquoid (10 µg/ml) or DS (200 µg/ml). After washing, the cells were tested for Fc (EIgG) and C3b (EIgMC1-C3b) receptor activities. Fig. 25 shows that treatment of macrophages with Liquoid and DS reduced the Fc receptor activity by as much as 90%, whereas the C3b receptor activity of these cells was not affected; it was actually increased 20% compared to the control cells incubated in M 199. The binding of

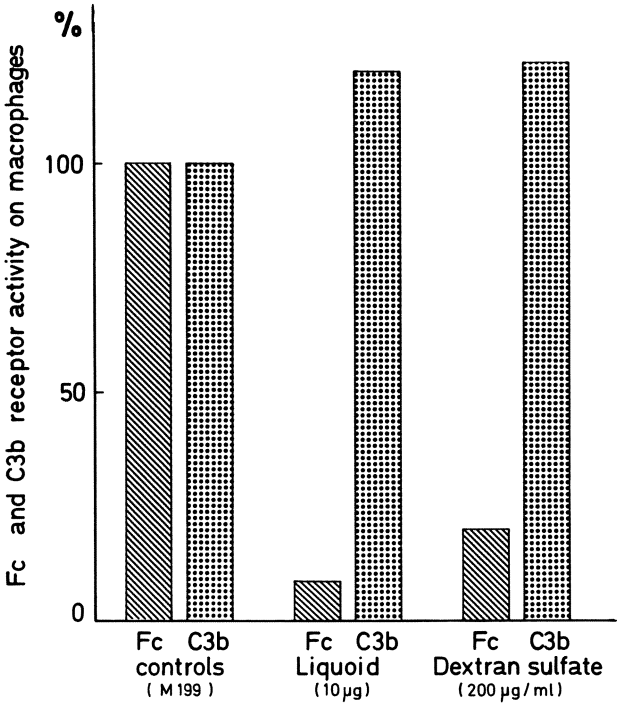

Fig. 25. Effect of incubation (30 min, 37 °C) with Liquoid (10 μg/ml) and dextran sulfate (200 μg/ml) on the expression of Fc and C3b receptor activity of mouse peritoneal macrophages

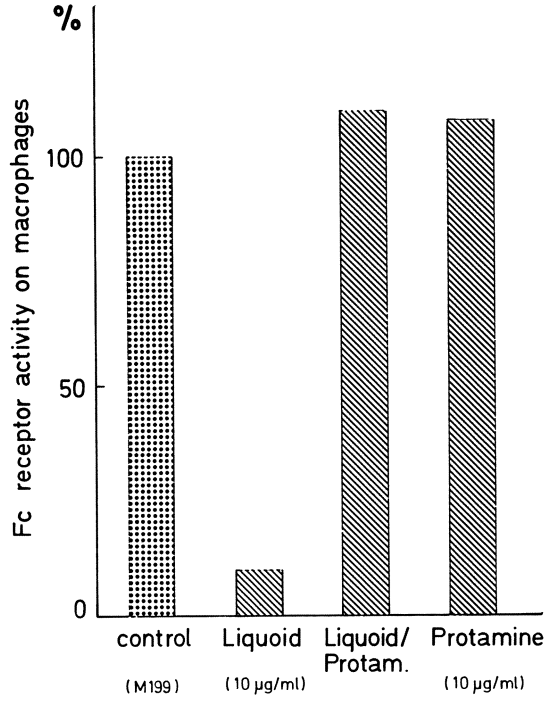

Fig. 26. Neutralizing effect of incubation (30 min, 37 °C) with protamine (10 μg/ml) on the Liquoid-induced inhibition of Fc receptor activity of mouse peritoneal macrophages. (Incubation with Liquoid (10 μg/ml) was for 30 min at 37 °C)

negatively charged PAs to C1q in the fluid phase is due to the basic nature of C1q (isoelectric point, pH 9.3). The inhibitory effect of Liquoid on the basic C1q molecule functioning as an Fc receptor should be reversed after incubating Liquoid-treated macrophages with protamine, another basic molecule. The results of such an experiment are shown in Fig. 26. Pretreatment of mouse macrophages with 10 µg/ml Liquoid leads to the inhibition of Fc receptor activity described above. However, this inhibition is totally reversed when the Liquoid-treated cells are incubated with protamine (10 µg/ml) for an additional 30 min at 37 °C. Protamine alone has no effect on the Fc receptor activity of macrophages. This experiment indicates that Liquoid, because of its polyanionic nature, binds to positively charged molecules.

Binding of optimally sensitized SRBCs (EIgG) to macrophages leads first to adherence, then to ingestion of the adherent cells (*Shaw* and *Griffin* 1981). Since PA treatment of macrophages prevents the adherence of EIgG, this may also result in decreased phagocytosis. Furthermore, since incubation of Liquoid-treated macrophages with protamine leads to reversal of the Liquoid effect, protamine treatment should also restore the phagocytic capacity of Liquoid-treated macrophages. The results shown in Table 3 confirm this interpretation. Liquoid-treated macrophages neither form rosettes with EIgG nor participate in phagocytosis. Protamine treatment restored both rosette formation and phagocytosis, indicating that the inhibitory affect of Liquoid is completely reversed in a short period of time.

In conclusion, polyanionic molecules trigger an increased release of lysosomal enzyme activity from macrophages by binding directly to the Fc receptor for EIgG, now shown to be identical with membrane-incorporated endogenous C1q. As a result of binding of polyanionic molecules to macrophages, Fc (EIgG)-mediated rosette formation and phagocytosis are inhibited. Both functions are restored by treatment of the macrophages with protamine, confirming the cationic character of C1q-associated Fc receptor activity.

Table 3. Effect of Liquoid on Fc-mediated rosette formation and phagocytosis and its reversal by protamine

Treatment		Fc rosettes per 200 macrophages[a]	Phagocytosis index[a]
1	2		
M 199	M 199	145.0	2.77
M 199	Liquoid (10 µg/ml)	32.8	0.94
Liquoid (10 µg/ml)	Protamine (10 µg/ml)	142.2	2.18
M 199	Protamine (10 µg/ml)	155.8	2.62

[a] Mean values of three different experiments

Peritoneal macrophages were treated with medium M 199 or with M 199 containing Liquoid (10 µg/ml) for 30 min at 37 °C (treatment 1). The cells were washed twice and incubated with either M 199, or M 199 containing Liquoid (10 µg/ml) or protamine (10 µg/ml) for an additional 30 min at 37 °C (treatment 2). After washing, the Fc receptor activity was tested by rosette formation with EIgG after 10 min at 37 °C and the Fc-mediated phagocytosis was determined after 60 min at 37 °C. The index of phagocytosis was determined as follows: 500 macrophages were examined under the microscope for the number of phagocytosed erythrocytes (E) per cell and divided into four groups: (a) no E, (b) 1–2 E, (c) 3–4 E, and (d) 5 and more E per cell. The ratio of number of phagocytosed E: total number of cells counted (500) was calculated and is given

8 Summary and Concluding Remarks

The macromolecular C1 complex is composed of three distinct subcomponents; C1r, C1s, and the collagen-like C1q. Biosynthetic studies with human, guinea pig, and mouse peritoneal macrophages revealed that the individual subcomponents are synthesized independently. De novo synthesis by macrophages was shown by:

1. A net increase of biologically active C1q and C1 in kinetic studies of synthesis and secretion. The C1q activity in the supernatants exceeded the activity of macromolecular C1 in ratios that varied from 15:1 to 30:1.
2. Incorporation of radiolabeled amino acids into immunochemically identified C1s and C1q proteins.
3. Inhibition of the appearance of functionally active C1q and C1 by inhibitors of protein synthesis, e.g., cycloheximide. The inhibition was reversed after removal of the inhibitor.
4. Double-labeled immunofluorescence studies, using RB 200-labeled anti-C1s and FITC-labeled anti-C1q, were consistant with the results of the functional studies. Individual cells were stained with anti-C1q, anti-C1s, or with both antisera. The number of cells stained with anti-C1q exceeded that of cells stained with anti-C1s.

The biosynthesis of the individual subcomponents was established after determining the proper culture conditions. Culture medium containing heat-inactivated FCS inhibited the functional analysis of C1, probably due to the presence of $C\bar{1}$ INH, which is not destroyed in FCS or GPS at 56 °C.

The secretion of functionally active C1q increased after in vivo i.p. stimulation of mice with Con A, and to a lesser extent with thioglycolate. In pulse-chase experiments it was found that ^{14}C-labeled proline was incorporated into the collagen-like C1q molecule. Under nonreducing conditions, SDS PAGE analyses revealed the existence of a pro-C1q which did not enter the gel.

Under reducing conditions the A-, B-, and C-chains had molecular weights similar those of the purified C1q chains. The gel pattern showed additional protein bands with [^{14}C]-proline incorporation. Since these bands were not detected upon analysis of fluid-phase C1q, it was considered likely that these molecules were linked to intracellular pro-C1q and were cleaved during or shortly after secretion (similar to the formation of collagen from procollagen).

Inhibitors of collagen biosynthesis, known to inhibit the post-translational hydroxylation of proline and lysine residues, were as effective inhibitors of C1q synthesis and secretion as described for collagen. Nonhydroxylated C1q was released at low temperature and found to be as hemolytically active as C1q in M 199-treated controls, indicating that hydroxylation of the lysine residues, and their glycosylation, are not important for the hemolytic activity of C1q. Based on the striking similarities between collagen and the collagen-like C1q molecule, different post-translational steps have been proposed (Fig. 17).

C1q, the Fc-recognizing subcomponent of C1, is synthesized by macrophages. It was detected in the cell membrane by immunofluorescence methods. Macrophages, on the other hand, also have Fc-recognizing functions by means of their Fc receptors. It was found that treatment of macrophages with inhibitors of C1q secretion results not only in a reduction of C1q release, but also in a decrease in Fc receptor activity. The C3b receptor

activity of these cells and the secretion of C2 and C4 were not affected. The inhibition of Fc receptor activity on macrophages by anti-C1q $F(ab')_2$, but not by anti-C3 $F(ab')_2$ or anti-IgM $F(ab')_2$, revealed antigenic identity between C1q and the Fc receptor of macrophages. Since only certain types of immunoglobulin subclasses are recognized by C1q (see Table 1) and the heterogeneity of Fc receptors for immunoglobulin subclasses is well established, it is proposed that endogenous C1q functions in the membrane of macrophages as an Fc receptor only for those immunoglobulins which are also recognized by C1q in the fluid phase.

Polyanions, known to bind directly to the basic C1q molecule, stimulated the release of lysosomal enzymes from macrophages, as described by other investigators. In addition, they completely inhibited the Fc receptor activity for EIgG. PA treatment, however, did not affect the C3b receptor activity of these cells. The inhibition of the Fc receptor

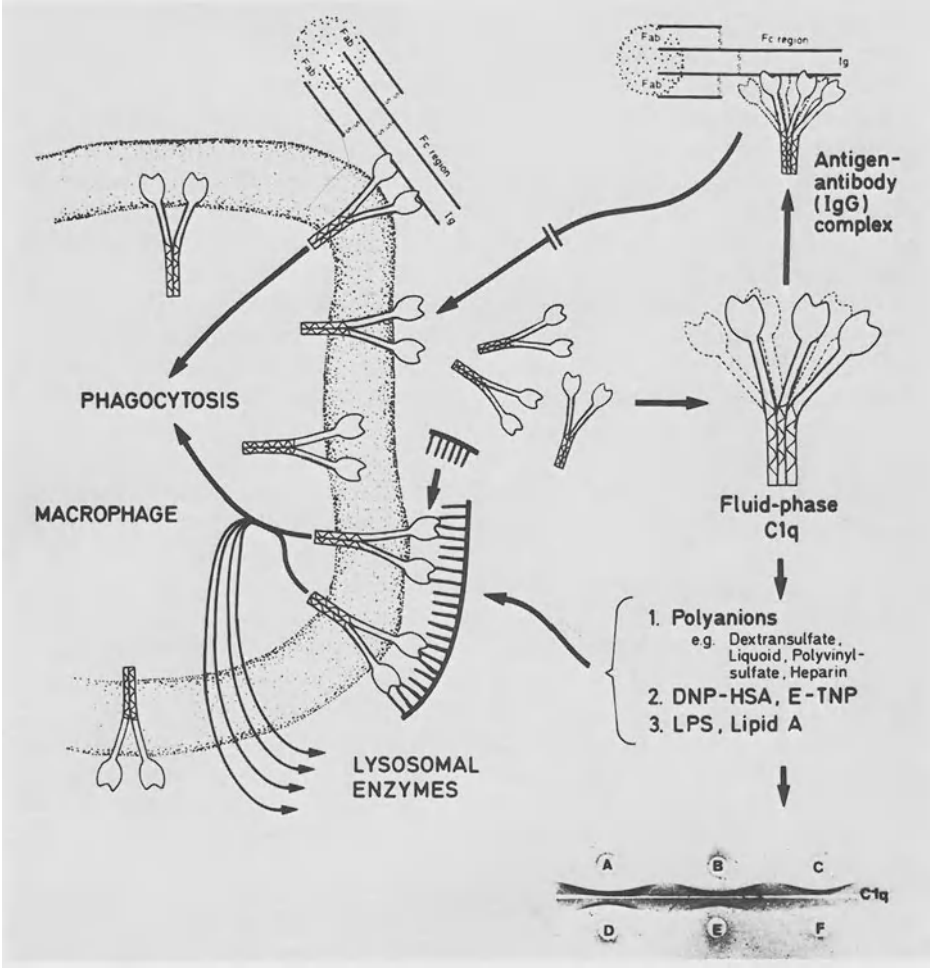

Fig. 27. Proposed functions of endogenous C1q as a receptor for Fc (EIgG) and polyanionic molecules on the macrophage membrane

activity and the release of lysosomal enzymes, however, is triggered only by high-molecular-weight PAs. Smaller sulfated molecules, such as heparin, neither inhibited Fc receptor activity nor induced lysosomal enzyme release. However, they prevented DS-induced enzyme release, indicating that they do bind to macrophages. Inhibition of DS-induced enzyme release by pretreating macrophages with anti-C1q $F(ab')_2$ confirmed the assumption that the Fc receptor and the receptor for PAs on macrophages are antigenically identical. PAs not only prevented Fc (EIgG)-mediated rosette formation, but also reduced the subsequently occurring phagocytosis of IgG-coated erythrocytes. However, treatment for a short time of PA-treated cells with the basic molecule protamine fully restored both rosette formation and phagocytosis. This indicated that PAs bind directly to C1q, their negatively charged sulfate groups binding to the positively charged membrane-associated C1q molecules.

Since C1q binds directly to lipid A and LPS of gram-negative bacteria, it may as a macrophage receptor also play an important role in triggering the uptake and the phagocytosis of these bacteria.

The proposed receptor functions of endogenous C1q for Fc and polyanionic molecules are summarized diagrammatically in Fig. 27.

Evidence is shown for the first time that C1q on macrophages has a receptor function of biological importance even before becoming part of macromolecular C1 in the humoral classical complement pathway. Since macrophages are essential for antigen processing and presentation to lymphocytes, as well as in triggering lymphocyte functions, the knowledge of the receptor functions of C1q in the membrane of macrophages aids in elucidating these important biological events. Preliminary results demonstrated that C1q in the membrane of macrophages may be involved in the early steps of antigen presentation of T-lymphocytes (*Hünig, Loos,* and *Schimpel*, manuscript in preparation).

Therefore, with the current knowledge of complement components, particularly in relation to macrophages, we can now add new information to *Metchnikoff's* early statement (1905) that "Alexine [complement] is nothing but a leucocytic product."

Acknowledgment. I thank my skilled co-workers, Drs. W. Müller, Rosemarie Storz, M.D. Golan, H.-P. Heinz and Felizitas Clas, as well as T. Hitschold, U. Rabs, and H. Hanauske-Abel, and my colleagues Drs. K. Reske and H.-U. Schorlemmer, all of whom made significant contributions to the data presented in this review.

In particular, the excellent technical assistance of Ms. Doris Bauer, Ms. Ingrid Alsenz, Ms. Gabriele Berg, Ms. Maria Latsch, and Ms. Heike Laubenheimer is gratefully acknowledged.

I also extend my deep appreciation to Professor Duane R. Schultz (Miami, Florida, USA) for our many fruitful discussions, for his suggestions, and for his thorough reading of this manuscript, and to Professor Paul Klein (Mainz, FRG) for his support and stimulating discussions. Finally, I wish to thank Professor Fritz Melchers (Basel, Switzerland) for encouraging me to write this review.

The research was supported by grants of the Deutsche Forschungsgemeinschaft, SFB 107, A2, Mainz, FRG.

References

Agnello V, Winchester RJ, Kunkel HG (1970) Precipitin reactions of the C1q component of complement with aggregated γ-globulin and immune complexes in gel diffusion. Immunology 19: 909–919

Al-Adnani MS, McGee JO'D (1976) C1q production and secretion by fibroblasts. Nature 263: 145–146

Almeda S, Rosenberg RD, Bing DH (to be published) The binding properties of human complement component C1q; interaction with mucopolysaccharides. J Biol Chem
Andrews JM, Baillie RD (1979) The enzymatic nature of human C1r: a subcomponent of the first component of complement. J Immunol 123:1403–1408
Arlaud GJ, Meyer CM, Colomb MG (1976) Use of an IgG fragment prepared with particulate plasmin to study the C1 binding and activation. FEBS Lett 66:132–136
Arlaud G, Reboul A, Colomb MG (1977a) Proenzymic C1s associated with catalytic amounts of C1r. Study of the activation process. Biochim Biophys Acta 485:227–235
Arlaud GJ, Reboul A, Meyer CM, Colomb MG (1977b) Purification of proenzymic and activated human C1s free of C1r. Effects of calcium and ionic strength on activated C1s. Biochim Biophys Acta 458:215–226
Arlaud GJ, Sim RB, Duplaa AM, Colomb MG (1979) Differential elution of C1q, $C1\bar{r}$ and $C1\bar{s}$ from human $C\bar{1}$ bond to immune aggregates. Use in the rapid purification of $C\bar{1}$ subcomponents. Mol Immunol 16:445–450
Asofsky R, Thorbecke GJ (1961) Sites of formation of immune globulins and of a component of C′3. J Exp Med 114:471–483
Assimeh SN, Bing DH, Painter RH (1974) A simple method for the isolation of the subcomponents of the first component of complement by affinity chromatography. J Immunol 113:225–234
Assimeh SN, Chapuis RM, Isliker H (1978) Studies on the precursor form of the first component of complement. II. Proteolytic fragmentation of C1r. Immunochemistry 15:13–17
Augener W, Grey HM, Cooper NR, Müller-Eberhard HJ (1971) The reaction of monomeric and aggregated immunoglobulins with C1. Immunochemistry 8:1011–1020
Bartholomew RM, Esser AF (1980) Mechanism of antibody-independent activation of the first component of complement (C1) on retrovirus membranes. Biochemistry 19:2847–2853
Bartholomew RM, Esser AF, Müller-Eberhard HJ (1978) Lysis of oncornaviruses by human serum. Isolation of the viral complement (C1) receptor and identification as p15E. J Exp Med 147: 844–853
Becker EL (1956) Concerning the mechanism of complement action. I. Inhibition of complement activity by diisopropyl fluophosphate. J Immunol 77:462–468
Becker EL (1967) The relationship of the structure of phosphonate esters to their ability to inhibit chymotrypsin, trypsin, acetylcholinesterase, and C′1a. Biochim Biophys Acta 147:289–296
Becker EL, Austen KF (1964) A comparison of the specificity of inhibition by phosphonate esters of the first component of complement and the antigen-induced release of histamine from guinea pig lung. J Exp Med 120:491–506
Bentley C, Zimmer B, Hadding U (1981) The macrophage as a source of complement components. In: Pick E (ed) Lymphokines 4. Academic Press, New York, pp 197–230
Berkel AI, Loos M, Sanal Ö, Mauff G, Güngen Y, Örs Ü, Ersoy F, Yegin O (1979) Clinical and immunological studies in a case of selective complete C1q deficiency. Clin Exp Immunol 38:52–63
Berkel AI, Loos M, Sanal Ö, Ersoy F, Yegin O (1981) Selective complete C1q deficiency. Report of two new cases. Immunol Lett 2:263–267
Betz S, Isliker H (1981) Antibody-independent interactions between Escherichia Coli J5 and human complement components. J Immunol 127:1748–1754
Betz S, Page N, Estrade C, Isliker H (1982) The effect of specific antibody on antibody-independent interactions between E. coli J5 and human complement. J Immunol 128:707–711
Bing DH (1969) Nature of the active site of a subunit of the first component of human complement. Biochemistry 8:4503–4510
Bing DH (1971) Purification of the first component of human complement by affinity chromatography on human γ-globulin linked to sepharose. J Immunol 107:1243–1249
Bing DH, Spurlock E, Bern MM (1975) Synthesis of the first component of complement by primary cultures of human tumors of the colon and urogenital tract and comparable normal tissue. Clin Exp Immunol 4:341–351
Bing DH, Andrews JM, Morris KM, Cole E, Irish V (1980) Purification of subcomponent C1q, C1r, and C1s of the first component of complement from Cohn fraction I by affinity chromatography. Prep Biochem 10:269–296
Bing DH, Almeda S, Isliker H, Lhav J, Hynes RO (to be published) Fibronectin binds to the C1q component of complement. Proc Natl Acad Sci USA

Bloksma N, Reuver de MJ, Willers JMN (1980) Influence on macrophage functions as a possible basis of immunomodification by polyanions. Ann Immunol (Paris) 131D:225–265

Bon S, Massoulie J (1978) Collagenase sensitivity and aggregation properties of Electrophorus acetylcholinesterase. Eur J Biochem 89:89–94

Bon S, Cartaud J, Massoulie J (1978) The dependence of acetylcholinesterase aggregation at low ionic strength upon a polyanionic component. Eur J Biochem 85:1–14

Bordet J (1896) Sur le mode d'action des sérums préventifs. Ann Immunol, Paris 10:193–219

Borsos T, Rapp HJ (1965a) Complement fixation on cell surfaces by 19S and 7S antibodies. Science 150:505–506

Borsos T, Rapp HJ (1965b) Hemolysin titration based on fixation of the activated first component of complement: Evidence that one molecule of hemolysin suffices to sensitize an erythrocyte. J Immunol 95:559–566

Borsos T, Rapp HJ, Mayer MM (1961) Studies on the second component of complement. I. The reaction between EAC′1,4 and C2: Evidence on the single site mechanism of immune hemolysis and determination of C′2 on a molecular basis. J Immunol 87:310–325

Borsos T, Rapp HJ, Walz UL (1964) Action of the first component of complement. Activation of C′1 to C′1a in the hemolytic system. J Immunol 92:108–112

Borsos T, Rapp HJ, Crisler C (1965) The interaction between carrageenan and the first component of complement. J Immunol 94:662–666

Borsos T, Loos M, Chapuis RM, Medicus R, Isliker H (1980) A novel way of relating the structure of C1q to the hemolytic activity of the first component of complement. Mol Immunol 17: 1415–1421

Brade V, Bentley C (1980) Synthesis and release of complement components by macrophages. In: van Furth R (ed) Molecular Phagocytes. Martinus Nijhoff Publ, pp 1385–1413

Bredt W, Wellek B, Brunner H, Loos M (1977) Interactions between *Mycoplasma pneumoniae* and the first component of complement. Infect Immunity 15:7–12

Bubb MO, Conradie JD (1976) The importance of quaternary structure in the expression of the C1-binding site of IgM. Immunology 31:893–902

Buchner H (1889a) Über die nähere Natur der bakterientötenden Substanz im Blutserum. Zentralbl Bakteriol 6:561–565

Buchner H (1889b) Über die bakterientötende Wirkung des zellfreien Blutserums. Zentralbl Bakteriol 5:817–823

Budzko DB, Müller-Eberhard HJ (1970) Cleavage of the fourth component of human complement (C4) by C1-esterase: Isolation and characterization of the low molecular weight product. Immunochemistry 7:227–234

Burger R, Loos M, Kossorotow A, Hadding U, Bitter-Suermann D (1977) Dual function of insoluble polyanions. In: Kallos P et al. (eds) Monographs in allergy. Karger, Basel, 12:78–81

Burton DR, Boyd J, Brampton AD, Easterbrook-Smith SB, Emanuel EJ, Novotny J, Rademacher TW, van Schravendijk MR, Sternberg MJE, Dwek RA (1980) The C1q receptor site on immunoglobulin G. Nature 288:338–344

Calcott MA, Müller-Eberhard HJ (1972) C1q protein of human complement. Biochemistry 11: 3443–3450

Chapuis RM, Isliker H, Assimeh SN (1977) Studies on the precursor form of the first component of complement. I. Isolation of the proenzyme forms of C1r and C1s. Immunochemistry 14: 313–317

Circolo A, Borsos T (1982) Lysis of hapten-labeled cells by anti-hapten IgG and complement: Effect of cell surface hapten density. J Immunol 128:1118–1121

Clas F, Loos M (1980) Killing of the S and Re forms of Salmonella minnesota via the classical pathway of complement activation in guinea pig and human sera. Immunology 40:547–556

Clas F, Loos M (1981) Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S- and Re-forms of Salmonella minnesota. Infect Immun 41:1138–1144

Clas F, Loos M (1982) The role of components of the outer membrane of gram-negative bacteria in the serum-bactericidal effect. In: Peters H (ed) Protides of the Biological Fluids. Pergamon Press, Oxford U.K., vol. 29, pp 317–320

Claus DR, Siegel J, Petras K, Osmand AP, Gewurz H (1977) Interactions of C-reactive protein with the first component of human complement. J Immunol 119:187–192

Colten HR (1972a) In vitro synthesis of a regulator of mammalian gene expression. Proc Natl Acad Sci 69:2233–2236
Colten HR (1972b) Ontogeny of the human complement system: In vitro biosynthesis of individual complement components by fetal tissues. J Clin Invest 51:725–730
Colten HR (1976) Biosynthesis of complement. Adv Immunol 22:67–118
Colten HR, Wyatt HV (1972) Biosynthesis of serum complement. In: Ingram DG (ed) Biological activities of complement. Karger, Basel, pp 244–255
Colten HR, Borsos T, Rapp HJ (1966) In vitro synthesis of the first component of complement by guinea pig small intestine. Proc Natl Acad Sci 56:1158–1163
Colten HR, Borsos T, Rapp HJ (1967) Efficiency of the first component of complement (C′1) in the hemolytic reaction. Science 158:1590–1592
Colten HR, Borsos T, Rapp HJ (1968a) Ultracentrifugation of the first component of complement: effects of ionic strength. J Immunol 100:808–813
Colten HR, Borsos T, Rapp HJ (1968b) Reversible loss of activity of the first component of complement (C′1) as a function of ionic strength. J Immunol 100:799–807
Colten HR, Gordon JM, Rapp HJ, Borsos T (1968c) Synthesis of the first component of guinea pig complement by columnar epithelial cells of the small intestine. J Immunol 100:788–792
Colten HR, Gordon JM, Borsos T, Rapp JH (1968d) Synthesis of the first component of human complement in vitro. J Exp Med 128:595–604
Colten HR, Silverstein AH, Borsos T, Rapp HJ (1968e) Ontogeny of the first component of sheep complement. Immunology 15:459–461
Conradie JD, Volanakis JE, Stroud RM (1975) Evidence for a serum inhibitor of C1q. Immunochemistry 12:967–971
Cooper NR, Morrison DC (1978) Binding and activation of the first component of human complement by the lipid A region of lipopolysaccharides. J Immunol 120:1862–1868
Cooper NR, Jensen FC, Welsh RM, Oldstone MBA (1976) Lysis of RNA tumor viruses by human serum: direct antibody-independent triggering of the classical complement pathway. J Exp Med 144:970–984
Csákó G, Suba EA, Herp A (1981) Similarities and dissimilarities between the binding ability of C1q and collagen. Clin Exp Immunol 44:181–190
Day NK, Gewurz H, Pickering PJ, Good RA (1970) Ontogenetic Development of C1q synthesis in the piglet. J Immunol 104:1316–1319
Day NK, Geiger H, Stroud RM, De Bracco ME, Mancado B, Windhorst D, Good RA (1972) C1r deficiency: An inborn error associated with cutaneous and renal disease. J Clin Invest 51: 1102–1108
DeBracco MME, Stroud RM (1971) C1r, subunit of the first complement component: Purification, properties, and assay based on its linking role. J Clin Invest 50:838–848
Dickler HB (1976) Lymphocyte receptors for immunoglobulin. Adv Immunol 24:167–215
Dodds AW, Sim RB, Porter RP, Kerr MA (1978) Activation of the first component of human complement (C1) by antibody-antigen aggregates. Biochem J 175:383–390
Einstein LP, Schneeberger EE, Colten HR (1976) Synthesis of the second component of complement by long-term primary cultures of human monocytes. J Exp Med 143:114–126
Fessler LI, Fessler JH (1974) Protein assembly of procallagen and effects of hydroxylation. J Biol Chem 249:7637–7646
Fessler JH, Fessler LI (1978) Biosynthesis of procollagen. Ann Rev Biochem 47:129–162
Fiedel BA, Rent R, Myhrman R, Gewurz H (1976) Complement activation by interaction of polyanions and polycations. II. Precipitation and role of IgG, C1q, and C1-INH during heparin-protamin-induced consumption of complement. Immunology 30:161–169
von Fodor J (1887) Die Fähigkeit des Blutes, Bakterien zu vernichten. Dtsch Med Wochenschr 13:745–747
Foster JA (1982) Elastin structure and biosynthesis: An overview. In: Cunningham LW, Frederiksen DW (eds) Methods in enzymology: structural and contractile proteins, part A 82: 559–570
Fothergill JE, Anderson WHK (1978) A molecular approach to the complement system. Curr Top Cell Regul 13:259–311
Frank M, Gaither TA (1970) Complement fixation by a single molecule of γG hemolysin. J Immunol 104:1458–1466

Ghebrehiwet B (1981) C1q inhibitor (C1gINH): Functional properties and possible relationship to a lymphocyte membrane-associated C1q precipitin. J Immunol 126:1837–1842

Gigli I, Austen KF (1969) Fluid phase destruction of $C2^{hu}$ by $C1^{hu}$. I. Its enhancement and inhibition by homologous and heterologous C4. J Exp Med 129:679–696

Gigli I, Ruddy S, Austen KF (1968) The stoichiometric measurement of the serum inhibitor of the first component of complement by the inhibition of immune hemolysis. J Immunol 100: 1154–1164

Gigli I, Porter RR, Sim RB (1976) The unactivated form of the first component of human complement, C1. Biochem J 157:541–548

Golan MD, Hitschold T, Loos M (1981) The reconstitution of human C1, the first complement component. Binding of C1r and C1s to C1q influences the C1q conformation. FEBS Lett 128:281–285

Golan MD, Burger R, Loos M (1982) Conformational changes in C1q after binding to immune complexes: detection of neoantigens with monoclonal antibodies. J Immunol 129:445–447

Grant ME, Kefalides NA, Prockop DJ (1972) The biosynthesis of basement membrane collagen in embryonic chick lens. J Biol Chem 247:3539–3544

Hadding U, Dierich M, König W, Limbert M, Schorlemmer HU, Bitter-Suermann D (1973) Ability of the T-cell replacing polyanion to trigger the alternate pathway of complement activation. Eur J Immunol 3:527–529

Hahn H (1974) Effects of dextran sulfate 500 on cell-mediated resistance to infection with *Listeria monocytogenes* in mice. Infect Immun 10:1105–1109

Hahn H, Bierther M (1974) Morphological changes induced by dextran sulfate 500 in mononuclear phagocytes of Listeria-infected mice. Infect Immun 10:110–119

Haines AL, Lepow IH (1964a) Studies on human C′1 esterase. I. Purification and enzymatic properties. J Immunol 92:456–467

Haines AL, Lepow IH (1964b) Studies on human C′1 esterase. II. Function of purified C′1 esterase in the human complement system. J Immunol 92:468–478

Harwood R, Grand ME, Jackson DS (1976) The route of secretion of procollagen. The influence of α,α′-bipyridyl, colchicine and antimycin A on the secretory process in embryonic-chick tendon and cartilage cells. Biochem J 156:81–90

Hitschold T, Golan MD, Loos M (1981) Guinea pig-C1q: Purification, physicochemical and functional characterization, and differences to human C1q. Immunobiol 160:44 (abstr)

Hitschold H, Golan MD, Rabs U, Loos M (to be published) Purification and physicochemical properties of C1q from guinea pig serum. Mol Immunol

Hochwald GM, Thorbecke GJ, Asofsky R (1961) Sites of formation of immune globulins and of a component of C3. J Exp Med 114:459–470

Hughes-Jones NC, Gardner B (1978) The reaction between the complement subcomponent C1q, IgG complexes and polyionic molecules. Immunology 34:459–463

Humphrey JH, Dourmashkin RR (1965) Electron microscope studies of immune cell lysis. In: Wolstenholme, Knight (eds) Complement, Ciba Foundation Symp. Little, Brown, Boston, pp 175–186

Hurst MM, Volanakis JE, Hester RB, Stroud RM, Bennett JC (1974) The structural basis for binding of complement by immunoglobulin M. J Exp Med 140:1117–1121

Hurst MM, Volanakis JE, Stroud RM, Bennett JC (1975) C1 fixation and classical complement pathway activation by a fragment of the Cμ4 domain of IgM. J Exp Med 142:1322–1326

Hyslop NE, Dourmashkin RR, Green NM, Porter RR (1970) The fixation of complement and the activated first component (C1) of complement by complexes formed between antibody and divalent hapten. J Exp Med 131:783–802

Ishizaka T, Ishizaka K, Borsos T, Rapp H (1966) C′1 fixation by human isoagglutinins: Fixation of C′1 by γG and γM but not by γA antibody. J Immunol 97:716–726

Isliker H, Jocot-Guillermod H, Waldesbühl M, von Fellenberg R, Cerrotini JC (1967) Complement fixation by different IgG proportions and fragments. In: Miescher and Graber (eds) Fifth International Symposium on Immunopathology. Mechanisms of inflammation induced by immune injury. Schwabe, Basel, pp 197–206

Isliker H, Bing DH, Hynes RO (1981) Interactions of fibronectin with C1q, a subcomponent of the first component of complement. The Immune System 2:231–238

Isliker H, Bing DH, Lahan J, Hynes RO (1982) Fibronectin interacts with C1q, a subcomponent of the first component of complement. Immunology Lett 4:39–43
Karnovsky ML, Lazdins JK (1978) Biochemical criteria for activated macrophages. J Immunol 121:809–813
Kay NE, Douglas StD (1981) Detection of shedding of human blood monocyte Fc receptor during in vitro culture. In Arch Allergy Appl Immunol 66:131–135
Kehoe JM, Fougereau M (1969) Immunoglobulin peptide with complement fixing activity. Nature 224:1212–1213
Kerwar SS, Felix AM (1976) Effect of L-3,4-deydropoline on collagen synthesis and prolyl hydroxylase activity in mammalian cell cultures. J Biol Chem 251:503–509
Kivirikko KI, Myllylä R (1982) Posttranslational enzymes in the biosynthesis of collagen: Intracellular enzymes. In: Cunningham LW, Frederiksen DW (eds) Methods in enzymology: structural and contractile proteins, part A, 82:245–304
Knobel HR, Villiger W, Isliker H (1975) Chemical analysis and electron microscopy studies of human C1q prepared by different methods. Europ J Immunol 5:78–82
Kolb WP, Kolb LM, Podack ER (1979) C1q: isolation from human serum in high yield by affinity chromatography and development of a highly sensitive hemolytic assay. J Immunol 122: 2103–2111
Lai A Fat RFM, van Furth R (1975) In vitro synthesis of some complement components (C1q, C3 and C4) by lymphoid tissues and circulating leucocytes in man. Immunology 28:359–368
Landen B, Dierich MP, Schmitt M (1979) Complement receptor analogous factors in human serum. I. Isolation of a molecule inhibitory for complement dependent rosette formation, its identification as a α1-antitrypsin and its functional characterization. Immunobiology 156:153–167
Laurell AB, Mårtensson U (1974) Interaction between C1q, C1r, and C1s from human serum. Acta Pathol Microbiol Scand [B] 82:585–589
Laurell AB, Mårtensson U, Sjöholm AG (1976) C1 subcomponent complexes in normal and pathological sera studied by crossed immunoelectrophoresis. Acta Pathol Microbiol Scand [C] 84:455–464
Laurell AB, Sjöholm A, Johnson U, Mårtensson U (1978) Circulating complexes between C1r and C1s and between C1r, C1s and $C\bar{1}$ inactivator. In: Opferkuch W, Rother K, Schulz DR (eds) Clinical aspects of the complement system. Thieme, Stuttgart, pp 21–26
Laurell AB, Mårtensson U, Sjöholm AG (1979) Quantitation of $C1\bar{r}$-$C1\bar{s}$-$C\bar{1}$ inactivator complexes by electroimmunoassay. Acta Pathol Microbiol Scand [C] 87:79–81
Lee JP, Painter RH (1980) Complement binding properties of two peptides from the C2 region of human IgG 1. Mol Immunol 17:1155–1162
Lepow IH, Naff GB, Todd EW, Pensky J, Hinz CF (1963) Chromatographic resolution of the first component of human complement into three activities. J Exp Med 117:983–1008
Lepow IH, Naff GB, Pensky J (1965) Mechanisms of activation of C′1 and inhibition of C′1 esterase. Ciba Foundation Symposium: "Complement" Churchill, London, pp 74–90
Levy LR, Lepow JH (1959) Assay and properties of serum inhibitor of C′1-esterase. Proc Soc Exp Biol Med 101:608–611
Lin TY, Fletcher DS (1978) Interaction of human C1q with insoluble immunoglobulin aggregates. Immunochemistry 15:107–117
Linscott WD, Hansen SS (1969) Non-complement-fixing IgM antibodies in the guinea pig. J Immunol 103:423–428
Littleton C, Kessler D, Burkholder PM (1970) Cellular basis for synthesis of the fourth component of guinea-pig complement as determined by a haemolytic plaque technique. Immunology 18:693–704
Littman BH, Ruddy S (1977) Production of the second component of complement by human monocytes: stimulation by antigen-activated lymphokines. J Exp Med 145:1344–1352
Loos M (1982a) The classical complement pathway: mechanism of activation of the first component by antigen-antibody complexes. Prog Allergy 30:135–192
Loos M (1982b) Antibody independent activation of C1, the first component of complement. Ann Immunol (Paris) 133C:165–179
Loos M (1982c) The functions of endogenous C1q, a subcomponent of the first component of complement, as receptor on the membrane of macrophages. Mol Immunol 19:1229–1238

Loos M, Bitter-Suermann D (1976) Mode of interaction of different polyanions with the first (C1, $C\bar{1}$) the second (C2), and the fourth (C4) component of complement. IV. Activation of C1 in serum by polyanions. Immunology 31:931–934

Loos M, König W (1977) Interaction of DNP-antigens with the first component of complement ($C\bar{1}$). J Immunol 118:223–225

Loos M, Thesen R (1978) Trinitrophenylated red cells (E-TNP) as a model for antibody-independent activation of the complement system via the classical pathway. J Immunol 121:24–28

Loos M, Schorlemmer HU (1981) The function of endogenous C1q, The Fc recognizing subcomponent of the first component of complement, in the membrane of peritoneal macrophage as Fc receptor and as trigger in polyanion-mediated macrophage stimulation. In: Resch K, Kirchner H (eds) Mechanisms of lymphocyte activation. Biomedical Press, Elsevier/North-Holland, pp 313–315

Loos M, Schorlemmer HU (1982) The receptor functions of endogenous C1q, a subcomponent of the first component of complement, on peritoneal macrophages. In: Peeters H (ed) Prot Biol Fluids. Pergamon Press, Oxford UK, vol 29:439–442

Loos M, Opferkuch W, Ringelmann R (1971) $C\bar{1}$-inactivator of guineapig serum: Titration, purification and characterization of the inactivator. Z Med Mikrobiol Immunol 156:194–207

Loos M, Borsos T, Rapp HJ (1972a) Activation of the first component of complement. Evidence for an internal activation step. J Immunol 108:683–688

Loos M, Wolf HU, Opferkuch W (1972b) The $C\bar{1}$ inactivator from guinea pig serum. III. Characterization and kinetic data of the reaction between the inactivator and $EAC\bar{1}$ and EAC14. Immunochemistry 9:451–459

Loos M, Vadlamudi S, Meltzer M, Shifrin S, Borsos T, Goldin A (1972c) Detection of endotoxin in commercial L-asparaginase preparations by complement fixation and separation by chromatography. Cancer Res 32:2292–2296

Loos M, Borsos T, Rapp HJ (1973) The first component of complement in serum: Evidence for a hitherto unrecognized factor in C1 necessary for internal activation. J Immunol 110:205–212

Loos M, Bitter-Suermann D, Dierich M (1974) Interaction of the first ($C\bar{1}$), the second (C2) and the fourth (C4) component of complement with different preparations of bacterial lipopolysaccharides and with lipid A. J Immunol 112:935–940

Loos M, Brunner H (1979) Complement components (C1, C2, C3, C4) in bronchial secretions after intranasal infection of guinea pigs with *Mycoplasma pneumoniae:* Dissociation of unspecific and specific defence mechanisms. Infect Immun 25:583–585

Loos M, Volanakis JE, Stroud RM (1976a) Mode of interaction of different polyanions with the first (C1, $C\bar{1}$) the second (C2) and the fourth (C4) component of complement. III. Inhibition of C4 and C2 binding site(s) on C1s by polyanions. Immunochemistry 13:789–791

Loos M, Hill HU, Wellek B, Heinz HP (1976b) Ultracentrifugation studies on the native form of the first component of human complement (C1). FEBS Lett 64:341–345

Loos M, Wellek B, Thesen R, Opferkuch W (1978) Antibody-independent interaction of the first component of complement with gram-negative bacteria. Infect Immun 22:5–9

Loos M, Müller W, Boltz-Nitulescu G, Förster O (1980a) Evidence that C1q, a subcomponent of the first component of complement, is an Fc receptor of peritoneal and alveolar macrophages. Immunobiol 157:54–61

Loos M, Storz R, Müller W, Lemmel EM (1981) Immunofluorescence studies on the subcomponents of the first component of complement (C1): Detection of C1q and C1s in different cells of biopsy material and on human as well as on guinea pig peritoneal macrophages. Immunobiol 158:213–224

Loos M, Laurell AB, Sjöholm AG, Mårtensson U, Berkel AI (1980b) Immunochemical and functional analyses of a complete C1q deficiency in man: Evidence that C1r and C1s are in the native form, and that they reassociate with purified C1q to form macromolecular C1. J Immunol 124:59–63

Lukas TJ, Munoz H, Erickson BW (1981) Inhibition of C1-mediated immune hemolysis by monomeric and dimeric peptides from the second constant domain of human immunoglobulin G. J Immunol 127:2555–2560

McKay EJ, Laurell AB, Mårtensson K, Sjöholm AG (1981) Activation of C1, the first component of complement, the generation of C1r-C1s and $C\bar{1}$ inactivator complexes in normal serum, by heparin-affinity chromatography. Mol Immunol 18:349–357

McKenzie MR, Creevy N, Heh M (1971) The interaction of human IgM and C1q. J Immunol 106:65–68
Mayer MM (1961) Development of the one-hit theory of immune hemolysis. In: Heidelberger M, Plescia OJ (eds) Immunochemical approaches to problems in microbiology. Rutgers University Press, New Brunswick, N. J., pp 268–279
Medicus RG, Chapius RM (1980) The first component of complement. I. Purification and properties of native C1. J Immunol 125:390–395
Melchers F (1971) Biosynthesis of the carbohydrate portion of immunoglobulins. Biochem J 125: 241–347
Menzel EJ, Smolen JS, Liotta LA, Reid KBM (1981a) Competition between antifibronectin-antibody, C1q and collagens for the collagen-binding region of fibronectin. Immunobiology 160:72 (abstr)
Menzel EJ, Smolen JS, Reid KBM (1981b) Immunological cross-reactivity between the collagen-like fragment of human C1q and human type I collagen. Mol Immunol 18:765–771
Metchnikoff E (1889) Sur le propriété bactéricide des humeurs. Revue critique. Ann Immunol, Paris 3:664–671
Metchnikoff E (1905) Immunity in infective diseases. Cambridge University Press, London New York, p 195
Miller EJ, Gay S (1982) Collagen: An overview. In: Cunningham LW, Frederiksen DW (eds) Methods in enzymology structural and contractile proteins, part A 82:3–32
Molenaar JL, van Galen M, Hannema AJ, Zeijlemaker W, Pondman KW (1977) Spontaneous release of Fc receptor-like material from human lymphoblastoid cell lines. Eur J Immunol 7:230–236
Moore DL, Vas SI (1968) Studies of the production of complement. Immunology 15:185–195
Morris KM, Colten HR, Bing DH (1978) The first component of complement. A quantitative comparison of its biosynthesis in culture by human epithelial and mesenchymal cells. J Exp Med 148:1007–1019
Müller W, Hanauske-Abel H, Loos M (1978a) Biosynthesis of the first component of complement by human and guinea pig peritoneal macrophages: Evidence for an independent production of the C1 subunits. J Immunol 121:1578–1584
Müller W, Hanauske-Abel H, Loos M (1978b) Reversible inhibition of C1q release from guinea pig macrophages by 2,2′-dipyridyl. FEBS Lett 90:218–222
Müller-Eberhard HJ (1969) Complement. Ann Rev Biochem 38:389–414
Müller-Eberhard HJ (1975) Complement. Ann Rev Biochem 44:697–724
Müller-Eberhard HJ, Kunkel HG (1961) Isolation of a thermolabile serum protein which precipitates γ-globulin aggregates and participates in immune hemolysis. Proc Soc Exp Biol Med 106:291–295
Müller-Eberhard HJ, Lepow IH (1965) C′1 esterase effect on activity and physicochemical properties of the fourth component of complement. J Exp Med 121:819–833
Müller-Eberhard HJ, Schreiber RD (1980) Molecular biology and chemistry of the alternative pathway of complement. Adv Immunol 29:1–53
Müller-Eberhard HJ, Polley MJ, Calcott MA (1967) Formation and functional significance of a molecular complex derived from the second and the fourth component of human complement. J Exp Med 125:359–380
Müller-Eberhard HJ, Bokisch VA, Budzko DB (1970) In: Mischer PA (ed) Immunopathology. VI. International Symposium. Grune and Stratton, New York, pp 191–200
Naff GB, Ratnoff OD (1968) The enzymatic nature of C′1r. Conversion of C′1s to C′1 esterase and digestion of amino acid esters by C′1r. J Exp Med 128:571–593
Naff GB, Pensky J, Lepow IH (1964) The macromolecular nature of the first component of human complement. J Exp Med 119:593–613
Nagaki K, Stroud RM (1969) The relationship of the hemolytic activity of active C′1s to its TAME esterase action: A new method of purification and assay. J Immunol 102:421–430
Nagasawa S, Takahashi K, Koyama J (1974) Isolation of a complex of the subcomponents of the activated first component of complement, $C1\bar{r}$-$C1\bar{s}$, from acid-human plasma. FEBS Lett 41: 280–282
Nolan JC, Ridge S, Oronsky AL, Kerwar SS (1978) Studies on the mechanism of reduction of prolyl hydroxylase activity by D,L-3,4-dehydroproline. Arch Biochem Biophys 189:448–453

Nuttall G (1888) Experimente über die bakterienfeindlichen Einflüsse des tierischen Körpers. Z Hyg Infektionskrankh 4:353–394
Painter RH, Foster DB, Gardner B, Hughes-Jones NC (1982) Functional affinity constants of subfragments of immunoglobulin G for G1q. Mol Immunol 19:127–131
Parkhouse RME, Melchers F (1971) Biosynthesis of the carbohydrate portions of immunoglobulin M. Biochem J 125:235–240
Patrick RA, Taubman SB, Lepow IH (1970) Cleavage of the fourth component of human complement (C4) by activated C1s. Immunochemistry 7:217–225
Pearlstein E, Sorvillo J, Gigli I (1982) The interaction of human plasma fibronectin with a subunit of the first component of complement, C1q. J Immunol 128:2036–2039
Peltier AP, Cyna L, Dryll A (1978) 'In vitro' study of a reaction between the complement system and cellular DNA. Immunology 35:779–784
Pfeiffer R, Issaeff R (1894) Über die specifische Bedeutung der Choleraimmunität. Z Hyg Infektionskr 17:355–400
Pickering RJ, Naff GB, Stroud RM, Good RA, Gewurz H (1970) Deficiency of C1r in human serum. Effects on the structure and function of macromolecular C1. J Exp Med 141:803–815
Pillemer L (1943) Recent advances in the chemistry of complement. Chem Rev 33:1–26
Pillemer LE, Ecker E, Oncley JL, Cohn EJ (1941) The preparation and physicochemical characterization of the serum protein components of human complement. J Exp Med 74:297–308
Plaut AG, Cohen S, Tomasi TB (1972) Immunoglobulin M: Fixation of human complement by the Fc fragment. Science 176:55–56
Porter RR, Reid KBM (1979) Activation of the complement system by antibody-antigen complexes: the classical pathway. Adv Protein Chem 33:1–71
Prockop DJ, Tuderman L (1982) Posttranslational in the biosynthesis of collagen: extracellular enzymes. In: Cunningham LW, Frederiksen DW (eds) Methods in enzymology Structural and contractile proteins. part A 82:305–319
Prockop DJ, Berg RA, Kivirikko KI, Uitto J (1976) Intracellular steps in the biosynthesis of collagen. In: Ramachandran GN, Reddi AH (eds) Biochemistry of Collagen. Plenum Press, New York, pp 163–273
Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA (1979) The biosynthesis of collagen and its disorders. N Engl J Med 301:13–85
Rabs U, Hitschold T, Golan MD, Loos M (1981) Isolation and characterization of guinea pig macrophages derived C1q and its similarities with guinea pig serum derived C1q. Immunobiology (abstr) 160:87
Raepple E, Hill HU, Loos M (1976) Mode of interaction of different polyanions with the first (C1, C1), the second (C2) and the fourth (C4) component of complement. I. Effect on fluid phase C1 and on C1 bound to EA or to EAC4. Immunochemistry 13:251–255
Rapp HJ, Borsos T (1970) Molecular basis of complement action. Appleton-Century-Crofts, New York
Ratnoff OD, Pensky J, Ogston D, Naff GB (1969) The inhibition of plasmin, plasma kallikrein, plasma permeability factor, and the C'1r subcomponent of the first component of complement by serum C'1 esterase inhibitor. J Exp Med 129:315–331
Regelson W (1968a) The growth-regulating activity of polyanions: A theoretical discussion of their place in the intercellular environment and their role in cell physiology. Adv Cancer Res 11: 223–304
Regelson W (1968b) The antimitotic activity of polyanions. Adv Chemother 3:303–371
Reid KBM (1974) A collagen-like amino acid sequence in a polypeptide chain of human C1q (a subcomponent of the first component of complement). Biochem J 141:189–203
Reid KBM (1977) Amino acid sequence of the N-terminal forty-two amino acid residues of the C chain of subcomponent C1q of the first component of human complement. Biochem J 161: 247–251
Reid KBM (1981) Preparation of human C1q, a subcomponent of the first component of the classical pathway of complement. In: Lorand L (ed) Methods in enzymology, proteolytic enzymes, part C, Academic Press, London 80:16–25
Reid KBM (1982) C1q. In: Cunningham LW, Frederiksen DW (eds) Methods in enzymology structural and contractile proteins. Academic Press, London, part A 82:319–324

Reid KBM, Porter RR (1976) Subunit composition and structure of subcomponent C1q of the first component of human complement. Biochem J 155:19–23
Reid KBM, Solomon E (1977) Biosynthesis of the first component of complement by human fibroblasts. Biochem J 167:647–660
Reid KBM, Lowe DM, Porter RR (1972) Isolation and characterization of C1q, a subcomponent of the first component of complement, from human and rabbit sera. Biochem J 130:749–763
Reid KBM, Sim RB, Faiers AP (1977) Inhibition of the reconstitution of the haemolytic activity of the first component of complement by a pepsin-derived fragment of subcomponent C1q. Biochem J 161:239–245
Rent R, Ertel N, Eisenstein R, Gewurz H (1975) Complement activation by interaction of polyanions and polycations. I. Heparin-protamine induced consumption of complement. J Immunol 114:120–124
Riches DWH, Stanworth DR (1981) Studies on the possible involvement of complement component C3 in the initiation of acid hydrolase secretion by macrophages. I. Correlation between enzyme-releasing and complement-activating capacities of several secretagogues. Immunology 44:29–39
Rosenberry TL, Barnett P, Mays C (1982) Acetylcholinesterase. In: Cunningham LW, Frederiksen DW (eds) Methods in enzymology: Structural and contractile Proteins, Part A 82:325–339
Sakai K, Stroud RM (1973) Purification, molecular properties, and activation of C1 proesterase, C1s. J Immunol 110:1010–1020
Sakai K, Stroud RM (1974) The activation of C1s with purified C1r. Immunochemistry 11:191–196
Sandberg AL, Oliveira B, Osler AG (1971) Two complement interaction sites in guinea pig immunoglobulins. J Immunol 106:282–285
Sassano FG, Colten HR, Borsos T, Rapp HJ (1972) Resolution of the first component of guinea pig complement into three subunits, C1q, C1r and C1s and their hybridization with human C1 subunits. Immunochemistry 9:405–412
Schorlemmer HU, Bitter-Suermann D, Allison AC (1977a) Complement activation by the alternative pathway and macrophage enzyme secretion in the pathogenesis of chronic inflammation. Immunology 32:929–940
Schorlemmer HU, Burger R, Hylton W, Allison AC (1977b) Induction of lysosomal enzyme release from cultured macrophages by dextran sulfate. Clin Immunol Immunopathol 7:88–96
Schultz DR, Arnold PI, Wu MC, Lo TM, Volanakis JR, Loos M (1979) Isolation and partial characterization of a polysaccharide in ant venom (Pseudomyrmex sp.) that activates the classical complement pathway. Mol Immunol 16:253–264
Schultz DR, Loos M, Bub F, Arnold PI (1980) Differentiation of hemolytically active fluid-phase and cell-bound human C1q by an ant venom-derived polysaccharide. J Immunol 124:1251–1257
Shaw DR, Griffin FM jr (1981) Phagocytosis requires repeated triggering of macrophage phagocytic receptors during particle ingestion. Nature 289:409–411
Shelton E, Yonemasu K, Stroud RM (1972) Ultrastructure of the human component C1q. Proc Nat Acad Sci USA 69:65–68
Siboo R, Vas SI (1965) Studies on in vitro antibody production III. Production of complement. Can J Microbiol 11:415–425
Siegel J, Claus D, Retras K (1976) Interaction of C-reactive protein (CRP) and C1. Fed Proc 35:493 (abstr)
Siegel RC, Schumaker VN, Poon PH (1981) Stoichiometry and sedimentation properties of the complex formed between the C1q and $C1r_2C1s_2$ subcomponent of the first component of complement. J Immunology 127:2447–2452
Silvestri L, Baker JR, Roden L, Stroud RM (1981) The C1q inhibitor in serum is a chrondroitin 4-sulfate proteoglycan. J Biol Chem 256:7383–7387
Sim RB (1981) The human complement system serine proteases C1r and C1s and their proenzyms. In: Lorand L (ed) Methods in Enzymology. Proteolytic enzymes. Academic Press, London, part C 80:26–42
Sim RB, Porter RR, Reid KBM, Gigli I (1977) The structure and enzymatic activities of the C1r and C1s subcomponents of C1, the first component of complement. Biochem J 163:219–227
Skok J, Solomon E, Reid KBM, Thompson RA (1981) Distinct genes for fibroblast and serum C1q. Nature 292:549–551

Sledge CR, Bing DH (1973) Binding properties of the human complement protein C1q. J Biol Chem 248:2818–2823

Sobel AT, Bokisch VA (1975) Receptors for C4b and C1q on human peripheral lymphocytes and lymphoblastoid cells. In: Seligman M, Preud'Homme, Kourilsky FM (eds) Membrane Receptors of lymphocytes. North Holland Publishing Co, Amsterdam, pp 151–162

Spiegelberg HL (1974) Biological activities of immunoglobulins of different classes and subclasses. Adv Immunol 19:259–294

Stecher VJ, Thorbecke GJ (1967a) Sites of synthesis of serum proteins. II. Medium requirements of serum protein production by rat macrophages. J Immunol 99:653–659

Stecher VJ, Thorbecke GJ (1967b) Sites of synthesis of serum proteins. III. Production of β1C, β1E, and transferrin by primate and rodent cell lines. J Immunol 99:660–668

Stecher VJ, Morse JH, Thorbecke GJ (1967c) Sites of production of primate serum proteins associated with the complement system. Proc Soc Exp Biol Med 124:433–438

Strang JC, Siegel RC, Phillips LM, Poon PH, Schumaker VN (1982) Ultrastructure of the first component of human complement: Electron microscopy of the cross-linked complex. Proc Natl Acad Sci USA 79:586–590

Stroud RM, Nagaki K, Pickering RJ, Gewurz H, Good RA, Cooper MD (1970) Sub-units of the first complement component in immunologic deficiency syndromes: Independence of C1s and C1q. Clin Exp Immunol 7:133–137

Suba EA, Csákó G (1976) C1q (C1) receptor on human platelets: inhibition of collagen induced platelet aggregation by C1q (C1) molecules. J Immunol 117:304–309

Takahashi K, Nagasawa S, Koyama J (1975a) A gross structure of an activated form of a subunit of the first component of human complement, $C1\bar{r}$. FEBS Lett 55:156–160

Takashashi K, Nagasawa S, Koyama J (1975b) The NH_2-terminal sequence of a subunit of the first component of human complement, C1s, and its activated form, $C1\bar{s}$. FEBS Lett 50:330–333

Tamura N, Nelson RA jr (1968) The purification and reactivity of the first components of complement from guinea pig, human, and canine sera. J Immunol 101:1333–1345

Taranta A, Weiss HS, Franklin EC (1961) Precipitating factor for aggregated γ-globulin in normal human sera. Nature 189:239–240

Taubman SB, Lepow IH (1971) Cleavage of human $C1\bar{s}$ by human lysosomal enzymes, plasmin, and trypsin. Immunochemistry 8:951–961

Tenner AJ, Cooper NR (1980) Analysis of receptor-mediated C1q binding to human peripheral blood mononuclear cells J Immunol 125:1658–1664

Tenner AJ, Cooper NR (1981) Identification of types of cells in human peripheral blood that bind C1q. J Immunol 126:1174–1179

Thesen R, Back W, Loos M (1978) Preparation of trinitrophenylated red cells for antibody independent lysis by complement. J Immunol Methods 20:201–209

Thompson RA, Haeney M, Reid KBM, Davies JG, White RHR, Cameron AH (1980) A genetic defect of the C1q subcomponent of complement associated with childhood (immune complex) nephritis. N Engl J Med 303:22–24

Thorbecke GJ, Hochwald GM, van Furth R, Müller-Eberhard HJ, Jakobson EB (1965) Problems in determining the sites of synthesis of complement components. In: Wolstenholme GEW, Knight J (eds) Ciba-Symposium "Complement" J and A Churchill, London, pp 99–119

Tsukamoto Y, Helsel WE, Wahl SM (1981) Macrophage production of fibronectin, a chemoatractant for fibroblasts. J Immunol 127:673–678

Unkeless JC, Fleit H, Mellman IS (1981) Structural aspects and heterogeneity of immunoglobulin Fc receptors. Adv Immunol 31:247–270

Valet G, Cooper NR (1974a) Isolation and characterization of the proenzyme form of the C1r subunit of the first complement component. J Immunol 112:1667–1673

Valet G, Cooper NR (1974b) Isolation and characterization of the proenzyme form of the C1s subunit of the first complement component. J Immunol 112:339–350

Valet G, Kahle H, Timpl R (1978) Inhibition of the C1 complement molecule by collagen and procollagen peptides. Immunobiology 155:71 (abstr)

Volanakis JE, Kaplan MH (1974) Interaction of C-reactive protein complexes with the complement system. II. Consumption of guinea pig complement by CRP complexes: requirement for human C1q. J Immunol 113:9–17

Volanakis JE, Stroud RM (1972) Rabbit C1q: Purification, functinal and structural studies. J Immunol Methods 2:25–34

Wautier JL, Souchon H, Reid KBM, Peltier AP, Caen JP (1977) Studies on the mode of reaction of the first component of complement with platelets. Interaction between collagen-like portion of C1q and platelets. Immunochemistry 14:763–766

Wautier JL, Reid KBM, Legrand Y, Caen JP (1980) Region of the C1q molecule involved in the interaction between platelets and subcomponent C1q of the first component of complement. Mol Immunol 17:1399–1405

Winkelhake JL (1978) Immunoglobulin structure and effector functions. Immunochemistry 15: 695–714

Wyatt HV (1974) Synthesis of the second and fourth components of complement by tissue culture cell lines. Eur J Immunol 4:34–38

Wyatt HV, Colten HR, Borsos T (1972) Production of the second (C2) and fourth (C4) components of guinea pig complement by single peritoneal cells: Evidence that one cell may produce both components. J Immunol 108:1609–1614

Yachnin S, Rosenblum D, Chatman D (1964) Biological properties of polynucleotides. V. Studies on the inhibition of the first component of complement by polyionsinic acid; the interaction with C1q. J Immunol 93:542–548

Yonemasu K, Stroud RM (1971) C1q: Rapid purification method for preparation of monospecific antisera and for biochemical studies. J Immunol 106:304–313

Yonemasu K, Stroud RM (1972) Structural studies on human C1q: non-covalent and covalent subunits. Immunochemistry 9:545–554

Yonemasu K, Stroud RM, Niedermeier W, Butler WT (1971) Chemical studies on C1q, a modulator of immunoglobulin biology. Biochem Biophys Res Comm 43:1388–1394

von Zeipel G, Sjöholm A, Laurell AB (1973) Analysis of C1 subcomponents in cell culture media of HeLa cells and diploid human fetal lung fibroblasts. Acta Pathol Microbiol Scand [B] 81: 259–263

Ziccardi RJ (1981) Activation of the early components of the classical complement pathway under physiologic conditions. J Immunology 126:1769–1773

Ziccardi RJ, Cooper NR (1976a) Physicochemical and functional characterization of the C1r subunit of the first complement component. J Immunol 116:496–503

Ziccardi RJ, Cooper NR (1976b) Activation of C1r by proteolytic cleavage. J Immunol 116: 504–509

Ziccardi RJ, Cooper NR (1977) The subunit composition and sedimentation properties of human C1. J Immunol 118:2047–2052

Changes Induced in Cell Membranes Adsorbing Animal Viruses, Bacteriophages, and Colicins

PINHAS FUCHS* AND ALEXANDER KOHN*

* Department of Virology, Israel Institute for Biological Research, Ness Ziona 70450, Israel

1 Introduction

Binding of various ligands (hormones, neurotransmitters, immunological stimuli) to membrane receptors induces the following changes:

1. Receptor redistribution (clustering, "capping")
2. Conformational changes that can be detected by fluorescent probes
3. Alteration in membrane fluidity (spin label and fluorescence polarization probes)
4. Changes in fluxes of ions and metabolites
5. Increased phospholipid turnover (especially of phosphatidyl inositol)
6. Activation of membrane-bound enzymes (adenyl cyclase, ATPase, transmethylases).

Some of the early changes resulting from or associated with the binding (adsorption) of virions to the host cell membrane are of the same type.

Adsorption of animal viruses to cells is the first step in a chain of events resulting in the production of progeny virus on the one hand and in damage to cells and tissues on the other. In the classical studies of viral infection, cells are adsorbed with virus, usually for 60 min, and the changes induced by the virus in the host cell are recorded thereafter. In the past decade, more and more studies have been aimed at the events occurring in these first 60 min of the so-called adsorption period. These studies deal with the nature of adsorption, e.g., the ligand-receptor type of interaction between the virus and the cell membrane. Many receptors for viruses were identified and so were the viral proteins which take part in adsorption.

In all systems studied, bacterial as well as animal, the changes are very fast, mostly transient, and occur seconds to minutes from the onset of virus-cell interaction. The changes which deserve consideration are in the microscopic morphology of the cell membrane, in its fluidity and in its permeability to ions and small molecules.

Changes in membrane permeability induced by viruses may also result in the uptake of molecules of high molecular weight; therefore "microinjection" studies are included in this review. Because of the effects listed at the beginning, which are shared by viruses and nonviral ligands, a comparison between them should be made.

2 Morphological Changes in Cell Membranes Induced by Viruses

Extracellular materials can enter the cells by a specific transport mechanism, passive flux, or by endocytosis and fusion. Viruses penetrate the cells by one of two possible mechanisms: (a) endocytosis and (b) fusion. The interactions of viruses with cells leading to virus penetration have been the subject of numerous reviews. The topics covered by these reviews are: host and tissue specificities in virus infection (*Smith* 1977), the interaction of viruses with model membranes (*Tiffany* 1977), the cellular receptors for different groups of viruses (*Kohn* 1979), the virus-erythrocyte membrane interaction (*Bächi* et al. 1977), structural studies of the surface of virus-infected cells (*Dubois-Dalcq* and *Rentier* 1980), and the virus-induced fusion reaction (*Poste* 1972; *Kohn* and *Fuchs* 1974; *Hosaka* and *Shimizu* 1977; *Knutton* 1978). In this chapter we will summarize studies dealing with the early morphological changes in the membranes of the virus-infected cells as revealed by light, electron, and scanning electron microscopy. Changes due to interaction of antiviral antibodies with viral antigens on the cell surface, recently reviewed by *Oldstone* et al. (1980), will not be discussed.

2.1 Endocytosis

Endocytosis is a temperature dependent process by which exogenous substances, soluble or particulate, are taken from the cellular environment via plasma membrane-derived vesicles or vacuoles (*Allison* and *Davies* 1974; *Silverstein* et al. 1977). Viruses are taken up by an adsorptive type of pinocytosis called micropinocytosis. This mode of entry is best exemplified by Semliki Forest virus (SFV). Following adsorption, this virus is rapidly internalized and is found in coated vesicles. It is then transferred to secondary lysosomes via endosomes (*Helenius* et al. 1980; *Marsh* and *Helenius* 1980). Field emission scanning electron microscopy of the entry of vesicular stomatitis virus (VSV), which is also endocytized, shows a progressive engulfment of the virions into an annular depression appearing in the cell surface membrane at the site of virus attachment. When virus entry is completed this membranal depression undergoes repair (*Dubois-Dalcq* and *Rentier* 1980). Recent electron-microscopic studies on the entry of fowl plaque virus (an avian influenza A virus) demonstrated that at 0 °C viruses were primarily bound to the microvilli of MDCK cells (*Matlin* et al. 1981). Upon warming to 37 °C the viruses were endocytosed in coated pits, coated vesicles, and large smooth surface vacuoles. Fusion was only observed when prebound virus was incubated at pH 5.5 or below, for 1 min at 37 °C. *Matlin* et al. (1981) therefore suggest that SFV and fowl plaque virus (and probably other influenza viruses) enter the cells by endocytosis and are then transported into lysosomes, where the low pH induces a fusion reaction resulting in the transfer of the viral genome into the cytoplasm. Micropinocytosis of influenza virus was also observed with chicken or human RBCs (*Danon* et al. 1961; *Bossart* et al. 1973). Following a 5-min incubation of the virus with the RBCs, pinocytic vesicles containing virus were detected in the cells. Some viruses were engulfed by the cell membrane but still outside the cells. The vesicles varied considerably in size and usually contained one virus. No uncoating was observed during 30 min incubation. It is interesting to note that similar events also occur in invasion of RBCs by malaria merozoites (*Dvorak* et al. 1975).

The experiments of *Stephenson* and *Dimmock* (1975) and *Stephenson* et al. (1978) demonstrated that influenza, Newcastle disease, and SF viruses, as well as some enteroviruses, "penetrate" the cells at 4 °C (as evident by resistance to pH 3 or to antibodies). This was taken to indicate that micropinocytosis, unlike fusion or phagocytosis, does not require metabolic energy. Similar results were also obtained by *Kato* and *Eggers* (1969). The experiments of *Bossart* et al. (1973, see above) and of *Okada* et al. (1975b) may provide another possible explanation. Studying the Sendai virus-induced hemadsorption reaction in Ehrlich ascites tumor cells, *Okada* et al. (1975b) found that at low temperatures the adsorbed viruses distorted the cell membrane so that it surrounded the viruses almost completely while leaving them topologically outside. Electron-microscopic studies by *Dourmashkin* and *Tyrell* (1974) show no evidence for influenza virus entry at 4 °C.

Other viruses which penetrate cells via endocytosis are adenoviruses 1, 5, 7, and 12 (*Dales* and *Chardonnet* 1973), BK (a papova) virus (*Maraldi* et al. 1975), and reoviruses (*Silverstein* and *Dales* 1968). In herpes virus and cytomegalovirus (CMV) infection of WI-38 cells, both endocytosis and fusion occur within 3 min of adsorption (*Smith* and *DeHarven* 1974).

According to *Steinman* et al. (1976) the interiorized vacuole membrane is recycled back to the cell surface. Electron-microscopic evidence for this suggestion was provided

by *Kim* et al. (1976). When Sendai virus was endocytized by Ehrlich ascites tumor cells, fusion of cell membrane occurred to form closed vesicles. The viral envelope antigens remaining in the vesicle membrane were discarded by exocytosis. In regular endocytosis (not with fusing viruses) the endocytized substances do not remain in the membrane, but are metabolized by the cells with the aid of lysosomes.

2.2 Fusion

Fusion of the viral envelope with the cell membrane during penetration seems to be the reverse of the process of virus maturation, in which viral nucleoproteins are enclosed in budding plasma membrane. The best known fusing viruses are the paramyxoviruses such as Sendai, Newcastle disease, SV5, and measles viruses (*Okada* 1958, 1969; *Kohn* 1965). Other fusing viruses are vaccinia (*Hanafusa* 1960), encephalic Germiston virus (*Olson* 1976), VSV (*Heine* and *Schnaitman* 1971; *Nishiyama* et al. 1976; *Chany-Fournier* et al. 1977), herpesviruses types 1 and 2 (*Roizman* 1962a, b; *Kousoulas* et al. 1978), and CMV (*Vonka* et al. 1976; *Booth* et al. 1978).

Virus-induced fusion occurs in two manners: (a) fusion from within (FFWI) and (b) fusion from without (FFWO). FFWI occurs at the end of the infecting cycle when maturating viruses modify the host cell membrane to induce its fusion with a neighboring cell (*Bratt* and *Gallaher* 1969; *Gallaher* and *Bratt* 1974). FFWO occurs when large quantities of infectious or inactive fusing virus are added to cells (*Okada* 1958; *Kohn* 1965). FFWO was considered for many years to be an amplified virus-cell reaction. Today, however, it is well established that the virus-cell fusion, although a prerequisite for cell-cell fusion, is a separate event from the latter (*Knutton* 1977, 1980; *Knutton* and *Pasternak* 1979; *Knutton* et al. 1977; *Miyake* et al. 1978; *Okada* et al. 1975c; *Maeda* et al. 1977b). Recently, using the double-label immunofluorescence technique coupled with light and electron microscopy, *Büechi* and *Bächi* (1979) followed virus-cell fusion, observing it "from within". Sendai virus, adsorbed to the ES (external surface) of the membrane at 4 °C, was not accessible to fluorescein-labeled antibodies applied from the plasma surface (PS) side. At 37 °C viral antigens were detected at the PS.

The first membranal change observed with fusing viruses during the virus-cell fusion process, however, is a change in the structure of the viral envelope. Using freeze-fracture technique, *Knutton* (1976) showed that Sendai virus adsorbed to the cell membrane lost its 14-nm-diameter intramembranal particles (believed to be the intraenvelope glycoprotein HN) from E faces and showed an appearance of 9-nm-diameter particles on both E and P faces. One of the early changes in the membrane of cells adsorbing virus is related to the distribution of the intramembranal particles (IMP). Just before or during fusion an aggregation of the IMP was observed. This aggregation (named also "destabilization" by *Maeda* et al. (1977a)) was described not only with virus-infected cells (*Bächi* et al. 1973; *Lalazar* et al. 1976; *Zakai* et al. 1977; *Volsky* and *Loyter* 1977; *Gazitt* et al. 1977; *Hennache* et al. 1979), but also with other fusogenic agents (*Lucy* 1970; *Elgsaeter* and *Branton* 1974; *Robinson* et al. 1979). As a result of this aggregation, parts of membrane areas become clear of IMP. These parts seem to be phospholipidic (*Gazitt* et al. 1976a, b). It has thus been proposed that direct membrane fusion occurs between IMP-denuded regions of closely adjacent membranes (*Volski* and *Loyter* 1977; *Bächi* et al. 1977; *Hosaka* and *Shimizu* 1974). *Knutton* (1978), however, is of the opinion that the above suggestion for

virus-induced fusion seems unlikely since IMP aggregation occurs at a later stage, mainly when cell fusion has already occurred. His suggestion is that IMP aggregation is a consequence, rather than a cause, of the fusion reaction. The experimental data seems to be in favor of the first suggestion, since in erythrocyte ghosts Sendai-virus-induced IMP aggregation is accompanied by depolymerization of spectrin (*Milner* et al. 1978) and its becoming accessible to proteases (*Lalazar* and *Loyter* 1979). The newly created protein-free phospholipid layer (*Ahkong* et al. 1975) is freely exposed to the ES, and it may fuse with the opposing (viral or cellular) phospholipids. Fusion of bare lipid bilayers is known to occur spontaneously (*Papahadjopoulos* et al. 1974c).

Sendai-virus-induced IMP aggregation and fusion of RBC ghosts were both inhibited by antispectrin antibodies previously entrapped in the ghosts. It therefore seems that these two reactions require the cross-linking of membrane proteins (*Sekiguchi* and *Asano* 1978). The entrapped antibody has to be complete; Fab fragments of the immunoglobulin had no effect.

For a number of years the prevailing view was that the virus itself provided the bridge for the fusion of two adjacent cells. The virus fuses on each side to a different cell. Due to enhanced ion fluxes (see Sect. 4) the cells swell to produce a polykaryocyte (*Apostolov* and *Almeida* 1972; *Meiselman* et al. 1967; *Okada* 1969). More recent evidence shows that linkage between membranes to produce fusion occurs in regions of membrane which become modified globally as a result of a local cell-virus interaction (see *Bächi* et al. 1977). The mechanism of virally induced cell fusion is thus analogous to that suggested for chemically induced fusion (*Lucy* 1970), the main reason being that neither viral particles nor viral fragments could be recognized at areas where membrane fusion occurred. However, *Knutton* and co-workers (*Knutton* 1977, 1980; *Knutton* and *Pasternak* 1979; *Knutton* et al. 1977) showed by electron microscopy that virus particles participate in every fusion event, and thus backed the "classical" view of virus-induced fusion. Similar observations were also reported by *Hosaka* et al. (1978).

In scanning electron microscopy of Hela cells adsorbing Sendai (infective or inactive) virus at 37 °C, considerable morphological changes were seen. These changes, which occurred 2–5 min post adsorption at 37 °C or 2–5 min after the transfer of virus-adsorbed cells from 4 °C to 37 °C, were characterized by the appearance of bridging microvilli, resembling a zipper structure, which interconnected the cells. The number of these connections increased with time and was followed by their widening, leading finally to the fusion of the cells into polykaryocytes (*Shahar* et al. 1976; *Fuchs* et al. 1978). These results are contradictory to those obtained by *Knutton* et al. (1976, 1977) who observed, using a similar technique, osmotic swelling of cells and loss of microvilli. This difference in results may be due to the differences in the experimental systems, i.e., in our experiments (*Shahar* et al. 1976; *Fuchs* et al. 1978). Hela cells were grown and infected in monolayers, whereas in *Knutton*'s experiments (1976, 1977) the cells (*Hela* and *Lettrée*) were grown and infected in suspension.

3 Changes in Membrane Fluidity

The dynamic behavior of biological membranes is determined to a large extent by the degree of mobility of membrane proteins (*Inbar* et al. 1973; *Shinitzky* and *Inbar* 1976) and by the degree of fluidity of the lipid bilayer (*Shinitzky* and *Inbar* 1974, 1976). These

dynamic features are interrelated, and thus adsorption of viruses which introduce protein(s) and phospholipids (in case of enveloped viruses) into the cell membrane may change the structure and function of the cell membrane.

The current concepts of cell membrane fluidity were recently reviewed by *Shinitzky* and *Henkart* (1979) and *Shinitzky* (1982). In this section we will summarize some of the studies which deal with changes in the fluidity of cell membrane induced by virus adsorption. The methods used for such studies were summarized by *Kohn* (1979) and will not be discussed here.

3.1 Role of Lipids

It is generally assumed that the host cells determine the lipid composition of enveloped viruses (*Hirschberg* and *Robbins* 1974; *Blough* and *Tiffani* 1973; *Kates* et al. 1961; *Barenholz* et al. 1976; *Klenk* 1974; *Moore* et al. 1977a, b). However, by using the fluorescence polarization technique (*Shinitzky* and *Inbar* 1974; *Shinitzky* and *Barenholz* 1974) it was found that as a rule, the envelopes of toga-, rhabdo-, myxo-, and paramyxoviruses were more viscous (less fluid) than the membranes of the cells from which they emerged (*Barenholz* et al. 1976; *Moore* et al. 1976; *Patzer* et al. 1978; *Levanon* and *Kohn*, 1978). The same virus grown in different hosts also had a different fluidity, which was related to that of the host. Influenza, SV5, and VSV studied by the electron spin resonance method (ESR) show a higher rigidity in relation to the rigidity of their host cell membrane. This is due to their higher cholesterol:phospholipid ratio. Nevertheless, artificial membranes made of lipids extracted from viruses are less rigid than the native viral envelope (*Stoffel* et al. 1976), indicating a strong involvement of viral proteins in the determination of viral membrane fluidity (*Tanaka* and *Ohnishi* 1976; *Landsberger* et al. 1978; *Sefton* and *Gaffney* 1974; *Landsberger* and *Compans* 1976; *Scheid* and *Choppin* 1974).

Measurements of changes in fluidity during the adsorption of enveloped viruses must take into account the possibility that the label used for these measurements (be it spin label in the case of ESR measurements or DPH in the case of fluorescence polarization) can be transferred from the labeled cells to the virus, thus introducing an artifact into the measurements. The fluorescence label DPH embedded in the cell membrane can also be transferred between cells during cell-cell contact in suspension (*Collard* et al. 1978). Such transfer can also occur between enveloped viruses and cells, even if the virus is noninfectious. Similar exchange of cholesterol may occur between vesicles and the envelope of influenza virus (*Lenard* and *Rothman* 1976). *Moore* et al. (1978) have shown that during the interaction of VSV with unilamellar phosphatidylcholine (PC) vesicles, the virus lost its cholesterol in a temperature-dependent reaction. After the interaction, the fluidity of the viral membrane increased. The cholesterol translocation occurs only during contact between the virus and the vesicle. DPH, on the other hand, can be translocated even through water. This was demonstrated by *Nicolau* et al. (1979a, b) who enclosed a labeled virus suspension within a dialysis bag and put the susceptible cells outside. Such a transfer of label (DPH) was also reported by *Levanon* and *Kohn* (1978), who observed that Sendai and ND viruses (fusing viruses), distinct from other viruses, induced an increase in the viscosity of the cell membranes as probed by the DPH label. However, when the cells were adsorbed with the virus at 4 °C and the unbound virus removed by centrifugation in the cold, the fluorescence polarization measurements per-

formed after a shift to 37 °C showed an increase in fluidity (or a decrease in microviscosity) (*Levanon* et al. 1979). Moreover, measurements of cell-virus complexes without the removal of unbound virus also present another source of experimental artifact. While the optical density of cell suspensions has little effect on fluorescence polarization measurements, as was shown by *Johnson* and *Nicolau* (1977), the turbidity of viral suspensions has a considerable effect due to light scattering. Removal of unbound virus from virus-cell suspensions eliminates this artifact.

Fluorescence polarization measurements, performed by *Levanon* et al. (1977, 1979), demonstrate that increased cell membrane fluidity, induced by virus adsorption, is not restricted to fusing viruses. A variety of naked and enveloped viruses such as picorna-, myxo-, toga- and papovaviruses produced the same effect. The fluidization of cell membranes became apparent within 1 min of adsorption at 37 °C and reached a peak at 5–10 min post adsorption (at 37 °C). This change occurred in different types of cells to which viruses adsorbed, but not in cells that did not adsorb virus, or in cells that had their viral receptors blocked or destroyed. The change was dependent, to a certain degree, on multiplicity of infection and was independent of the infective status of the virus; UV-inactivated viruses produced the same effect as infectious virions.

3.2 Effects of Viral and Cellular Proteins on Membrane Fluidity

Intermixing of lipids between cells and viruses is strongly dependent on the fluidity of the membranes. The higher the fluidity, the better is the exchange. Intermixing of lipids is therefore a measure of membrane fluidity. Fluidization of virus-adsorbed cell membranes was also demonstrated by the ESR method. *Maeda* et al. (1975) used a Sendai virus preparation which was spin-labeled with PC*. Within 5–8 min of incubation of human red blood cells (RBC) with the PC*-Sendai virus, the ESR spectrum underwent a change, indicating the intermixing of phospholipids. When fusion was prevented, either by fixation of cells with gluteraldehyde or by treating the virus with proteolytic enzymes, no fluidity change was observed (*Maeda* et al. 1977a). Fusion conditions and an intact F protein therefore, seem, to be essential for the changes in fluidity. Also, *Homma* et al. (1976) have shown that nonhemolytic, infectious Sendai virus, harvested after only 1 day (early harvest virus), induced fusion and was capable of inducing fluidization of the RBC membrane. Similarly, in a cell mutant (*sil* mutant of KB cells) which is not fused by Sendai virus, the adsorption of PC*-Sendai had no effect on membrane fluidity, whereas in wild-type KB cells a considerable change occurred within 10 min of adsorption (*Koyama* et al. 1978). The importance of F protein is strengthened by the finding of *Koyama* et al. (1978) that adsorption of influenza virus (nonfusing) to wild-type KB cells did not alter the ESR spectrum (*Maeda* et al. 1975). However, when the hemagglutinins of influenza virus were cleaved by proteolytic treatment, the virions containing these hemagglutinins were able to induce the fluidity change (*Nicolau* et al. 1979b) (See also Sect. 6).

The presence or absence of microtubules, microfilaments, or spectrin are also of importance in the changes in fluidity discussed above. *Landsberger* et al. (1978) and *Lyles* and *Landsberger* (1976, 1978) have shown that chicken RBC which possess microtubules had an increased fluidity when adsorbed with myxo- or paramyxoviruses. Mammalian RBCs were not affected. These results are in contrast to the findings of *Maeda* et al. (1975, 1977a), *Homma* et al. (1976), and *Koyama* et al. (1978), which were discussed above. The

difference may be due to the different spin labels used, though both studies were done with the ESR method. The same changes were observed when chicken RBC were treated with lectins such as concanavalin A and wheat germ hemagglutinin instead of virus (*Lyles* and *Landsberger* 1976, 1978). In chicken RBC pretreated with the microtubule-disrupting drug colchicine, there was no change in the membrane bilayer fluidity. The changes described by *Lyles* and *Landsberger* appeared to require cross-linking of the receptor molecules, be it virus receptors or lectin receptors. Hemagglutinin glycoproteins isolated from influenza virus by bromelin treatment (*Brand* and *Skehel* 1972) and succinylated concanavalin A (con A) (*Gunther* et al. 1973), which bind to receptors but do not cross-link them, had no effect on fluidity. On the other hand, multivalent aggregates of influenza virus hemagglutinins (prepared by Triton X-100 extraction), which cross-link receptors, were able to induce changes in fluidity. The importance of cross-linking of spectrin in RBC was demonstrated by *Sekiguchi* and *Asano* (1978). They showed that fusion of RBC ghosts by Sendai virus was prevented if the ghosts were filled beforehand with antispectrin antibodies. Fab fragments of these antibodies which would not cross-link spectrin had no effect on fusion.

A marked increase in fluidity also occurs in plasma membranes of phagocytizing polymorphonuclear leukocytes (*Berlin* and *Fera* 1977). This increase was due to a microtubule-dependent reorganization of membrane lipids. Such an effect can also be expected to occur when viruses are phagocytized or pinocytized (*Stephenson* et al. 1978). Ca^{++} ions play an important role in the assembly and disassembly of microtubules (*Blomberg* et al. 1977; *Welsh* et al. 1978; *Marcum* et al. 1978). Virus-induced increase in intracellular Ca^{++} (*Volski* and *Loyter* 1978b; *Hallett* et al. 1982) might affect the microtubule system, thus bringing about the various effects which were described above (see also Sect. 4.3.3).

3.3 Lateral Mobility of Macromolecules

The lateral movements of molecules during virus-induced cell fusion were demonstrated by *Keller* et al. (1977). They measured resonance energy transfer between two different fluorescent molecules (fluorescein and rhodamin coupled to 18-carbon-long hydrocarbon chains, F18 and R18) embedded in the lipid bilayer of the membrane. When F18-labeled HEL cells were adsorbed at 38 °C with the syncytial (*syn*) mutant of herpesvirus labeled with R18, fluorescence was enhanced at 5–10 min post-adsorption. Such enhancement indicates energy transfer between the different fluorescent probes, which involves an intimate mixing of viral and cell membrane components. Nonfusing herpesvirus did not induce such a transfer (*Keith* and *Snipes* 1977).

The mobility of membrane proteins is also strongly affected at the early stages of virus infection. Mengovirus receptors on Ehrlich ascites tumor cells were evenly distributed (as small clusters) when virus was adsorbed at 0 °C. When the cells were transferred to 37 °C, patching was observed within 5 min, and on some of the cells complete capping was detected. Capping was completed after 60 min at 37 °C (*Gschwender* and *Traub* 1979). Fluorescein isothiocyanate-labeled band-3 protein of human RBC was mobile during both Sendai virus- and polyethylene glycol-induced fusion. This mobility change was evident when no intramembrane particle aggregation was observed (*Fowler* and *Branton* 1977). This change was strongly temperature dependent and was completely prevented at 0 °C.

Maeda et al. (1979) used the fluorescence photobleaching technique to study the changes in membrane receptor mobility induced by Sendai virus. Treatment of 3T3 and KB cells with UV-inactivated Sendai virus significantly increased the mobility of con A receptors and of human β2-microglobulin (associated with histocompatibility antigens) in KB cells, but lipid molecules were less mobile. This is in contrast to the findings of *Levanon* and *Kohn* (1978), *Levanon* et al. (1977, 1979), and *Lyles* and *Landsberger* (1976, 1978) which were discussed above. The difference may be due to the different methods employed in these studies (ESR, fluorescence polarization, fluorescence photobleaching recovery), each one measuring a different type of mobility or fluidity. The experiments of *Maeda* et al. (1979) provide additional information on the importance of F protein in the virus-induced changes in mobility (see above). Mild treatment of Sendai virus with trypsin, which inactivated the fusion and hemolytic activities of the virus without affecting the hemagglutination or neuraminidase activities, did not induce changes in mobility.

4 Membrane Permeability to Ions, Phosphorylated Metabolities, and Macromolecules in Animal Cells

4.1 Induced by Viruses

The first observation of permeability changes in animal cells adsorbing virus was made by *Klemperer* (1960), who studied hemolysis induced by NDV in fowl erythrocytes, Hela cells, and Krebs 2 ascites tumor cells. He observed that at the early stage of hemolysis (3–5 min after addition of the virus at 37 °C) there was an increase in efflux of potassium (K^+) ions from the cells. Nonhemolytic viruses such as influenza, fowl plague, adeno-, EMC, and vaccinia viruses did not induce K^+ release from Hela or Krebs 2 ascites cells although these cells were susceptible to these viruses. Antibodies against NDV abolished the reaction of the virus with the cells, as did calcium (Ca^{++}) ions. According to *Klemperer* (1960), the K^+ release reflected an increased cation permeability of the cell membrane. This in turn caused a rise in the concentration of ions inside the cell, which led to cell swelling and lysis. Cell swelling was indeed observed by *Knutton* (1978) in scanning electron microscope studies (see also Sect. 2.2). Following the report by *Klemperer* (1960), many observations on loss of the ionic barrier in a variety of cells adsorbing different viruses were described (*Fuchs* and *Giberman* 1973; *Negreanu* et al. 1974; *Pasternak* and *Micklem* 1973, 1974a, b; *Okada* et al. 1975b; *Genty* 1975; *Fuchs* et al. 1978, 1980; *Spiegelstein* et al. 1976).

The change in transport can be measured directly by kinetic determination of ions and radioactive isotopes inside and outside the virus-treated cells (*Fuchs* and *Giberman* 1973; *Pasternak* and *Micklem* 1973, 1974a, b; *Fuchs* et al. 1978, 1980) or indirectly by electrophysiological measurements (*Okada* et al. 1975b, *Spiegelstein* et al. 1976; *Fuchs* et al. 1978). Direct measurements of ^{42}K efflux in chicken RBC (preloaded with ^{42}K) adsorbing Sendai virus led *Fuchs* and *Giberman* (1973) to two main conclusions:

1. The virus induced an increased K^+ efflux even under conditions where the virus was rendered nonhemolytic by mild heat inactivation (45 °C for 30 min). This damage to the erythrocyte membrane lasted for a long period of time and was not repaired by the RBC.

2. The enhanced K^+ efflux took place when Ca^{++} (1 m*M*) was present in the medium.

These results are in contrast to the conclusions of *Klemperer* (1960, see above) and also of *Pasternak* and *Micklem* (1973, 1974a, b), who studied the leakage of intracellular [^{3}H]deoxyglucose and [^{14}C]choline from Lettrée cells adsorbing Sendai virus. These latter authors reported that 0.1–1 m*M* Ca^{++} inhibited the virus-induced leakage and, in contrast to the findings of *Fuchs* and *Giberman* (1973) and *Spiegelstein* et al. (1976), that heat-inactivated Sendai virus did not induce K^+efflux in Lettrée cells (*Pasternak* and *Micklem* 1974a).

The immediate effect of virus adsorption is the leakage of K^+ from cells preloaded with ^{42}K (*Fuchs* et al. 1978). The rate of this leakage is much higher than that of free diffusion in the normal nontreated cells. This difference is most pronounced from 5 to 30 min post adsorption, when the efflux reaches its plateau value. At about 60 min post adsorption the total efflux in virus-treated and in control cells is about the same. An interesting concomitant effect was observed with the influx of ^{42}K. The rate of influx into virus-treated cells was considerably enhanced during the first 7–10 min after virus adsorption. It then reached a plateau, and in some experiments even became negative. At 20–30 min post adsorption and thereafter, the rate of ^{42}K influx equaled that of control cells (*Fuchs* et al. 1978). These changes may reflect a repair mechanism by which the cells restore their ion concentration gradient. Also, electrophysiological measurements indicated a sharp drop in the membrane resting potential of virus-treated cells, which peaked at about 8 min post adsorption. Thereafter, within 15–20 min, the resting potential rose to its original value (and in some experiments beyond it). Similar changes were observed in FL cells by *Okada* et al. (1975b) and in Hela cells by *Fuchs* et al. (1978).

Adsorption of Sendai virus also induced an increased influx of sodium (Na^{++}) (*Fuchs* et al. 1978) and calcium (Ca^{++})ions (*Impraim* et al. 1979; *Fuchs* et al. 1980; *Hallett* et al. 1982) and an efflux of Ca^{++} and deoxyclucose (but not fluorescein) from preloaded cells (*Fuchs* et al. 1980). *Impraim* et al. (1980) studied the nature of virally mediated changes in membrane permeability to small molecules. They concluded that the uptake of molecules that were concentrated within cells, such as choline, deoxyglucose, aminoisobutyrate, and glycin, was inhibited by virus adsorption. This inhibition is due either to the leakage of phosphorylated materials (choline, 2-deoxyglucose) from the cells, or to the collapse of the Na^+ gradient resulting from an increased permeability to Na^+ (*Fuchs* et al. 1978) for compounds like glycin or aminoisobutyrate (which are accumulated in the cells by a Na^+-linked mechanism). The uptake of a substance under conditions where its diffusion across the membrane is rate-limiting (such as amino acids at high concentrations or a low Na^+ medium) is stimulated by the adsorption of a virus.

Changes in permeability were also observed under conditions where cell-cell fusion was inhibited (*Impraim* et al. 1979). This may indicate that it is the net entry of water, consequent to the leakage of Na^+ and K^+, which is required for fusion [similar to what was suggested by *Klemperer* (1960) for virus-induced RBC hemolysis (see above)]. *Foster* et al. (1980) studied permeability changes in a variety of cell types using different viruses. Sendai virus induced leakage of [^{3}H]choline from preloaded cells when added to a variety of freshly isolated brain cells or to organ cultures of ferret lung or nasal turbinate. Influenza, rabies, and vesicular stomatitis virus, however, did not cause such a change.

It is possible that virus-induced changes in ion fluxes might be a separate event from the leakage of phosphorylated substances. Some viruses (e.g., Sendai) might cause both, whereas other, e.g., EMC (*Fuchs* and *Kohn,* unpublished results), cause only changes in

ion fluxes. Influenza virus did not cause any change in permeability to [^{3}H]choline in ferret lungs and nasal turbinates (*Foster* et al. 1980). These results were attributed to the inability of influenza virus to induce fusion. The fusion capacity, as well as the infectivity of paramyxoviruses, was attributed to the cleaved form of F protein (*Homma* and *Ohuchi* 1973; *Scheid* and *Choppin* 1974; *Nagai* et al. 1976; *Choppin* and *Scheid* 1980). Similarly, the proteolytic cleavage of the orthomyxovirus HA protein (which appears to exert the function of the F protein of paramyxoviruses) is needed for infectivity (*Klenk* et al. 1975; *Choppin* and *Scheid* 1980). In a recent study, *Huang* et al. (1981) demonstrated that influenza viruses which possess cleaved HA were able to induce both hemolysis and cell fusion under suitable pH conditions. Thus, it is reasonable to assume that given the same conditions, influenza virus would also induce changes in permeability of phosphorylated substances.

During the early period of incubation of Sendai virus with FL cells, the cells become permeable to fragment A of diphtheria toxin. Maximum permeability was observed at the time when the change in ionic flux was at its peak (7 min after transfer from 4 °C to 37 °C). The enhanced permeability for the toxin persisted for about 15 min at 37 °C (*Yamaizumi* et al. 1979), i.e. the exact time for Sendai virus-induced ion permeability change (*Fuchs* et al. 1978). Similarly, Hela, BHK-21 and L cells became permeable to plant toxin at the early stages of their interaction with EMC, Semliki forest virus, or adenovirus (*Fernandez-Puentes* and *Carrasco* 1980). Although the relation of such permeabilization to the changes discussed above is not yet understood, it seems that the ability to induce changes in permeability is not restricted to Sendai virus but is a more general phenomenon. Introduction of macromolecules into cells via virus-induced changes in permeability is discussed in detail in Sect. 6.

4.2 Induced by Lectins

Although earlier reports by *Pasternak* and *Micklem* (1974a, b) showed that con A-induced agglutination had no effect on cell permeability, in a later study they stated that the lectin was active in inducing ion exchange (*Micklem* and *Pasternak* 1977). It is now well documented that a variety of lectins indeed induce changes in cell permeability similar to those induced by viruses. *Quastel* and *Kaplan* (1970) noted an increased K^+ uptake in lymphocytes treated with the mitogen phytohemagglutinin (PHA). *Averdunk* (1972, 1976) reported that the increased rate of ^{42}K uptake in PHA-treated lymphocytes was evident as early as 30 s after addition of the lectin to the cells. These results were confirmed by *Segel* and *Lichtman* (1976). In this system changes in permeability for Na^+ were also reported (*Averdunk* and *Lauf* 1975). PHA also caused an increased efflux of ^{42}K and ^{86}Rb from preloaded rat thymic and human lymphocytes (*Segel* et al. 1976). Similarly, an increase in K^+ efflux was observed in Ehrlich ascites tumor cells treated with con A (*Aull* and *Nachbar* 1973; *Aull* et al. 1977), in direct correlation to the degree of agglutination produced by the lectin (*Inoue* et al. 1975). Aminoisobutyric acid and cycloleucin uptake was inhibited in a number of tissue culture cell lines treated with wheat germ agglutinin (WGA) (*Li* and *Kornfeld* 1977). This inhibition is very similar to that reported by *Impraim* et al. (1980) for Sendai virus-treated Lettree cells and occurred also within minutes of WGA addition (*Li* and *Kornfeld* 1977). On the other hand, *Van den Berg* and *Betel* (1972) demonstrated an increased transport of aminoisobutyrate 5 min after the addition of con

A. This increase, however, required protein synthesis, whereas we have found that changes in K^+ flux induced by virus were not sensitive to cycloheximide (unpublished results).

4.3 Mechanism of Permeability Changes

As for the mechanisms by which Sendai virus induces changes in membrane permeability, the following alternatives can be considered:

1. According to *Shimizu* et al. (1976), the Sendai virus envelope is usually incomplete and has a number of discontinuities. During the fusion of the viral envelope with the cell membrane, these discontinuities produce "pores" or "microholes" in the membrane through which molecules can pass freely.
2. Viruses change the activity of Na^+/K^+ ATPase either by a direct or indirect action on the cellular enzyme or by the insertion of the viral ATPase (*Kohn* 1970; *Fuchs* and *Giberman* 1973) into the cellular membrane.
3. Ca^{++} ions serve as a mediator or a messenger in cellular metabolic processes leading to changes in permeability.
4. Permeability changes are the result of a virus-induced change in the physical properties of the cell membrane or of changes in the fluidity of membrane lipids (see also Sect. 3).
5. Viruses affect membranal enzymes involved in phosphate metabolism, such as phosphatases or kinases, which in turn activate another protein responsible for the permeability changes.

4.3.1 Microholes

The possibility that viruses fused to the cell membrane produce pores or microholes which would allow for the free passage of ions and small molecules has been suggested by many investigators (*Appostolov* and *Almeida* 1972; *Pasternak* and *Micklem* 1973, 1974a, b; *Micklem* and *Pasternak* 1977; *Poste* and *Pasternak* 1978; *Impraim* et al. 1979, 1980; *Wyke* et al. 1980). In a close analogy with intercellular communication ("gap") junctions (*Pitts* 1975) the viral pores would thus be considered as a half junction. Both pores or channels allow free passage of ions and phosphorylated compounds such as nucleotides and sugar phosphates, and both types of channel are closed by Ca^{++} (*Rose* and *Loewenstein* 1975; *Loewenstein* 1979; *Pasternak* and *Micklem* 1974a, *Wyke* et al. 1980). These virus-induced changes also resemble those occurring after immune cytolysis of cells (*Holmes* et al. 1969; *Iles* et al. 1973; *Seeman* 1974, *Hallett* et al. 1981; *Campbell* and *Luzio* 1981). In this case the membrane behaves like a sieve, permitting ions and molecules up to a certain size to pass by diffusion. This would permit free movement of K^+ in both directions, to the outside along the concentration gradient and to the inside by solvent drag with water, because of the change in osmotic pressure. Production of "holes" in artificial membranes adsorbing viruses has recently been described by *Durham* (1978). He used lipid vesicles which contained the Ca^{++}-sensitive probe arsenazo III. Attachment of some plant viruses, as well as vaccinia or frog 3 viruses, to such vesicles in buffer containing Ca^{++} resulted in a chemoluminescent reaction, indicating damage to the vesicle membrane.

The idea of virus-induced "permanent holes" in the membrane which might result

from the clustering of viral proteins is somewhat analogous to hydrophilic channels formed by polypeptide ionophores (*Carrasco* 1977; *Pressman* 1976; *Ball* and *Medzon* 1976; *Baxt* and *Bablanian* 1976). In the case of virus-induced permeability changes, this idea is backed by two types of experiment. One, performed by *Okada* et al. (1975b), showed that the changes in the resting potential induced by Sendai virus (see above) also occurred at low temperatures (10°–15 °C). These results might be taken to indicate that once a structural pore is produced it stays "opened", also at low temperatures. The second type of experiment is that reported by *Wyke* et al. (1980). They have shown that Sendai virus grown in embryonated eggs for 20 h instead of 48–72 h – "1-day virus" – did not cause permeability changes. Such a virus has an undamaged envelope: it is impermeable to heavy metal ions used for negative staining, and, though it fuses with the cell membrane, it does not cause hemolysis (*Shimizu* et al. 1976). It was thus concluded (*Wyke* et al. 1980) that only a virus with a damaged ("leaky") envelope can induce permeability changes. On the other hand, when we studied the virus-induced K^+ flux in Hela cells (*Fuchs* and *Kohn*, unpublished results), we found no difference between 1-day and 2-day virus. *Wyke* et al. (1980) have also shown that the presence of intact F protein (which 1-day virus contains) is essential for the induction of permeability changes, since F protein induces cell-virus fusion.

By using peptides of different sizes labeled with fluorescent compounds, *Wyke* et al. (1980) could estimate the size of Sendai virus-induced pores. Peptides having a molecular weight larger than 1000 did not enter the cells; this suggested a pore diameter of about 1 nm. The ability of Sendai virus to introduce macromolecules into cells (*Tanaka* et al. 1975; *Yamaizumi* et al. 1979; see also above and in Sect. 6) was interpreted by *Wyke* et al. (1980) as being due to larger pores produced at a high multiplicity of infection.

Several other reports, however, do not favor the pores or microholes theory. *Okada* et al. (1975b) claimed that virus-induced membrane potential changes were independent of temperature. *Fuchs* et al. (1980), however, as well as *Micklem* and *Pasternak* (1977) found that virus-induced K^+ flux and leakage of choline were temperature dependent. Both changes were evident only at temperatures higher than 20 °C. Moreover, in a temperature shift experiment, *Fuchs* et al. (1980) showed that when the primary interaction of the virus with the cells (in both chicken RBC and Hela cells) was performed at 37 °C for 2–3 min and the cells were then transferred to 4 °C, no efflux or influx of ions or lysis of RBC occurred. Treatment for 2–3 min at 37 °C is sufficient for changes in cell morphology, fluidity, and permeability to occur (*Fuchs* et al. 1978; *Levanon* et al. 1977; *Bächi* et al. 1973). In similar experiments testing the leakage of phosphorylated materials or Ca^{++} uptake, however, *Impraim* et al. (1979, 1980) reported opposing results. This discrepancy can be explained by the different experimental protocols or by the assumption (as suggested above) that virally mediated change in ion fluxes is an event separate from changes in permeability of phosphorylated metabolites. The theory of the viral "leaky" envelope being the site of the pore is also contradicted by the experiments of *Hosaka* and *Shimizu* (1972b) and *Volsky* and *Loyter* (1978a). Both groups have prepared reassembled viral envelopes, devoid of the viral nucleocapsid, which were capable of inducing hemolysis (and thus changes in permeability) and cell-cell fusion. *Wyke* et al. (1980) attributed this capacity to residual trace amount of detergents in the reassembled-envelope preparations. Finally, since the virus-induced pores theory is based mainly on paramyxovirus-induced permeability, it is not sufficient, by itself, to explain permeability changes induced by nonenveloped viruses (*Fernandez-Puertes* and *Carrasco* 1980; see also above).

4.3.2 Na^+/K^+ ATPase

The involvement of Na^+/K^+ ATPase in virus-induced ion fluxes was suggested by *Fuchs* and *Giberman* (1973). Involvement of Na^+/K^+ ATPase was also reported for lectin-induced K^+ flux (*Kaplan* 1978; *Quastel* and *Kaplan* 1975; *Quastel* et al. 1970) and for increased K^+ influx in a variety of virus-transformed animal cell lines (*Elligsen* et al. 1974; *Graham* et al. 1973; *Kasarov* and *Friedman* 1974; *Kimelberg* and *Mayhew* 1975, 1976).

Fuchs et al. (1980), however, demonstrated that virus induced K^+ influx was insensitive to ouabain. This seems to rule out the involvement of Na^+/K^+ ATPase in the virus-induced damage.

4.3.3 Ca^{++}

Calcium ions (Ca^{++}) may play a role in the effects described here. It is now well established that both virus-induced and naturally occurring fusion processes are strongly Ca^{++}-dependent (*Okada* and *Murayama* 1966; *Poste* and *Reeve* 1972; *Fuchs* and *Levanon* 1978; *Shainberg* et al. 1969). However, there is contradictory evidence as to the molecular events in which Ca^{++} participates. *Poste* (1970, 1972) and *Poste* and *Allison* (1971) suggested that the displacement of Ca^{++} from sites within the surface membrane is an essential step in fusion. *Loyter* et al. (1976) and *Zakai* et al. (1976) suggested that during fusion of RBC, Ca^{++} penetrates to the cytoplasmic side of the membrane and causes polymerization of spectrin and consequently the aggregation of the inner membrane particles (see also Sect. 2.2). Membrane phospholipids that become exposed in the two adjacent cells intermix, and the cell membranes fuse. *Volsky* and *Loyter* (1977) have shown that fusion is indeed accompanied by a transient influx of Ca^{++}. Similar observations were made by *Impraim* et al. (1979) and by *Fuchs* et al. (1980), who also demonstrated a transient virus-induced efflux of Ca^{++} from preloaded cells. Transient influx of Ca^{++} also occurs in *Urechis caupo* eggs during fertilization (*Paul* and *Randal* 1976), as well as within seconds after the addition of complement to an immune cytolysis system (*Campbell* et al. 1979). On the basis of the existing evidence it is difficult to decide whether Ca^{++} is involved in producing or repairing the virus-induced leakage. The addition of Ca^{++} to Sendai virus-infected RBC and Lettree cells decreased the virus-induced leakage of metabolites (*Klemperer* 1960; *Pasternak* and *Micklem* 1974a). Addition of Ba^{++}, Mn^{++}, and UO_2^{++} to NDV- or Sendai-infected cells exerted the same effects (*Zakai* et al. 1974b; *Apostolov* and *Sawa* 1979). On the other hand, *Impraim* et al. (1979) showed that virally mediated increase in Ca^{++} movement was a consequence and not a cause of membrane fusion (virus-cell) and that an increase in intracellular Ca^{++} was unlikely to be necessary for cell-cell fusion. The well-established connection between Ca^{++} and K^+ flux does not favor the above conclusions. *Lew* and *Ferreira* (1976) reported a direct correlation between the intracellular level of Ca^{++} and the rate constant for equilibrium of ^{42}K across the membrane. They concluded that permeability of K^+-selective channels is controlled by Ca^{++} concentration inside the cell. An increase of intracellular free Ca^{++} was indeed observed in human and pigeon RBC ghosts treated with Sendai virus, even in low Ca^{++} medium or with 1-day virus (*Hallett* et al. 1982). A strong linkage between Ca^{++} concentration and K^+ or ^{86}Rb flux was also reported by *Wenner* and *Hackney* (1976) and *Reed* (1976).

In view of this contradictory evidence as to the role of Ca^{++}, direct measurements of Ca^{++} concentrations inside and outside the cell during the first minutes of virus adsorp-

tion might shed more light on its involvement in virus-induced permeability changes and in the fusion reaction.

4.3.4 Fluidity

The change in membrane fluidity and in the mobility of membrane proteins is discussed in detail in Sect. 3. Fluidity change can be responsible for formation of channels allowing the passage of ions and small molecules, or it can alter the activity of the existing ion pumping site; finally, it can increase the rate of pinocytosis, thus allowing the uptake of molecules. The specific activity of membrane-bound enzymes, such as Ca^{++}-ATPase or Na^+/K^+-ATPase, was shown to be indeed dependent on membrane fluidity (*Warren* et al. 1974; *Kimelberg* 1975). Treatment of cells with unsaturated fatty acids led to an increase in the fluidity of the cell membrane (*Orly* and *Schramm* 1975; *Hoover* et al. 1977; *Kohn* et al. 1980a). *Fuchs* et al. (1980) incubated Hela cells for 6 h with linoleic acid (C18:2) and then followed the uptake of ^{45}Ca into these cells. The increased fluidity induced by linoleic acid had no effect on ^{45}Ca uptake, nor was there a synergetic effect between linoleic acid and the virus. It seems, therefore, that fluidization of cell lipids per se, either by adsorption of virus or by unsaturated fatty acids, was not the common cause of the changes in permeability.

4.3.5 Phosphorylating Enzymes

Let us now consider the fifth possibility suggested above, namely, that membranal enzymes involved in phosphate metabolism play a role in the permeability changes induced by virus. The uptake of Ca^{++} by chick fibroblasts in the presence of ATP was interpreted by *Perdue* (1971) as due to phosphorylation of carrier protein in the cell membrane. This uptake was inhibited by oligomycin but not by ouabain. The temperature dependence of the virus-induced ^{42}K uptake by Hela cells (*Fuchs* et al. 1980) suggested the involvement of an enzyme. Independent evidence for such involvement was provided by *Rozengurt* and *Heppel* (1975), who showed that exposure of SV40-transformed, but not normal, mouse 3T3 cells to external ATP dramatically changed their permeability to *p*-nitrophenylphosphate, a molecule that ordinarily does not permeate cells. Intracellular pools labeled with radioactive uridine, adenosine, deoxyglucose, or ^{86}Rb were depleted within minutes upon addition of as little as 0.2 m*M* ATP (but not other nucleotide triphosphates) (*Rozengurt* et al. 1977). Treatment of Ehrlich ascites tumor cells with ATP induced an increased Ca^{++} uptake (*Landry* and *Lehninger* 1976) and increased the K^+ flux (*Aiton* and *Lamb* 1975). The K^+ flux was insensitive to ouabain (compare to *Fuchs* et al. 1980) but was blocked by the nonspecific enzyme inhibitor tetramethylammonium ions. *Mastro* and *Rozengurt* (1975) attributed these changes to endogenous plasma membrane kinase. In transformed cells the activity of this kinase was five- to tenfold higher than that of untransformed cells. These studies were extended by *Makan* (1978), who showed that an ATP-requiring protein kinase and a fluoride-inhibitable protein phosphatase might be involved in the control of permeability in transformed cells. Phosphorylation of membrane proteins may be involved in the control of ion fluxes in astrocytes (*Trams* 1974) and perhaps also in RBC (*Park* and *Snow* 1972). In Hela cells labeled with [^{3}H]deoxyglucose, where glucose efflux did not respond to treatment with ATP, addition of Sendai virus plus ATP increased the membrane permeability severalfold above that induced by the

virus alone (*Fuchs* et al. 1980). There are several possible explanations why ATP in the presence of the virus was very active whereas ATP alone was not:

a) The virus itself provides a protein kinase activity catalyzing the transfer of phosphorus to a specific protein(s), which in turn increases membrane permeability. Indeed, Sendai virus was found to contain a high activity of protein kinase (*Kohn* et al. 1980b).
b) Since according to *Makan* (1978) NaF (an inhibitor of protein phosphatase) enhanced the ATP-induced permeability in Hela cells, the possibility exists that the virus also inactivates such a phosphatase.
c) A kinase is present in Hela cell membranes in a cryptic form. Adsorption of virus induces a conformational change which makes this kinase accessible to ATP. This possibility was studied by *Kohn* et al. (1980b), who found that adsorption of virus in the presence of $^{32}\gamma$-ATP did not increase the labeling of any cell membrane protein.
d) In the presence of virus, ATP may enter the cells and produce some of the observed effects from the inside. An increase in phosphorylation of some intracellular proteins was indeed observed by *Kohn* et al. (1980b).

4.3.6 Transmethylation

Another possible mechanism, which may explain the virus-induced changes in permeability, comes from studies on the interaction of hormones, neurotransmitters, lectins, and immunoglobulins with cells (*Hirata* and *Axelrod* 1980). Many cell types were found to contain in their membranes two phospholipid methyltransferases (MT). The first (MTI) converts phosphatidylethanolamine to phosphatidyl-*N*-monoethylethanolamine. The second (MTII) catalyzes the stepwise methylation of the product of MTI to phosphatidylcholine (*Crews* et al. 1980; *Hirata* and *Alexrod* 1978a; *Hirata* et al. 1979a, b, 1980). In RBC (as probably in all cells) MTI is located on the cytoplasmic side of the membrane whereas MTII is on its outer face (*Hirata* and *Axelrod* 1978b). During methylation a facilitated flip-flop occurs, and the methylated phospholipids are translocated from the cytoplasmic side of the membrane to the exterior surface in less than 2 min (*Hirata* and *Axelrod* 1980). When a methyl donor was introduced into RBC ghosts the fluidity of the membrane increased, and this change was inhibited when methylation inhibitors were introduced into the system (*Hirata* and *Axelrod* 1978b).

Con A induced mast cells to release histamine (*Ishizaka* and *Ishizaka* 1969). Such a treatment also resulted in an increased methylation of phospholipids which preceded the histamine release. Both were inhibited by methyltransferase inhibitors (*Hirata* et al. 1979a). Increase in fluidity, phospholipid methylation, and Ca^{++} influx followed by histamine release also occur when mast cells are treated with antibodies or $F(ab')_2$ fragments of antibodies against immunoglobulin E. The phospholipid methylation reaction seems to be a prerequisite for Ca^{++} influx, since its inhibition abolished all the effects described above (*Ishizaka* et al. 1980). Similarly Ca^{++} influx, which is a major event in mitogenic processes in lymphocytes, was blocked when phospholipid methylation was inhibited (*Hirata* and *Axelrod* 1980). In studies with rat and human RBCs *Strittmatter* et al. (1979) found that phospholipid methylation increased the efflux of Ca^{++} by increasing the Ca^{++}-ATPase activity. These changes were attributed to the increase in membrane fluidity induced by phospholipid methylation. Hela cells (which contain β-adrenergic receptors)

responded to binding of catecholamines by increased phospholipid methylation and increased levels of cyclic AMP. β-Adrenergic antagonists (but not α-adrenergic antagonists) blocked both the methylation and cyclic AMP formation (*Hirata* and *Axelrod* 1980). Changes in ion fluxes as a primary mode of action also occur in interferon-treated cells (*Grollman* et al. 1978). Interferon changes the membrane potential and its action is potentiated by dibutyryl cyclic AMP. Ouabain, however, blocks the antiviral action of interferon.

The correlation of virus-induced changes with increased phospholipid methylation is at this stage speculative and awaits further experimentation. It is of interest, however, to mention here two types of experiment. One, reported by *Sugiyama* (1977), indicated that Sendai virus induced histamine release in mast cells. The second, reported by *Ohki* et al. (1975, 1978) showed that one of the very early effects in the interaction of Sendai virus with Ehrlich ascites tumor cells (2 min post adsorption) was a twofold increase in the intracellular concentration of cyclic AMP. Moreover, inhibitors of cyclic nucleotide phosphodiesterase increased the frequency of cell-cell fusion by a factor of 2. This effect was Ca^{++}-dependent (*Ohki* et al. 1978).

4.4 Role of Permeability Changes in Viral Infection

Whereas changes in permeability induced by neurotransmitters or hormones are of significance because of their essential physiological role, the significance of virus-induced permeability changes in virology or clinical virology is less obvious. Although virus induces in vitro release of histamine from mast cells (*Sugiyama* 1977), γ-aminobutyrate from brain slices, and corticotropin from pituary cells (*Pasternak* 1980), the in vivo significance of this is also not clear.

Although this review deals with the early interaction of viruses with cells, it is of interest to mention in this section the work of *Carrasco* and co-workers. In a series of papers (*Carrasco* and *Smith* 1976; *Carrasco* 1977, 1978; *Contereras* and *Carrasco* 1979; *Alonso* and *Carrasco* 1981) they have shown that during infection of cells with a variety of enveloped and nonenveloped RNA or DNA viruses, the cells become permeable to ions and small molecules at the time of onset of late viral protein synthesis. Molecules of molecular weight below 750 entered the infected cells, whereas molecules of molecular weight 10 000 and above were excluded. These findings led *Carrasco* and co-workers to the hypothesis that it is the increase in the monovalent ion concentration inside the cells (as well as the divalent ions concentration see *Durham* 1977) that preferentially blocked initiation of host protein synthesis while promoting viral protein synthesis. This increase in intracellular ion concentration is induced throughout the infection by viral coat protein(s) embedded in the membrane. At the penetration stage it is induced by the proteins of the infecting virus, while in the later stages of infection it is induced by the coat proteins synthesized in the infected cell. This hypothesis correlates the changes in permeability induced by viruses in the early and late stages of infection with the well-documented effects of elevated Na^+ and K^+ concentrations on virus multiplication (*Egberts* et al. 1977; *Garry* et al. 1979; *Nair* et al. 1978).

During the virus-induced permeability changes in the later stages of infection, the cells became permeable to a number of translation inhibitors which are impermeable to normal uninfected cells (*Carrasco* 1978; *Contereras* and *Carrasco* 1979). These findings

may open the way to design new types of antiviral agents which will penetrate and kill only cells infected by viruses (*Carrasco* 1978).

5 Changes in Permeability in Bacteria

5.1 Induced by Bacteriophages

Doerman (1948) observed that bacteria which adsorbed phages swelled within a few minutes and eventually lysed. In later studies, *Puck* and *Lee* (1954) traced these events in infected bacteria to an increase in host cell permeability to cations. *Hessler* et al. (1967) observed that bacteria which adsorbed phages, unlike uninfected bacteria, accumulated acridine. These observations were later confirmed by *Duckworth* (1970). *Sekiguchi* (1966) noted the loss of ATP and NAD from bacteria infected with T4 rII mutant bacteriophages. An explanation for the leakage of ions and accumulation of other molecules by bacteria adsorbing bacteriophages was proposed by *Buller* and *Astrachan* (1968) and by *Furrow* and *Pizer* (1968), who claimed that the permeability changes were due to a changed pattern of incorporation of membrane phosphatidyl-ethanolamine and phosphatydylcholine, and that in the normal course of infection repair of the damaged membrane, involving synthesis of membrane lipids, was required.

Also *Shapira* et al. (1974) showed that *E. coli* cells, adsorbing osmotically shocked T4 phage ghosts, lost their intracellular potassium within 1 min of ghost attachment. However, in bacteria which adsorbed intact infective phages and which had their protein synthetizing machinery unimpaired, the potassium level also dropped within 1 min but it was restored in the next 2 min, so that the bacteria could resume their productive interaction with the phage. The restoration of the pumping activity of the bacterial membrane required protein synthesis, and the new protein must have been coded by the phage DNA, since in bacteria which adsorbed formalin-treated phages (so that phage DNA cannot be injected), or phage ghosts (which lack DNA entirely), the loss of potassium was irreversible and led to bacterial death. However, when the ghost-treated bacteria were superinfected with infectious phage (which was able to inject its DNA) within 30 s before or after adsorption of the ghosts, the transport activities of the bacterial membrane were restored, and the bacteria were now able to produce progeny infectious phage. According to *Mathews* (1971), the proteins responsible for repairing the damage to the membrane are coded by the rII region of the bacterial chromosome, presumably by genes rIIA, rIIB, 39, and 52 (*Takats* and *Rosenbush* 1975). The experiments of *Shapira* et al. (1974) implicated a protein coded by the bacteriophage DNA as responsible for the repair. It is probable therefore that the page-coded protein derepresses the bacterial repair genes. *Shapira* et al. (1974) also showed that the permeability change, induced soon after adsorption of phage ghosts, must have been brought about by a component of the bacteriophage tail, probably a protein related to the E1 group of colicins.

In addition to T4, described above, *Ponta* et al. (1976) and *Herrlich* et al. (1974) showed that T7 phage also caused increased permeability in *E. coli*. This effect seems to be dependent on phage protein M, which by altering the cellular phospholipid synthesis kills the bacteria. Alteration of the bacterial membrane by the attachment of T7 can be monitored by ANS binding. *Condit* (1975) demonstrated that male *E. coli* cells became permeable to *o*-nitrophenyl-β-galactopyranoside (in the presence of lactose transport inhibitor), and

this was accompanied by a decrease of ATP at 8–12 min after infection at 30 °C. He also showed that this change in permeability required the presence of genes for F factor (male) and phage-specific protein synthesis. Also *Britton* and *Haselkorn* (1975) observed T7-induced permeability to phosphorylated compounds, as well as loss of ability to accumulate amino acids in bacterial cells.

5.2 Induced by Colicins

Colicins are bactericidal proteins produced by Enterobacteriaceae under the control of genes localized on plasmids (*Nomura* 1964; *Holland* 1975). Plasmids are self-replicating entities of DNA, potentially infectious. Colicin-producing plasmids (Col) are in a way analogous to viruses because (a) they adsorb to the same receptors as related phages, (b) they replicate in "infected" hosts, and (c) they produce proteins that are lethal to other hosts.

Colicins may be grouped into two classes (Table 1): Colicins in one group (B) act as nucleases, while the others (A) affect the structure and function of the bacterial membrane, so as to deenergize it and make it permeable to ions and small molecules. Colicins of group A interact with the bacterial membrane from the outside, whereas those in group B have to gain entry to the cytoplasm of the bacteria in order to express their nucleolytic activity.

Of particular interest in the context of this review are the colicins of group A. Colicins E1 and K, which have been studied most, affect active transport in *E. coli*, as well as macromolecular synthesis, and kill the bacteria (*Nomura* 1963; *Fields* and *Luria* 1969; *Feingold* 1970; *Wendt* 1970; *Lusk* and *Nelson* 1972; *Phillips* and *Cramer* 1973; *Plate* et al. 1974; *Hirata* et al. 1969; *Nieva-Gomez* et al. 1976; *Tokuda* and *Konisky* 1978a; *Kopecky* et al. 1975). Killing of the bacterial cells has a single-hit character and requires that the colicins interact with specific receptors on the outer membrane of the bacteria. Bacteria lacking

Table 1. Colicins

Group	Designation	Mol. Wt. (daltons)	Function	Target	Reference
A	E_1	56 000	Two-step action	Inner plasma membrane	*Schwartz* and *Helinski* (1971)
	K	45 000	Changes in inner membrane; formation of ion permeable channels (?)		*Jesaitis* (1970)
	Ia	80 000			*Konisky* and *Richards* (1970)
	Ib				*Konisky* and *Tokuda* (1979)
	A				*Jetten* and *Vogels* (1973)
	L				*Foulds* and *Chai* (1978)
B	E2	60 000	DNase	DNA	*Herschman* and *Helinski* (1967)
	E3	60 000	RNase	16-S ribosomal RNA	*de Graaf* (1979)
	E4				*Cavard* and *Lazdunski* (1979)
	I-JF246				
	V	92 000			*Hutton* and *Goebel* (1962)

such receptors are not sensitive to the action of colicins. After adsorption of colicin to the receptor, in the presence of Mg^{++} (stage I), there is an energy-dependent interaction of the colicin with the cytoplasmic membrane (stage II) which results in the alteration of membrane permeability and eventually in cell death. Colicin E1- or K-treated bacteria become permeable to potassium, magnesium, and cobalt ions (*Jetten* and *Vogels* 1973; *Fields* and *Luria* 1969); their intracellular potassium concentration drops below a level compatible with the normal physiological functions of the bacterial cells, and they die. Adsorption of 1 killing unit colicin K empties the cell of its potassium content. Plating of bacteria treated with colicin K or E1 (but not E2 or E3), on agar supplemented with a high concentration of potassium (100 m*M*) and Mg^{++} (1 m*M*), so as to restore the freely diffusing cations to their original intracellular physiological levels, allowed the survival of bacteria which would otherwise have died (*Tokuda* and *Konisky* 1978a). *Shapira* et al. (1974) also showed that when bacteria adsorbing T4 ghosts, which would otherwise die, were resuspended in suitable concentrated lysates of other bacteria (thus restoring the concentration of all solutes in the medium to that normally prevailing in the bacteria), they would survive, in spite of their leakiness.

Bacteria may be sensitive, resistant, or tolerant to the action of colicin. In resistant strains receptors are either missing or defective, and such bacteria do not adsorb colicin; in tolerant mutants colicin is bound, but there is an intracellular barrier to cell killing (*Holland* 1975). According to the chemiosmotic theory (*Mitchell* 1961), a gradient of electrochemical potential for protons is generated across the bacterial membrane by extrusion of protons to the cell exterior coupled with respiration and/or ATP hydrolysis (*Gould* 1979). This gradient is composed of the chemical gradient (ΔpH) and the gradient of electric potential ($\Delta\psi$), and is thought to be the major driving force for active transport (*Ramos* and *Kaback* 1977). Uncoupling agents such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) or 2,4-dinitrophenol collapse the gradient and block active transport of ions such as K^+ and Mg^+. *Gould* and *Cramer* (1977) showed that in colicin K- or E_1-treated bacteria the loss of potassium paralleled an increased rate of proton efflux. Since colicin-treated bacteria or their vesicles can generate ΔpH, but not transmembrane $\Delta\psi$, the depolarization is due to increased permeability to ions other than protons (*Tokuda* and *Konisky* 1978a). Nevertheless, the extent of electrogenic uptake of potassium is not sufficient to explain the colicin-dependent membrane depolarization. *Tokuda* and *Konisky* (1978a), as well as *Weiss* and *Luria* (1978), used a lipophilic cation, triphenylmethylphosphonium ($TPMP^+$), which equilibrated across the bacterial membrane according to the transmembrane potential. When colicin E_1 (*Tokuda* and *Konisky* 1978a), or K (*Weiss* and *Luria* 1978), was added to *E. coli* loaded with $TPMP^+$, the membrane potential dropped considerably (to 50 mV) without affecting the intracellular pH; the authors concluded that colicin K or E_1 acted as an ion channel, i.e., that the cytoplasmic membrane became permeable to ions other than protons. Valinomycin mimicked the action of colicin by abolishing $\Delta\psi$ alone, and by allowing potassium to move as counter ions to protons. Colicins K and Ia inhibit transport of glutamine (*Plate* et al. 1974), proline, and thiomethyl galactoside (*Gilchrist* and *Konisky* 1975). These compounds, when preaccumulated in the cells, leak out following treatment with colicin. It is not, however, a general leakiness that colicin induces, since the uptake of α-methyglucoside is enhanced tenfold in cells treated with colicin Ia (presumably via the phosphotransferase system (*Gilchrist* and *Konisky* 1975). Glutamine transport, which requires both ATP and membrane potential (*Plate* 1979), is inhibited by colicin K as well as by valinomycin.

The channel hypothesis of colicin action is contested by *Kell* et al. (1981), since (a) it is not consistent with the single-hit character of colicin killing, (b) the proton uptake and the rapid dissipation of $\Delta\psi$ in bacteria treated with colicin does not fit the observed rates of K^+ efflux (*Schein* et al. 1978; *Gould* 1979), (c) colicin has a large effect on fluorescence changes of neutral probes (*Gould* 1979), and (d) colicin adsorbed on sepharose beads is fully active (*Lau* and *Richards* 1976). *Kell* et al. (1981) postulate that transducing membrane systems contain proteins which act as conformationally switchable proton conductors (*Changeux* et al. 1974), permitting lateral proton transfer along the surface of the membrane and acting as links between proton-active sources and sinks (*Williams* 1978). Such a "protoneural" network would be the target of colicin. Indeed, *Lieberman* and *Hong* (1974) and *Hong* (1977) isolated a *ts* mutant of *E. coli* ECF, in which the energy made available at the membrane was not coupled to active transport of amino acids and α-methyl-D-thiogalactoside or to ATP synthesis. It is possible that the protein encoded by the *ecf* gene (mapped in the 60 min region of the bacterial chromosome) might be the target for colicin. *Plate* (1976) isolated *ts* mutants B51 and B51-70 which, unlike ECF, were impaired in proline and methylglucoside transport but not in glutamine transport. *Plate's* findings, and the fact that his mutants were not sensitive to colicin K, provide evidence that there is a protein component in the membrane of *E. coli* which functions in energy coupling of the respiration-linked active transport system and is essential to the action of colicin K (but not E1, E2, or E3).

Another possible connection between the target site of colicin E1 and the site of origin of DNA replication in *E. coli* may be discerned from the results of the following experiments: *Lion* and *Bergmann* (1961) showed that freeze-dried *E. coli* bacteria are exponentially killed when exposed in the dry state to molecular oxygen. *Israeli* et al. (1975) found that the target site for oxygen was the DNA initiation complex bound to the bacterial membrane. *Israeli* et al. (1974) also demonstrated that oxygen injured the transport system for ONPG and potassium. Finally, *Israeli* and *Kohn* (1972) found that colicin E1, adsorbed to *E. coli* before freeze-drying, protected the cells in the dry state against the lethal effects of oxygen (a decrease in the survival decay constant from 3.5 to 2.0). The conclusion drawn was that the common target for oxygen and colicin was the membranal site which bound the DNA initiation complex.

The primary target proteins on the surface of colicin-sensitive bacteria may be the same proteins that interact with lactoperoxidase-thiocyanate peroxide (LPS) (*Law* and *John* 1981), because both colicins and LPS inhibit $\Delta\psi$ without affecting ΔpH (*Kell* et al. 1981). Against the concept of the same target for colicin and oxygen is the study by *Kopecky* et al. (1975) showing that colicin-treated bacteria can be rescued by restoring the ionic environment in the damaged cell.

What happens in the bacterial membrane upon adsorption of colicin? Some findings possibly relevant to the mechanism of action of group A colicins on bacteria were reported by *Knepper* and *Lusk* (1976). They found that upon adsorption of colicin E1 to sensitive or tolerant bacteria, there occurred a loss of several bacterial proteins of molecular weight 69 000, 30 000 and 25 000. Also, *Sabet* (1978) demonstrated the loss of six proteins from colicin K treated bacteria: at least two of them (molecular weight 122 000 and 62 000) seemed to serve as colicin receptors. *Cavard* et al. (1968), *Cramer* and *Keenan* (1974), and *Lusk* and *Park* (1975) found that colicins E1 and K induced considerable changes in the phospholipid composition of bacterial membranes (such as hydrolysis of phosphatidylethanolamine), but these changes did not seem to be the primary effects of colicin action

and not directly related to the changes in transport of K^+ and the subsequent death of the bacteria.

In order to further analyze the mode and site of action of group A colicins, two approaches were employed. In one, a comparison was made between the activity of colicins on intact bacteria, on the one hand, and on vesicles prepared from bacterial membranes or on liposomes incorporating colicin receptors, on the other. The second approach was based on controlled proteolysis of colicins and the study of the functional activity of the fragments.

5.2.1 Effect of Colicins on Bacterial Vesicles

Bacterial vesicles are bags which are devoid of cytoplasmic constituents and are made of bacterial membranes. They are produced by removal of the bacterial cell wall by lysozyme, followed by osmotic lysis of the resulting spheroplasts in the presence of chelates and nucleases. However, some cell wall components are still left on the vesicles. Vesicles retain the same polarity as the membranes of intact cells and catalyze active transport of various solutes.

Tokuda and *Konisky* (1978a, b) reported that colicin Ia-treated *E. coli* K12, as well as membrane vesicles prepared from such cells, were depolarized and were unable to generate membrane potential. However, when the vesicles were first prepared from intact bacteria and only then treated with colicin Ia, the colicin was totally inactive. On the other hand, when such vesicles were frozen and thawed in the presence of colicin Ia, colicin was retained inside the vesicles. Such vesicles lost their transport activity, their membranes became depolarized, and the ions leaked out. This phenomenon was observed in vesicles prepared from sensitive, resistant, or tolerant bacterial cells, and it occurred with as few a 900 colicin molecules per vesicle (of which about 230 are retained inside).

Why is there a difference between frozen and thawed vesicles and nontreated ones? It seems that the vesicles which were not frozen lacked colicin Ia receptors or had the receptors displaced; this would account for the lack of activity of colicin on such vesicles. However, when the vesicles are frozen and thawed, cracks and tears in the vesicles may permit the entry of colicins and their direct contact with the inner membrane, where colicin seemed to be acting. This would also explain the finding that in this situation, colicin Ia is active on vesicles prepared from sensitive, resistant, or tolerant bacteria. This explanation, however, should be viewed with caution, since in the case of other colicins of group I, such as E1 (*Bhattacharyya* et al. 1970), K (*Kabat* and *Luria* 1970), A (*Jetten* and *Vogels* 1973), and L (*Foulds* and *Chai* 1978), the inhibition of transport in vesicles occurred even without freezing and thawing (*Takagaki* et al. 1973).

The interpretation of the results obtained from the interaction of group A colicins with vesicles is based on a model which postulates that the colicin first binds to its specific receptor (complex I) and is then translocated to the inner plasma membrane, where it integrates (complex II) and forms a channel, in analogy to gramicidin (*Krasne* et al. 1971; *Weaver* et al. 1981). The shape of the colicin molecule, with its axial ratio of 11:1, is well suited to span the membrane.

The translocation of the Ia colicin from the outer membrane to the target on the cytoplasmic membrane is thought to depend on tol I gene function (*Tokuda* and *Konisky* 1978a). Figure 1 (*Konisky* and *Tokuda* 1979) represents schematically the relationship (to scale) between the colicin and the inner and outer membrane of energized and de-ener-

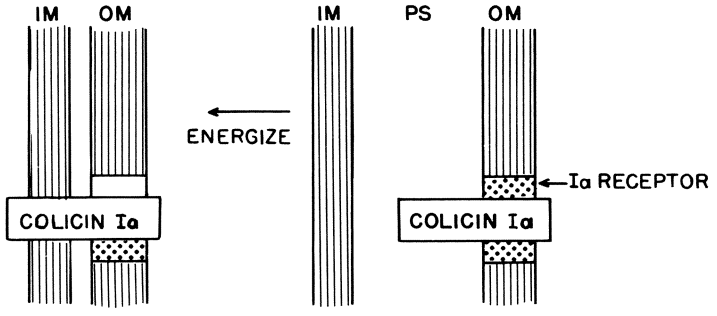

Fig. 1. Hypothetical mode of action of colicin Ia (according to *Konisky* and *Tokuda* 1979). *IM*, inner membrane; *OM*, outer membrane; *PS*, periplasmic space

gized cells. Indeed, the volume of periplasmic space is 20%–40% of the total cell volume in the de-energized cells, but only 1% in the energized cells (*Stock* et al. 1977). Translocation of the colicin molecule to the inner membrane becomes possible when energization causes the proper approach of the outer and inner membranes. It is possible, however, that the already existing adhesion spots are the site of translocation of colicin.

Another possible interpretation is that adsorption of colicin to its receptor alters the state (fluidity?) of the membrane phospholipid (*Cavard* et al. 1968; *Cramer* and *Keenan* 1974; *Lusk* and *Park* 1975), and thus affects the membrane permeability (*Plate* 1973). Experimental results of *Plate* (1973), on the effect of temperature changes and fatty acid substitutions on the activity of colicin K are compatible with the assumption that like valinomycin and nonactin (*Krasne* et al. 1971), colicin K, which is sensitive to changes in membrane fluidity, is not a channel but an ion carrier.

Lau and *Richards* (1976) showed that colicin attached to sepharose beads (in order to prevent its internalization) was also active. This result would refute the argument that colicin is either a channel or an ion carrier [unless the possibility is considered that colicin is split off the sepharose beads by a cellular protease (*Watson* and *Sherratt* 1979), and can thus gain entry into the cell] and would be compatible with the hypothesis of *Kell* et al. (1981).

Studies of glutamic acid transport were done with membrane vesicles made from colicin A-sensitive, -resistant, and -tolerant strains of *E. coli*. Colicin A caused leakage of preaccumulated ^{14}C-glutamic acid from vesicles made from sensitive and resistant, but not from tolerant, strains of *E. coli* (*Jetten* and *Vogels* 1973). The uptake of amino acids into vesicles was inhibited by colicin A, as well as by colicin Ia (*Bhattacharyya* et al. 1970). Since these colicins inhibited amino acid uptake irrespective of electron donors, and since in bacteria amino acid transport is coupled via dehydrogenases and transport carriers to the electron transport chain (*Kaback* 1971), colicins of group A may act as uncouplers of transport function from the electron transport chain.

Change in the membrane due to colicins E1 or K is also demonstrable by the increase of fluorescence of fluorescent membrane probes, such as 8-anilino-1-naphthtalene sulfonate (ANS), *N*-phenyl-1-naphtylamine (NPN), or 3′3-dihexyloxacarbocyanine (*Brewer* 1976), in sensitive and tolerant, but not resistant, bacterial cells (*Cramer* et al. 1973; *Cramer* and *Philips* 1970; *Philips* and *Cramer* 1973). Fluorescence changes in neutral dyes reflect conformational changes in the hydrophobic regions of proteins or phospholipids

brought about by change of potential in the membrane induced by colicin, whereas the charged probes react to changes in $\Delta\psi$. *Nieva-Gomez* and *Gennis* (1977) and *Gould* (1979), showed that neutral probes such as NPN, pyrene, and diphenylhexatriene (DPH) monitor structural changes in *E. coli* envelopes which accompany energization and de-energization of the cells. In membrane vesicles the fluorescence of incorporated ANS decreased when vesicles made of tolerant (but not sensitive or resistant) cells were treated with colicin A.

5.2.2 Effect of Colicins on Liposomes

The activity of colicins was also tested on artificial phospholipid membranes and on liposomes. Liposomes are prepared by sonication of acetone-washed phospholipids, in the presence of ions or molecules that would be enclosed in the liposomes. *Schein* et al. (1978) showed that colicins K, E1, and Ia formed voltage-dependent ion channels in planar artificial phospholipid membranes. *Weiss* and *Luria* (1978) and *Gould* and *Cramer* (1977) demonstrated that membranes of liposomes made of either *E. coli* or soybean phospholipids became depolarized under the influence of these colicins and permitted a free movement of ions across the membrane. The ion flux in vesicles or liposomes was not voltage-dependent (*Tokuda* and *Konisky* 1979); there was a rapid efflux of preloaded Rb^+, Na^+, $PO_4^{\bar{}}$, and choline, as well as of glucose-6-phosphate and sucrose, but not of inulin or dextran from liposomes treated with colicins of group A. Not only colicins affected transport: 0.4 *M* lysozyme also caused 40% efflux of total internal $^{22}Na^+$ from liposomes within 1 h (*Kimmelberg* and *Papahadjopoulos* 1971). A much greater effect was elicited by colicin Ia (within 15 S) at a concentration as low as 30 m*M*. Lysozyme, applied at such a low concentration, however, had no effect at all on the efflux of $^{86}Rb^+$.

When colicin E1 was incorporated into unilamellar membrane vesicles made of a single phospholipid, dimirystoylphosphatidylcholine (DMP), it acted as a channel for ions and molecules smaller than glycerol (e.g. CNS–, K^+, Na^+, Urea) (*Uratani* and *Cramer* 1981). In this study the channel function of colicin was clearly demonstrated. On the other hand, results of studies on liposomes or vesicles made of bacterial membranes did not permit the distinction between the channel or the ion-carrier function of colicins. The incorporation of colicin into a pure phospholipid vesicle did not affect its phase transition temperature which, for DMP, is 23°–24 °C. The fact that colicin depolarizes such a vesicle even at 12 °C (when the lipid is in a gel state) would indicate that it functions as a channel. ANS (8-anilino-1-naphthalene sulfate) entrapped in DMP vesicles is insulated at 12 °C from changes in ion concentration outside, but, when colicin is incorporated into the membrane, ANS responds to low-molecular-weight salts.

5.2.3 Role of Proteolytic Cleavage of Colicins

The proposal has been made that after adsorption to an outer membrane receptor, the colicin molecule is proteolytically cleaved, generating a fragment that is subsequently translocated to the cellular target, either in the inner membrane or in the cytoplasm.

Watson and *Sherratt* (1979) and *Watson* (1980) found that colicins Ia, E1, or K, when mixed with outer membranes of *E. coli* containing colicin receptors from sensitive (but not from resistant) cells, were cleaved into two fragments, one of which (molecular weight 24 000) retained the colicin activity when tested on membrane vesicles. Also in

the case of colicin E3, the contact with isolated receptors resulted in the cleavage of colicin, so that the C-terminal fragment had a specific activity 300–1000-fold that of intact colicin in vitro. Similar fragmentation of colicins was obtained by treatment with proteases (*Lau* and *Richards* 1976; *Ohno* et al. 1977; *Yamamoto* et al. 1978).

On the other hand, *Bowles* and *Konisky* (1981) showed that incubation of ^{124}I-labeled colicin with outer membrane preparations from both sensitive and resistant strains led to cleavage of the colicin, but that incubation of colicin with intact cells did not result in its degradation; they concluded that the interaction of the colicin with its specific receptor was not a sufficient condition for cleavage, and that the functional protease might have been provided by the membrane preparations (*MacGregor* et al. 1979). In the in vivo situation, the adsorption of colicin to the receptor would also result in cleavage, but this is not detectable because the C-terminal fragment is immediately translocated to the lesion site.

In a study by *Cavard* and *Lazdunski* (1979), colicin E4 was incubated with intact *E. coli* cells. In sensitive and tolerant cells, with functional receptors, colicin was cleaved into two fragments (molecular weight 27 000 and 26 000). This was not the case with resistant cells, which did not adsorb colicin. The conclusion that cleavage must therefore have occurred upon contact with the receptor was later retracted [*Watson* and *Sherratt* (1979); *Cavard* and *Lazdunski* (1979)]; also, it became apparent that E4 was actually colicin A (*J. Konisky,* personal communication).

Bacteria which upon adsorption of colicin are destined to die can be rescued by trypsin (*Nomura* and *Nakamura* 1962). The inhibition of transport of amino acids (*Dankert* et al. 1980), as well as the killing ability of colicin E1 or K (*Plate* 1973), can be arrested by trypsin applied to the colicin-bacteria complex at low temperature. The inhibition of transport need not necessarily be the cause of bacterial death because (a) bacteria are still viable after 10–15 min, even when transport is completely stopped 2–3 min after addition of colicin; (b) trypsin can restore viability even when transport is still inhibited; and (c) respiratory inhibitors and uncouplers block active transport but do not kill bacteria (*Holland* 1975).

Since interaction of colicin with cell receptors is followed by a proteolytic cleavage of the colicin (*Cavard* and *Lazdunsky* 1979; *Bowles* and *Konisky* 1981), the possibility has been raised that trypsin might inactivate the protease involved in this cleavage; the source of protease would thus seem to be cellular. If this were the case, one would expect that treatment of bacteria with trypsin, before the addition of colicin, would destroy such proteases and would thus also abolish the colicin effect. This is not the case.

It is therefore reasonable to assume that trypsin is able to act only on those colicin molecules which are adsorbed and which are at the stage of complex I (*Plate* and *Luria* 1972), whereas molecules already translocated to the inside (complex II) after an incubation at higher temperatures would be inaccessible to trypsin and therefore not affected by it. This hypothesis is supported by the finding by *Plate* (1973) that at low temperature a higher percentage of bacteria can be rescued by trypsin.

6 Microinjection: Introduction of Molecules into Animal Cells

The technique by which molecules and substances which are normally impermeable to cells are introduced into the cells is termed microinjection. In "classical" microinjection

methods the material is introduced directly with the aid of a capillary syringe into the cytoplasm. These methods are laborious, involve only a limited number of cells, and are suitable for large cells only (*Feldherr* 1969; *Lane* et al. 1971; *Graesmann* et al. 1974). During the last decade a different microinjection technique has been developed, enabling the simultaneous microinjection of a large number of cells irrespective of their size or growth characteristics (whether they grow as monolayers or in suspension). Of the several methods developed, one, namely the use of liposomes as the introducing vehicle (*Magee* and *Miller* 1972; *Gregoriadis* and *Buckland* 1973; *Papadjopoulos* et al. 1974a, b), is outside the scope of this review and will not be discussed here. The other methods are based on the phenomena described in the previous chapters, namely, virus-cell fusion, cell-cell fusion, and changes in the permeability of the cell membrane induced by virus.

Microinjection techniques can thus be divided into three main categories:

1. Virus-induced cell-cell fusion as a means for introduction of molecules
2. Use of the viral envelope as the introducing vehicle
3. Introduction of molecules due to changes in permeability during early stages of virus infection

6.1 Virus-Induced Cell-Cell Fusion

A better insight into gene regulation, gene mapping, and other related problems in eukaryotic cells can be gained by using techniques of fusion of isolated nuclei or micronuclei with enucleated cells. *Ege* et al. (1974), using the procedure for enucleation of cells developed by *Prescott* et al. (1972), succeeded in introducing isolated nuclei from L-6 cells into enucleated cells by means of Sendai-virus-induced fusion. Micronuclei obtained by colchicine treatment, the smallest of which had a DNA content equivalent to 1–2 chromosomes, were also successfully introduced into enucleated L-6 cells (*Ege* et al. 1974). Using the same fusion method, we have introduced Hela cell nuclei (prepared by detergent treatment) into mouse L-929 cells (*Fuchs* and *Kohn,* unpublished results). *Veomett* et al. (1974) introduced isolated nuclei into enucleated L cells and demonstrated that some of the reconstituted cells were able to undergo mitosis.

It should be noted, however, that the nuclei prepared by *Prescott's* method (*Prescott* et al. 1972), are surrounded by a small amount of cytoplasm and a plasma membrane. Thus although the probability of fusion is increased, interpretation of the results must take into consideration the participation of the donor cytoplasm and plasma membrane.

During gradual lysis of mammalian RBCs the hemoglobin can easily be replaced by foreign substances present in the medium. Resealing of the RBC ghosts thus obtained will entrap the substances within the ghosts. Such ghosts can readily be fused with other cells (using active or UV-inactivated Sendai virus), thus "injecting" these substances into the recipient cells. *Zakai* et al. (1974a) reported that by gradual hemolysis of human RBC in the presence of BSA, they were able to entrap within the ghosts macromolecules and substances such as ferritin, bacteriophage T2, and latex beads (with a diameter of up to 0.5 μm). Using Sendai-virus-mediated fusion, *Zakai* et al. (1974a) and *Loyter* et al. (1975) demonstrated the transfer of ferritin and latex beads into hepatoma tissue culture cells (HTC), subclone GM22-5. *Nishimura* et al. (1976) entrapped, within ghosts obtained by the method of *Seeman* (1967), FITC and IgG molecules (1.5×10^{-3} pg/ghost and 1.5×10^{-2} pg/ghost respectively). Sendai-virus-induced fusion of these ghosts to FL and Ehrlich

ascites tumor cells (ETC) resulted in the injection of the molecules into the recipient cells. The average frequency of intracellular injection was 41%, compared with 1% obtained when ghosts were prepared by a rapid one-step hemolysis (*Furusawa* et al. 1974).

Introduction of biochemical indicator molecules into cells by similar methods permits the study of biochemical "events" in the living cells. Using a modification of the "pre-swell" method of *Rechsteiner* (1975) for gradual hemolysis of human RBCs, *Hallett* and *Campbell* (1982) entrapped the photoprotein Obelin (*Campbell* 1974) and the chemoluminescent compound luminol in the ghosts. These molecules were introduced, via fusion, into rat polymorphonuclear leukocytes. The rate of hybrid formation was very high (99%) and the frequency of cellular injection reached 80%. *Hallett* and *Campbell* (1982) also followed the changes in free Ca^{++} in the hybrid cells and monitored radical formation of cytoplasmic oxygen as a response to a phagocytic stimulus. Similar experiments were reported by *Campbell* et al. (1980) for other chemoluminescent-labeled indicators.

RBCs also served as microinjection vehicles in the studies of *Schlegel* and *Rechsteiner* (1975), *Kruse* et al. (1981), and *Yamaizumi* et al. (1978a, b). *Schlegel* and *Rechsteiner* (1975) successfully introduced the enzyme thymidine kinase (TK) into the TK^- mouse cell line 3TB-4E. *Kruse* et al. (1981) were able to introduce another enzyme, arginase, into arginase-deficient fibroblasts. The arginase activity in the infected cells exceeded five- to tenfold that in the control cells, indicating a fusion ratio of 4–10 RBC ghosts per fibroblast. Fusion was induced not only by the intact Sendai virus but also by isolated viral glycoproteins. *Yamaizumi* et al. (1978a) showed that one molecule of fragment A of diphtheria toxin microinjected into a toxin-resistant mouse L cell was enough to kill the cell. Fragment A of diphtheria toxin is a 22 000 molecular weight polypeptide which is liberated from the 62 000 molecular weight diphtheria toxin molecule by proteolytic and reductive treatment. The remaining 40 000 molecular weight fragment (fragment B) recognizes specific receptor on susceptible cells and is necessary for binding, while fragment A is a very potent protein inhibitor. When susceptible FL cells were microinjected by the RBC ghost method with antifragment A IgG, the cells became resistant to diphtheria toxin. This IgG preparation neutralized the enzymatic activity of fragment A in vitro, but did not protect the cells, when present outside, against the complete toxin (*Yamaizumi* et al. 1978b).

6.2 Reconstituted Viral Envelopes

During virus infection, Sendai virus envelope fuses with the cell plasma membrane and the viral nucleocapsid is released into the cytoplasm. The viral envelope, which acts as a carrier of the nucleocapsid, can also serve as a carrier for other substances, thus providing a method whereby the viral nucleocapsid can be replaced by such substances. A simple method for such replacement was suggested by *Uchida* et al. (1979b). Sendai virus, sonicated in the presence of fragment A of diphtheria toxin, entrapped this fragment and was very effective in transfer of the toxin into toxin-resistant L cells. Such a method, though simple, cannot be used for macromolecules which are sensitive to prolonged (12 min) sonication (*Uchida* et al. 1979b). Therefore methods were developed for solubilization of viral envelopes, separation of the nucleocapsids, and reassembly of the envelopes in the presence of foreign macromolecules, which are then entrapped within the reassembled

envelopes. Sendai virus preparations were solubilized by the detergents Tween 80 or NP-40 (*Hosaka* and *Shimizu* 1972a; *Hosaka* et al. 1974; *Shimizu* et al. 1972; *Okada* et al. 1975a), and after centrifugation and removal of the detergents reconstituted viral envelopes free of viral nucleocapsids were obtained. These preparations retained their neuraminidase, hemagglutinating, hemolytic, and cell fusion activities. The mutant protein of diphtheria toxin CRM 45, present in the buffer during envelope reconstruction was trapped inside the viral envelopes (*Uchida* et al. 1977). The CRM 45 protein is nontoxic for animal cells because it lacks the C-terminal peptide necessary for the binding of the toxin to cell membranes. When viral envelopes containing CRM 45 protein were added to L cells, the cells were killed; fusion of empty envelopes in the presence of CRM 45 had no effect. This experiment indicated that only entrapped toxin fragment can be efficiently microinjected (*Uchida* et al. 1977).

A similar method for the reassembly of viral envelopes which gave a higher yield of viral proteins in the reconstituted envelopes was suggested by *Volsky* and *Loyter* (1978a), who used the nonionic detergent Triton X-100 for solubilization of the virus. The detergent was removed by dialysis against buffer containing SM-2 Bio-beads. Experimental conditions were worked out to obtain envelope preparations which fused with the cells, but did not induce cell-cell fusion, thus enabling efficient microinjection while preserving the viability of the cells. Using this method, *Volsky* et al. (1979) and *Cabantchik* et al. (1980) successfully introduced the human erythrocyte band 3 protein (ion channel, molecular weight 100 000) into reconstituted viral envelopes. Incubation of these envelopes with Friend erythroleukemic cells resulted in the functional insertion of this protein into the cell membrane.

Recently *Uchida* et al. (1979a) prepared lipid vesicles which contained Sendai virus spike proteins and fragment A of diphtheria toxin. This tailor-made preparation had the hemolytic and hemagglutinating activities but was devoid of cell fusion activity. Such a preparation is not only a very potent microinjection vehicle, but it also seems to be valuable in identification of the factor(s) or structure(s) which promotes cell fusion. Similar preparations of liposomes containing viral glycoproteins (HN and F) were described by *Hsu* et al. (1979).

Studies with influenza virus revealed that only viruses that possessed cleaved hemagglutinins were infectious (*Klenk* et al. 1975, 1977; *Bosch* et al. 1979). Such hemagglutinins were successfully incorporated into liposomes containing fluorescein isothiocyanate or fluorescein dextran (*Huang* et al. 1980). When these liposomes were mixed with chick fibroblasts, the fluorescent molecules were microinjected. Liposomes containing uncleaved hemagglutinins adsorbed to the fibroblasts but did not inject their contents.

6.3 Changes in Permeability

The use of inactivated Sendai virus to introduce molecules and substances into cells was first demonstrated by *Enders* et al. (1967), who introduced poliovirus into nonsusceptible cells. Although the mechanism of such introduction has not been elucidated, it is undoubtedly related to the virus-induced leakage of cell membranes discussed in detail in Sect. 4. *Tanaka* et al. (1975), using the same method, restored unscheduled DNA synthesis to Xenoderma pigmentosum (XP) cells, which lack the ability for excision

repair of UV-induced damage of DNA (*Cleaver* 1968). When UV-irradiated XP cells were treated simultaneously with Sendai virus and T4 endonuclease V, the enzyme was introduced into the cells and restored the unscheduled DNA synthesis to the level observed in control cells. Similarly, *Natarajan* and *Obe* (1978) and *Natarajan* et al. (1980) have shown that the Sendai-virus-mediated introduction of *Neurospora* endonuclease (NE) into Chinese hamster (CHO) cells effectively increased the frequencies of chromosomal aberrations induced by irradiation. NE is an enzyme which converts single-strand DNA breaks into double-strand breaks. In a comparative study, *Obe* and *Natarajan* (1980) demonstrated that permeabilization of CHO cells to NE using Sendai virus was much better than permeabilization using polyethylene glycol (*Davidson* and *Gerald* 1976; *Lau* et al. 1977), or Tween 80 (*Olson* and *Billen* 1978).

In a detailed study, *Yamaizumi* et al. (1979) showed that the introduction of fragment A of diphtheria toxin into FL and L cells was dependent on the concentrations of both the virus and the toxin. The penetration was most effective during the first 7 min incubation with the virus and the toxin, and it lasted for about 15 min. This is also the time when the virus-induced leakage of ions is at its peak (see Sect. 4.1).

The permeabilization of cell membranes to macromolecules during the very early stages of infection is not restricted to cells infected with Sendai virus. As was recently shown by *Fernandez-Puentes* and *Carrasco* (1980), other enveloped (Semliki Forest virus) or nonenveloped (EMC and adenovirus type 5) viruses have a similar effect. During the adsorption period of these viruses to Hela or BHK-21 cells, the membranes became permeable to several plant protein toxins, such as A chains of ricin and Abrin (molecular weight ca. 30 000), α-sarcin (molecular weight 16 800), and others. Such toxins, which are normally impermeable to mammalian cells, penetrated the membranes during the adsorption and effectively inhibited protein synthesis. The effect was dependent on both the amount of the virus and the concentration of the toxins, and there was no cellular or viral gene expression involved. Although the greatest effect was observed when the toxin and virus were added simultaneously, a considerable effect was still demonstrated when the toxin was added 2 h post infection. This is in contrast to the permeabilization induced by Sendai virus, where the toxin and virus had to be added simultaneously (*Yamaizumi* et al. 1979).

7 Concluding Remarks

It is a well-documented phenomenon that viruses induce changes in host cell permeability. It seems likely that this phenomenon is not restricted, as was thought before, to the paramyxo- and myxoviruses, but is rather more widespread; it occurs with many RNA and DNA, naked or enveloped viruses. It is possible, however, that different viruses vary in the onset time and the extent of this effect.

The following alternatives for the mechanism of the virus-induced changes were considered here: (a) changes in the organization of the membrane and in the phospholipid-protein interactions; (b) changes in the physical state of the membrane, affecting fluidity of phospholipids and mobility of membrane proteins; and (c) involvement of membranal enzymes in the virus-induced damage. Solid evidence for any of these possibilities is still lacking, and they can thus serve merely as a good indicator for further research. Nevertheless, even if the exact molecular mechanisms of virus-induced permeability

changes are not well understood, their significance for practical use in the fast-growing field of microinjection is now well recognized.

Acknowledgments. We thank Mrs. *J. Gitelman* and Mrs. *Orna Galan* for their bibliographical help.

References

Ahkong QF, Fisher D, Tampion W, Lucy JA (1975) Mechanisms of cell fusion. Nature 253:194–195

Aiton JF, Lamb JF (1975) The transient effect of ATP on K movement in Hela cells. J Physiol (Lond) 248:14

Allison AC, Davies P (1974) Mechanisms of endocytosis and exocytosis. Symp Soc Exp Biol 28:419–436

Alonso MA, Carrasco L (1981) Reversion by hypotonic medium of the shut off of protein synthesis induced by encephalomyocarditis virus. J Virol 37:535–540

Apostolov K, Almeida JD (1972) Interaction of Sendai (HVJ) virus with human erythrocytes: a morphological study of haemolysis and cell fusion. J Gen Virol 15:227–234

Apostolov K, Sawa MI (1979) Reversible inhibition of Newcastle disease virus (NDV) haemolysis by bivalent cations. Microbios 26:45–49

Aull F, Nachbar MS (1974) Concanavalin A-induced alterations in sodium and potassium content of Ehrlich ascites tumor cell. J Cell Physiol 83:243–250

Aull F, Nachbar MS, Oppenheim JD (1977) Nature of lectin-induced alteration of potassium transfer in Ehrlich ascites tumor cells. J Cell Physiol 90:9–14

Averdunk R (1972) The effect of phytohaemagglutinin and antilymphocyte serum on the transport of potassium ions, glucose and amino acids in human lymphocytes. Hoppe Seylers Z Physiol Chem 353:79–88

Averdunk R (1976) Early changes of "leak flux" and the cation content of lymphocytes by concanavalin A. Biochem Biophys Res Commun 70:101–109

Averdunk R, Lauf PK (1975) Effects of mitogens on sodium-potassium transport, ^{3}H-ouabain binding and adenosine triphosphatase activity in lymphocytes. Exp Cell Res 93:331–342

Bächi T, Auget M, Howe C (1973) Fusion of erythrocytes by Sendai virus studied by immunofreezeetching. J Virol 11:1004–1012

Bächi T, Deas JE, Howe C (1977) Virus-erythrocyte interactions. Cell Surf Rev 2:83–128

Ball FR, Medzan EL (1976) Evidence for an "Early Early" vaccinia virus-induced protein which causes a density change of infected L-M cells. J Virol 17:60–67

Barenholz Y, Moore NF, Wagner RR (1976) Enveloped viruses as model membrane systems: microviscosity of vesicular stomatitis virus and host cell membranes. Biochemistry 15: 3563–3570

Baxt B, Bablanian R (1976) Mechanisms of vesicular stomatitis virus-induced cytopathic effects. Early morphologic changes induced by infectious and defective-interfering particles. Virology 72:370–382

Berlin RD, Fera JF (1977) Changes in membrane microviscosity associated with phagocytosis: effects of colchicine. Proc Natl Acad Sci USA 74:1072–1076

Bhattacharyya P, Wendt L, Whitney E, Silver S (1970) Colicin-tolerant mutants of *Escherichia coli.* Resistance of membranes to colicin E1. Science 168:998–1000

Blomberg F, Cohen R, Siekevitz P (1977) The structure of postsynaptic densities isolated from dog cerebral cortex II. Characterization and arrangement of some of the major proteins within the structure. J Cell Biol 74:204–225

Blough HA, Tiffani JM (1973) Lipids in viruses. Adv Lipid Res 11:267–339

Booth JC, Beesley JE, Stern H (1978) Syncytium formation caused by human cytomegalovirus in human embryonic lung fibroblasts. Arch Virol 57:143–152

Bosch FX, Orlich M, Klenk HD, Rott R (1979) The structure of the hemagglutinin, a determination for the pathogenicity of influenza virus. Virology 95:197–207

Bossart W, Meyer J, Bienz K (1973) Electron microscopic study on influenza virus hemagglutination: pinocytosis of virions by red cells. Virology 55:295–298

Bowles LK, Konisky J (1981) Cleavage of colicin Ia by the *Escherichia coli* K-12 outer membrane is not mediated by the colicin Ia receptor. J Bacteriol 145:668–671
Brand CM, Skehel JJ (1972) Crystalline antigen from the influenza virus envelope. Nature New Biol 238:145–147
Bratt MA, Gallaher WR (1969) Preliminary analysis of the requirements for fusion from within and fusion from without by Newcastle disease virus. Proc Natl Acad Sci USA 64:536–543
Brewer GI (1976) The state of energization of the membrane of *Escherichia coli* as affected by physiological conditions and colicin K^+. Biochemistry 15:1387–1392
Britton JR, Haselkorn R (1975) Permeability lesions in male *E. coli* infected with bacteriophage T7. Proc Natl Acad Sci USA 72:2222–2226
Buechi M, Bächi T (1979) Immunofluorescence and electron microscopy of the cytoplasmic surface of the human erythrocyte membrane and its interaction with Sendai virus. J Cell Biol 83: 338–347
Buller CS, Astrachan L (1968) Replication of T4rII bacteriophage in *Eschericha coli* K 12 (λ). J Virol 2:298–307
Cabantchik ZI, Volsky DJ, Ginsburg H, Loyter A (1980) Reconstitution of erythrocyte anion transport systems, in vitro and in vivo approaches. Ann NY Acad Sci 341:444–454
Campbell AK (1974) Extraction, partial purification and properties of obelin, the calcium activated luminescent protein from the hydroid Obelia geniculata. Biochem J 143:411–418
Campbell AK, Luzio JP (1981) Intracellular free calcium as a pathogen in cell damage initiated by the immune system. Experientia 37:1110–1112
Campbell AK, Daw RA, Luzio JP (1979) Rapid increase in intracellular free Ca induced by antibody plus complement. FEBS Lett 107:55–60
Campbell AK, Davies EJ, Hart R, McCapra F, Patel A, Richardson A, Ryall ME, Simpson JSA, Woodhead JS (1980) Chemiluminescent labels in immunoassay. J Physiol (Lond) 306:3P–4P
Carrasco L (1977) The inhibition of cell function after viral infection. FEBS Lett 76:11–15
Carrasco L (1978) Membrane leakiness after viral infection and a new approach to the development of antiviral agents. Nature 272:694–699
Carrasco L, Smith AE (1976) Sodium ions and the shut off of host cell protein synthesis by picornaviruses. Nature 264:807–809
Cavard D, Lazdunski C (1979) Interaction of colicin E4 with specific receptor sites mediates its cleavage into 2 fragments inactive towards whole cells. Eur J Biochem 96:525–533
Cavard D, Rampini C, Barbu E, Polonovski J (1968) Activite phospholipidique et autres modifications du metabolisme des phospholipides consecutives a l'action des colicines sur *E. coli.* Bull Soc Chem Biol 50:1455–1471
Changeux JP, Thiery J, Tung Y, Kittel C (1974) On the cooperativity of biological membranes. Proc Natl Acad Sci USA 57:335–341
Chany-Fournier F, Chany C, Lafai F (1977) Mechanism of polykaryocyte induction by vesicular stomatitis virus in rat XC cells. J Gen Virol 34:305–314
Choppin PW, Scheid A (1980) The role of viral glycoproteins in adsorption penetration and pathogenicity of viruses. Rev Infect Dis 2:40–61
Cleaver JE (1968) Defective repair replication of DNA in xeroderma. Nature 218:652–656
Collard JG, De-Wildt A, Inbar M (1978) Translocation of a fluorescent lipid probe between contacting cells. Evidence for membrane lipid interactions. FEBS Lett 90:24–28
Condit RC (1975) F Factor-mediated inhibition of bacteriophage T7 growth: increased membrane permeability and decreased ATP levels following T7 infection of male Escherichia coli. J Mol Biol 98:45–56
Contreras A, Carrasco L (1979) Selective inhibition of protein synthesis in virus-infected mammalian cells. J Virol 29:114–122
Cramer WA, Keenan TW (1974) Phospholipase A activity is not associated with early effects of colicin E1. Biochem Biophys Res Commun 56:60–67
Cramer WA, Phillips SK (1970) Response of an Escherichia coli-bound fluorescent probe to colicin E1. J Bacteriol 104:819–825
Cramer WA, Phillips SK, Keenan TW (1973) On the role of membrane phase in the transmission mechanism of colicin E1. Biochemistry 12:1177–1181
Crews FT, Hirata F, Axelrod J (1980) Identification and properties of methyltransferase that synthesize phosphatidylcholine in rat brain synaptosomes. Neurochem Res 34:1491–1498

Dales S, Chardonnet Y (1973) Early events in the interaction of adenoviruses with Hela cells. IV. Association with microtubules and the nuclear pore complex during vectorial movement of the inoculum. Virology 56:465–483

Dankert J, Hammond SM, Cramer WA (1980) Reversal by trypsin of the inhibition of active transport by colicin E1. J Bacteriol 143:594–602

Danon D, Jerushalmy Z, Kohn A, De Vries A (1961) In vitro phagocytosis by leucocytes of influenza virus adsorbed onto erythrocytes. Electron microscopic and cinematographic observations. Virology 15:258–262

Davidson RL, Gerald PS (1976) Improved technique for the induction of mammalian cell hybridization by polyethylene glycol. Somatic Cell Genet 2:165–176

De Graaf FK (1979) Mode of action of colicin E2, colicin E3 and colicin DF13. Zentralbl Bakteriol Mikrobiol Hyg A244:121–134

Doerman AH (1948) Lysis and lysis inhibition with *Escherichia coli* bacteriophage. J Bacteriol 55:257–276

Dourmashkin RA, Tyrrell DAJ (1974) Electron microscopic observations on the entry of influenza virus into susceptible cells. J Gen Virol 24:129–141

Dubois-Dulcq M, Rentier B (1980) Structural studies of the surface of virus-infected cells. Prog Med Virol 26:158–213

Duckworth DH (1970) Biological activity of bacteriophage ghosts and "take-over" of host function of bacteriophage. Bact Rev 34:344–363

Durham ACH (1977) Do viruses use calcium ions to shut off host cell functions? Nature 267: 375–376

Durham ACH (1978) Viruses make holes in membranes. Proc 4th Int Congress Virol, Hague, p 178

Dvorak JA, Miller LH, Whitehouse WC, Shiroishi T (1975) Invasion of erythrocytes by malaria merozoites. Science 187:748–750

Egberts E, Hackett PB, Traub P (1977) Alteration of the intracellular energetic and ionic conditions by mengovirus infection of Ehrlich ascites tumor cells and its influence on protein synthesis in the midphase of infection. J Virol 22:591–597

Ege T, Krondahl U, Ringertz NR (1974) Introduction of nuclei and micronuclei into cells and enucleated cytoplasms by Sendai virus induced fusion. Exp Cell Res 88:428–432

Elgsaeter A, Branton D (1974) Intramembrane particle aggregation in erythrocyte ghosts. I. The effects of protein removal. J Cell Biol 63:1018–1036

Elligsen JD, Thompson JE, Frey HE, Kruuv J (1974) Correlation of (Na^{++}-K^{+}) ATPase activity with growth of normal and transformed cells. Exp Cell Res 87:233–240

Enders JF, Holoway A, Grogan EA (1967) Replication of poliovirus I in chick embryo and hamster cells exposed to Sendai virus. Proc Natl Acad Sci USA 57:637–644

Feingold DS (1970) The mechanism of colicin E1 action. J Membrane Biol 3:372–386

Feldherr CM (1969) A comparative study of nucleocytoplasmic interactions. J Cell Biol 42:841–845

Fernandez-Puentes C, Carrasco L (1980) Viral infection permeabilizes mammalian cells to protein toxins. Cell 20:769–775

Fields KL, Luria E (1969) Effects of colicins E1 and K on transport systems. J Bact 97:57–63

Foster KA, Gill K, Micklem KS, Pasternak CA (1980) Survey of virally mediated permeability changes. Biochem J 190:639–646

Foulds J, Chai T (1978) Defeat of colicin tolerance in Escherichia coli *ompA* mutants: evidence for interaction between colicin L-JF246 and the cytoplasmic membrane. J Bact 133:158–164

Fowler V, Branton D (1977) Lateral mobility of human erythrocyte integral membrane proteins. Nature 268:23–26

Fuchs P, Giberman E (1973) Enhancement of potassium efflux in baby hamster kidney cells and chicken erythrocytes during absorption of parainfluenza 1 (Sendai) virus. FEBS Lett 31:127–130

Fuchs P, Gruber E, Gitelman J, Kohn A (1980) Nature of permeability changes in membranes of Hela cells adsorbing Sendai virus. J Cell Physiol 103:271–278

Fuchs P, Levanon A (1978) Inhibition of adsorption of West-Nile and Herpes simplex viruses by procaine. Arch Virol 56:163–168

Fuchs P, Spiegelstein M, Haimsohn M, Gitelman J, Kohn A (1978) Early changes in the membranes of Hela cells adsorbing Sendai virus under conditions of fusion. J Cell Physiol 95: 223–234

Furrow MH, Pizer LI (1968) Phospholipid synthesis in *E. coli* infected with T4 bacteriophages. J Virol 2:294–605

Furusawa M, Nishimura T, Yamaizumi M, Okada Y (1974) Injection of foreign substances into single cells by cell fusion. Nature 249:449–450

Gallaher WR, Bratt MA (1974) Conditional dependence of fusion from within and other cell membrane alterations by Newcastle disease virus. J Virol 14:813–820

Garry RF, Bishop JM, Parker S, Westbrook K, Lewis G, Waite MRF (1979) Na^+ and K^+ concentrations and the regulation of protein synthesis in Sindbis virus infected chick cells. Virology 96:108–120

Gazitt Y, Loyter A, Ohad I (1976a) Phosphorylation and dephosphorylation of membrane proteins as a possible mechanism for structural rearrangement of membrane components. Biochim Biophys Acta 436:1–14

Gazitt Y, Reichler Y, Ohad I (1976b) Correlations between changes in the membrane organization and susceptibility to phospholipase C attack induced by ATP depletion of rat erythrocytes. Biochim Biophys Acta 419:479–492

Gazitt Y, Loyter A, Ohad I (1977) Induction of ATP depletion, intermembrane particle aggregation and exposure of membrane phospholipids in chicken erythrocytes by local anesthetics and tranquilizers. Biochim Biophys Acta 471:361–371

Genty N (1975) Analysis of uridine incorporation in chicken embryo cells infected by vesicular stomatitis virus and its temperature-sensitive mutants: uridine transport. J Virol 15:8–15

Gilchrist MJR, Konisky J (1975) Effects of colicin Ia on transport and respiration in *Escherichia coli.* J Biol Chem 250:2456–2462

Gould JM (1979) Respiration-linked proton transport, changes in external pH, and membrane energization in cells of *Escherichia coli.* J Bacteriol 138:176–184

Gould JM, Cramer WA (1977) Studies on the depolarization of the *E. coli* cell membrane by colicin E1. J Biol Chem 252:5491–5497

Graessmann A, Graessmann M, Hoffmann H, Niebel Y (1974) Inhibition by interferon of SV40 tumor antigen formation in cells injected with SV40 RNA transcribed in vitro. FEBS Lett 39:249–251

Graham JM, Summer MCB, Curtis DH, Pasternak CA (1973) Sequence of events in plasma membrane assembly during the cell cycle. Nature 246:291–295

Gregoriadis G, Buckland RA (1973) Enzyme-containing liposomes alleviate a model for storage disease. Nature 244:170–172

Grollman EF, Lee G, Ramos S, Lazo PS, Kaback HR, Friedman RM, Kohn LD (1978) Relationships of the structure and function of the interferon receptor to hormone receptors and establishment of the antiviral state. Cancer Res 38:4172–4185

Gschwender HH, Traub P (1979) Mengovirus induced capping of virus receptors in the plasma membrane of Ehrlich ascites tumor cells. J Gen Virol 42:439–442

Gunther GR, Wang JL, Yahara I, Cunningham BA, Edelman GM (1973) Concanavalin A derivatives with altered biological activities. Proc Natl Acad Sci USA 70:1012–1016

Hallett MB, Campbell AK (1982) Measurements of changes in cytoplasmic free Ca^{+2} in fused cell hybrids. Nature 295:155–158

Hallett MB, Luzio JP, Campbell AK (1981) Stimulation of Ca^{+2} dependent chemiluminiscence in rat polymorphonuclear leucocytes by polystyrene beads and the nonlytic action of complement. Immunology 44:569–576

Hallett MB, Fuchs P, Campbell AK (1982) Sendai virus causes a rise in intracellular Ca^{+2} before cell fusion. Biochem J 206:671–674

Hanafusa H (1960) Killing of L-cells by heat and U.V. inactivated vaccinia virus. Biken J 3:191–199

Heine JW, Schnaitman CA (1971) Entry of vesicular stomatitis virus into L cells. J Virol 8:786–795

Helenius A, Kartenbech J, Simmons K, Fries E (1980) On the entry of Semlike forest virus into BHK-21 cells. J Cell Biol 84:404–420

Hennache B, Torpier G, Bonlanger P (1979) Freeze-fracture study of adenovirus-induced KB cell surface alterations. Exp Cell Res 124:139–150

Herrlich P, Rahmsdorf HJ, Schweiger M (1974) Regulation of macromolecular synthesis by membrane changes. Adv Biosci 12:523–537

Herschman HR, Helinski DR (1967) Purification and characterization of colicin E_2 and colicin E_3. J Biol Chem 242:5360–5368

Hessler AM, Barnes MB, Baird JP (1967) Acridine sensitivity of bacteriophage T2H in *Escherichia coli*. J Virol 1:534–549
Hirata F, Axelrod J (1978a) Enzymatic synthesis and rapid translocation of phosphatidylcholine by two methyltransferase in erythrocyte membranes. Proc Natl Acad Sci USA 75:2348–2352
Hirata F, Axelrod J (1978b) Enzymatic methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity. Nature 275:219–220
Hirata F, Axelrod J (1980) Phospholipid methylation and biological signal transmission. Science 209:1082–1090
Hirata F, Axelrod J, Crews FT (1979a) Concanavalin A stimulates phospholipid methylation and phosphatidylserine decarboxylation in rat mast cells. Proc Natl Acad Sci USA 76:4813–4816
Hirata F, Corcoran BA, Venkatasubramanian K, Schiffmann E, Axelrod J (1979b) Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. Proc Natl Acad Sci USA 76:2640–2643
Hirata F, Toyoshima S, Axelrod J (1980) Phospholipid methylation: a biochemical signal modulating lymphocyte mitogenesis. Proc Natl Acad Sci USA 77:862–865
Hirata H, Fukui S, Ishikawa S (1969) Initial events caused by colicin K infection - cation movement and depletion of ATP pool. Biochem J 65:843–847
Hirschberg CB, Robbins PW (1974) The glycolipids and phospholipids of Sindbis virus and their relation to lipids of the host cell plasma membrane. Virology 61:602–608
Holland IB (1975) Physiology of colicin action. Adv Microb Physiol 12:55–139
Holmes KV, Klenk HD, Choppin PW (1969) A comparison of immune cytolysis and virus-induced fusion of sensitive and resistant cell types. Proc Soc Exp Biol Med 131:651–657
Homma M, Ohuchi M (1973) Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural difference of Sendai viruses grown in eggs and tissue culture cells. J Virol 12:1457–1465
Homma M, Shimizu K, Shimizu VK, Ishida N (1976) On the study of Sendai virus hemolysis. 1. Complete Sendai virus lacking in hemolytic activity. Virology 71:41–47
Hong J (1977) An ecf mutation of *Escherichia coli* pleiotropically affecting energy coupling in active transport but not generation or maintenance of membrane potential. J Biol Chem 252: 8582–8588
Hoover RL, Lynch RD, Kanovsky MJ (1977) Decrease of adhesion of cells cultured in polyunsaturated fatty acids. Cell 12:295–300
Hosaka Y, Shimizu YK (1972a) Artificial assembly of envelope particles of HVJ (Sendai virus). 1. Assembly of hemolytic and fusion factors from envelopes solubilized by nonidet P40. Virology 49:627–639
Hosaka Y, Shimizu YK (1972b) Artificial assembly of envelope particles of HVJ (Sendai virus). II. Lipid components for formation of the active hemolysin. Virology 49:640–646
Hosaka Y, Shimizu YK (1974) Cell fusion by the antigen-antibody complex of Sendai virus studied by electron microscopy. J Gen Virol 24:369–374
Hosaka Y, Shimizu YK (1977) Cell fusion by Sendai virus. Cell Surf Rev 2:129–156
Hosaka Y, Semba T, Fukai K (1974) Artificial assembly of envelope particles of HVJ (Sendai virus), fusion activity of envelope particles. J Gen Virol 25:391–404
Hosaka Y, Sugimoto Y, Fukai K (1978) Cell fusion of red blood cells with Sendai virus. Studies by scanning electron microscope. J Electron Microsc 27:390
Hsu MC, Scheid A, Choppin PW (1979) Reconstitution of membranes with individual paramyxovirus glycoproteins and phospholipid in cholate solution. Virology 95:476–491
Huang RTC, Wahn K, Klenk HD, Rott R (1980) Fusion between cell membranes and liposomes containing the glycoproteins of influenza virus. Virology 104:294–302
Huang RTC, Rott R, Klenk HD (1981) Influenza virus cause hemolysis and fusion of cells. Virology 110:243–247
Hutton JJ, Goebel WF (1962) The isolation of colicin V and a study of its immunological properties. J Gen Physiol 45:125–141
Iles GH, Seeman P, Naylor D, Cinader B (1973) Membrane lesions in immune lysis surface rings, globule aggregates, and transient openings. J Cell Biol 56:528–539
Impraim CC, Micklem KJ, Pasternak CA (1979) Calcium, cells and virus-alteration caused by paramyxoviruses. Biochem Pharmacol 28:1963–1969

Impraim CC, Foster KA, Micklem KJ, Pasternak CA (1980) Nature of virally mediated changes in membrane permeability to small molecules. Biochem J 186:847–860

Inbar M, Shinitzky M, Sachs L (1973) Rotational relaxation time of concanavalin A bound to the surface membrane of normal and malignant transformed cells. J Mol Biol 81:245–253

Inoue M, Utsumi K, Seno S (1975) Effect of concanavalin A and its derivative on the potassium compartmentation of Ehrlich ascites tumor cells. Nature 255:556–557

Ishizaka K, Ishizaka T (1969) Immune mechanisms of reversed type reaginic hypersensitivity. J Immunol 103:588–595

Ishizaka T, Hirata F, Ishizaka K, Axelrod J (1980) Stimulation of phospholipid methylation, Ca^{+2} influx and histamine release by bridging of IgE receptors on rat mast cells. Proc Natl Acad Sci USA 77:1903–1906

Israeli E, Kohn A (1972) Protection of lyophilized *E. coli* from oxygen by colicin E1 treatment. FEBS Lett 26:323–326

Israeli E, Giberman E, Kohn A (1974) Membrane malfunctions in freeze-dried *Escherichia coli.* Cryobiology 11:473–477

Israeli E, Kohn A, Gitelman J (1975) The molecular nature of damage by oxygen to freeze-dried *Escherichia coli.* Cryobiology 12:15–25

Jesaitis MA (1970) The nature of colicin K from Proteus mirabilis. J Exp Med 131:1016–1038

Jetten AM, Vogels JD (1973) Effects of colicin A and staphylococcin 1580 on amino acid uptake into membrane vesicles of *E. coli* and *Staph. aureus.* Biochim Biophys Acta 311:483–495

Johnson SMM, Nicolau C (1977) The distribution of 1,6 diphenylhexatriene fluorescence in normal human lymphocytes. Biochem Biophys Res Commun 76:869–879

Kaback HR (1971) Transport across isolated bacterial cytoplasmic membranes. Biochim Biophys Acta 265:367–416

Kabat JP, Luria SE (1970) Inhibition by colicins of the proline-uptake function of *Escherichia coli* membrane vesicles. Bacteriol Proc EP 85:62

Kaplan JG (1978) Membrane cation transport and the control of proliferation of mammalian cells. Annu Rev Physiol 40:19–41

Kasarov LB, Friedman H (1974) Enhanced Na^+-K^+ activated adenosine triphosphatase activity in transformed fibroblasts. Cancer Res 34:1862–1865

Kates M, Allison AC, Tyrrell D, James A (1961) Lipids of influenza virus and their relation to those of the host cell. Biochim Biophys Acta 52:455–466

Kato N, Eggers HJ (1969) Inhibition of uncoating of the fowl plague virus by L-adamantamine hydrochloride. Virology 37:632–641

Kayalar C, Luria SE (1976) Permeability to ions and organic molecules induced by colicin K on lysosomes. Bacterial Proc pp 146

Keith AD, Snipes W (1977) Dynamic aspects of biological membranes. Horiz Biochem Biophys 4:31–62

Kell DB, Clarke DJ, Morris JG (1981) On proton-coupled information transfer along the surface of biological membranes and the mode of action of certain colicins. FEMS Microbiol Lett 11:1–11

Keller PM, Person S, Snipes W (1977) A fluorescence enhancement assay of cell fusion. J Cell Sci 28:167–177

Kim J, Mitsui H, Okada Y (1976) Specific movement of cell membranes fused with HVJ (Sendai virus). II. Electron microscopic observations on the course of cap formation. Cell Struct Funct 1:237–249

Kimelberg HK (1975) Alteration in phospholipid-dependent Na^+, K^+ ATPase activity due to lipid fluidity. Effects of cholesterol and Mg^{++}. Biochim Biophys Acta 413:143–156

Kimelberg HK, Mayhew E (1975) Increased ouabain-sensitive $^{86}Rb^+$ uptake and sodium and potassium ion-activated adenosine triphosphatase activity in transformed cell lines. J Biol Chem 250:100–104

Kimelberg HK, Mayhew E (1976) Cell growth and ouabain-sensitive $^{86}Rb^+$ uptake and $(Na^+ + K^+)$-ATPase activity in 3T3 and SV40 transformed 3T3 fibroblasts. Biochim Biophys Acta 455: 865–875

Kimelberg HK, Papahadjopoulos D (1971) Interaction of basic proteins with basic phospholipid membranes. Binding and changes in the sodium permeability of phosphatidy l serine vesicles. J Biol Chem 246:1142–1148

Klemperer HG (1960) Hemolysis and the release of potassium from cells by Newcastle disease virus (NDV). Virology 12:540–552
Klenk HD (1974) Viral envelopes and their relationship to cellular membranes. Curr Top Microbiol Immunol 68:29–88
Klenk HD, Rott R, Orlich M, Blodorn J (1975) Activation of influenza A viruses by trypsin treatment. Virology 68:426–439
Klenk HD, Rott R, Orlich M (1977) Further studies on the activation of influenza virus by proteolytic cleavage of the hemagglutinin. J Gen Virol 36:151–161
Knepper JE, Lusk JE (1976) Disappearance of specific proteins from the membranes of colicin treated cells. J Biol Chem 25:7577–7580
Knutton S (1976) Changes in viral envelope structure preceding infection. Nature 264:672–673
Knutton S (1977) Studies on membrane fusion. II. Fusion of human erythrocytes by Sendai virus. J Cell Sci 28:189–210
Knutton S (1978) The mechanism of virus induced cell fusion. Micron 9:133–154
Knutton S (1980) Studies on membrane fusion. VI. Mechanism of the membrane fusion and cell swelling stages of Sendai virus-mediated cell fusion. J Cell Sci 43:103–118
Knutton S, Pasternak CA (1979) The mechanism of cell: cell fusion. TIBS 4:220–223
Knutton S, Jackson D, Graham M, Micklem KJ, Pasternak CA (1976) Microvilli and cell swelling. Nature 262:52–54
Knutton S, Jackson D, Ford M (1977) Studies on membrane fusion. I. Paramyxovirus-induced cell fusion, a scanning electron-microscope study. J Cell Sci 28:179–188
Kohn A (1965) Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells. Virology 26:228–245
Kohn A (1970) Fusion factor and ATPase in Newcastle disease strains and host differences. Isr J Med Sci 6:423–427
Kohn A (1979) Early interaction of viruses with cellular membrane. Adv Virus Res 24:223–276
Kohn A, Fuchs P (1974) Initial effects of viral infection in bacterial and animal host cells. Adv Virus Res 18:159–194
Kohn A, Gitelman J, Inbar M (1980a) Interaction of polyunsaturated fatty acids with animal cells and enveloped viruses. Antimicrob Agents Chemother 18:962–968
Kohn A, Wallach D, Fuchs P (1980b) Changes in permeability and phosphorylation in Hela cells adsorbing Sendai virus. Eur J Cell Biol 22:223
Konisky J, Richards FM (1970) Characterization of colicin Ia and colicin Ib. J Biol Chem 245: 2972–2978
Konisky J, Tokuda H (1979) Mode of action of colicins Ia, E1 and K. Zentralbl Bakteriol Mikrobiol Hyg A244:105–120
Kopecky AL, Copeland DP, Lusk JE (1975) Viability of *Escherichia coli* treated with colicin K. Proc Natl Acad Sci USA 72:4631–4634
Kousoulas KG, Person S, Holland TC (1978) Timing of some of the molecular events required for cell fusion induced by Herpes simplex virus type 1. J Virol 27:505–512
Koyama AH, Maeda T, Toyama S, Ohnishi SI, Uetake H, Toyama S (1978) Interaction between Sendai virus and KB cell lines with altered cell fusion ability: A study employing electron spin resonance. Biochim Biophys Acta 508:130–136
Krasne S, Eisenman G, Szabo G (1971) Freezing and melting of lipid bi-layers and the mode of action of nonactin, valinomycin and gramicidin. Science 174:412–415
Kruse CA, Spector EB, Cederbaum SD, Wisneiski BJ, Popjak G (1981) Microinjection of arginase into enzyme deficient cells with the isolated glycoproteins of Sendai virus as a fusogen. Biochim Biophys Acta 645:339–345
Lalazar A, Loyter A (1979) Involvement of spectrin in membrane fusion: Induction of fusion in human erythrocyte ghosts by proteolytic enzymes and its inhibition by antispectrin antibody. Proc Natl Acad Sci USA 76:318–322
Lalazar A, Reichler Y, Loyter A (1976) Reversible aggregation of intramembrane particles during virus induced fusion of human erythrocytes. Proc Sixth Europ Congress Elect Microsc, Jerusalem, pp 327–330
Landry Y, Lehninger AL (1976) Transport of Ca ions by Ehrlich ascites tumor cells. Biochem J 158:427–438
Landsberger FR, Compans RW (1976) Effect of membrane protein on lipid bi-layer structure:

a spin label electron spin resonance study of vesicular stomatitis virus. Biochemistry 15: 2356–2360
Landsberger FR, Lyles DS, Choppin PW (1978) Enveloped viruses: structure and interaction with cell membranes. In: Mahy BWJ, Barry RD (eds) Negative strand viruses and the host cell. Academic, New York, pp 787–800
Lane CD, Marbaix G, Gurdon JB (1971) Rabbit haemoglobin synthesis in frog cells: the translation of reticulocyte 9S RNA in frog oocytes. J Mol Biol 61:73–91
Lau C, Richards FM (1976) Proteolytic and chemical modification of colicin E3 activity. Biochemistry 15:3856–3863
Lau YF, Brown RL, Arrighi FE (1977) Induction of premature chromosome condensation in the CHO cells fused with polyethelene glycol. Exp Cell Res 110:57–61
Law BA, John P (1981) Effect of lactoperoxidase bactericidal system on the formation of the electrochemical proton gradient in *E. coli.* FEMS Microbiol Lett 10:67–70
Lenard J, Rothman JE (1976) Transbi-layer distribution and movement of cholesterol and phospholipids in the membrane of influenza virus. Proc Natl Acad Sci USA 73:391–395
Levanon A, Inbar M, Kohn A (1979) Fluorescence polarization of DPH labelled cells adsorbing viruses and its diagnostic potential. Arch Virol 59:223–230
Levanon A, Kohn A (1978) Changes in cell membrane microviscosity associated with adsorption of virus. FEBS Lett 85:245–248
Levanon A, Kohn A, Inbar M (1977) Increase in lipid fluidity of cellular membranes induced by adsorption of RNA and DNA viruses. J Virol 22:353–360
Lew VL, Ferreira HG (1976) Variable Ca sensitivity of a K selective channel in intact red-cell membranes. Nature 263:336–338
Li E, Kornfeld S (1977) Effects of wheat germ agglutinin on membrane transport. Biochim Biophys Acta 469:202–210
Lieberman MA, Hong J (1974) A mutant of *Escherichia coli* defective in the coupling of metabolic energy to active transport. Proc Natl Acad Sci USA 71:4395–4399
Lion MB, Bergmann ED (1961) The effects of oxygen on freeze-dried *Escherichia coli.* J Gen Microbiol 24:191–200
Loewenstein WR (1979) Functional intercellular communication and the control of growth. Biochim Biophys Acta 560:1–65
Loyter A, Zakai N, Kulka RG (1975) Ultramicroinjection of macromolecules or small particles into animal cells. A new technique based on virus induced cell fusion. J Cell Biol 66:292–304
Loyter A, Kulka RG, Zakai N, Ronn G, Reichler Y, Lalazar A (1976) Induction of reversible rearrangement of membrane proteins and phospholipids during fusion of human erythrocyte ghosts. Proc Sixth Europ Congr Elect Micros, Jerusalem, pp 33–38
Lucy AJ (1970) The fusion of biological membranes. Nature 227:815–817
Lusk JF, Nelson DL (1972) Effects of colicins E1 and K on permeability to magnesium and cobaltous ions. J Bacteriol 112:148–160
Lusk JF, Park MH (1975) Phospholipase activity plays no role in the action of colicin K. Biochim Biophys Acta 394:129–134
Lyles DS, Landsberger FR (1976) Virus and lectin agglutination of erythrocytes: Spin label study of membrane lipid-protein interaction. Proc Natl Acad Sci USA 73:3479–3501
Lyles DS, Landsberger FR (1978) Enveloped virus and lectin-induced structural change in erythrocyte lipid bi-layer: dependence on species of erythrocyte and on the multivalence of the agglutinin. Virology 88:25–32
MacGregor CH, Bishop CW, Blech JE (1979) Localization of proteolytic activity in the outer membrane of *Escherichia coli.* J Bacteriol 137:574–583
Maeda T, Asano O, Ohki K, Okada Y, Ohnishi SI (1975) A spin-label study on fusion of red blood cells induced by hemagglutinating virus of Japan. Biochemistry 14:3736–3741
Maeda T, Asano O, Okada Y, Ohnishi SI (1977a) Transmembrane phospholipid motions induced by F glycoprotein in hemagglutinating virus of Japan. J Virol 21:232–241
Maeda T, Kim J, Koseki I, Mekada E, Shikawa Y, Okada Y (1977b) Modification of cell membrane with viral envelope during fusion of cells with HVJ (*Sendai virus*). II. Effects of mono and disaccharides on cell fusion and membrane movement of fused cells. Exp Cell Res 108:95–106
Maeda T, Eldridge C, Toyama S, Ohnishi SI, Elson EL, Webb WW (1979) Membrane receptor mobility changes by Sendai virus. Exp Cell Res 123:333–343

Magee WE, Miller OV (1972) Liposomes containing antiviral antibody can protect cell from virus infection. Nature 235:339–340
Makan NR (1978) Induction of permeability change and restoration of membrane permeability barrier in transformed cell cultures. Exp Cell Res 114:417–427
Maraldi NM, Barbanti-Brodano G, Portolani M, La Placa M (1975) Ultra-structural aspects of BK virus uptake and replication in human fibroblasts. J Gen Virol 27:71–80
Marcum JM, Dedmar JR, Brinkley BR, Means AR (1978) Control of microtubule assembly-disassembly by calcium-dependent regulator protein. Proc Natl Acad Sci USA 75:3771–3775
Marsh M, Helenius A (1980) Adsorptive endocytosis of Semliki Forest Virus. J Mol Biol 142: 439–454
Mastro AW, Rozengurt E (1975) Endogenous protein kinase in outer plasma membrane of cultured 3T3 cells. J Biol Chem 251:7899–7906
Mathews CK (1971) Bacteriophage chemistry. Van Nostrand Reinhold, New York, chaps 4 and 7
Matlin KS, Reggio H, Helenius A, Simmons K (1981) Infectious entry pathway of influenza virus in canine kidney cell line. J Cell Biol 91:601–613
Meiselman N, Kohn A, Danon D (1967) Electron microscopic study of penetration of NDV into cells leading to formation of polykaryocytes. J Cell Sci 2:71–76
Micklem KJ, Pasternak CA (1977) Surface components involved in virally mediated membrane changes. Biochem J 162:405–410
Milner Y, Ben Zaquen R, Loyter A (1978) A possible correlation between the fusogenic activity of Sendai virus and its ability to dephosphorylate membrane proteins. In: Krause EG, Pinna L, Wollenberger. A Proc 12th FEBS Meeting, vol 54/S7. Pergamon, Oxford, pp 211–224
Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144–148
Miyake Y, Kim J, Okada Y (1978) Effect of cytochalasin D on fusion of cells by HVJ (Sendai virus). Cell: cell fusion is separable from cell: virus fusion. Exp Cell Res 116:167–178
Moore NF, Barenholz Y, Wagner RR (1976) Microviscosity of togavirus membrane studies by fluorescence depolarization: influence of envelope proteins and the host cell. J Virol 19:126–135
Moore NF, Patzer EJ, Barenholz Y, Wagner RR (1977a) Effect of phospholipase C and cholesterol oxidase on membrane integrity, microviscosity and infectivity of vesicular stomatitis virus. Biochemistry 16:4708–4715
Moore NF, Wagner RR, Yeagle PL, Hutton WC, Martin RB (1977b) The structure of vesicular stomatitis virus membrane. A phosphorus nuclear magnetic resonance approach. Biochim Biophys Acta 464:234–244
Moore NF, Patzer EJ, Shaw JM, Thompson TE, Wagner RR (1978) Interaction of vesicular stomatitis virus with lipid vesicles: Depletion of cholesterol and effect on virion membrane fluidity and infectivity. J Virol 27:320–329
Nagai Y, Ogura H, Klenk HD (1976) Studies in the assembly of the envelope of Newcastle disease virus. Virology 69:523–538
Nair CN, Stowers JW, Singfield B (1978) Guanidine sensitive Na^+ accumulation of poliovirus infected HeLa cells. J Virol 31:184–189
Natarajan AT, Obe G (1978) Molecular mechanisms involved in the production of chromosomal aberrations. I. Utilization of Neurospora endonuclease for the study of aberration production in G2 stage of the cell cycle. Mutat Res 52:137–149
Natarajan AT, Obe G, Van Zeeland AA, Palitti F, Meijers M, Verdegaal-Immerzeed EAM (1980) Molecular mechanisms involved in the production of chromosomal aberrations. II. Utilization of Neurospora endonuclease for the study of aberration production by X-rays in G1 and G2 stages of the cell cycle. Mutat Res 69:293–305
Negreanu Y, Reinhertz Z, Kohn A (1974) Effects of adsorption of U.V. inactivated parainfluenza 1 (Sendai) virus on the incorporation of amino acids in animal host cells. J Gen Virol 22:265–270
Nicolau C, Klenk HD, Hildebrand K, Reimann B, Reimann A, Bauer M (1979a) Early molecular events in the interaction of enveloped riboviruses with cells. I. A fluorescence and radioactivity study. Biophys Struct Mech 5:11–23
Nicolau C, Klenk HD, Reimann A, Hildebrand K, Bauer H (1979b) Molecular events during the interaction of envelopes of myxo and RNA tumor viruses with cell membranes. A 270 MHZ 'H nuclear magnetic resonance study. Biochim Biophys Acta 511:83–92
Nieva-Gomez D, Gennis RB (1977) Affinity of intact *Escherichia coli* for hydrophobic membrane

probes is a function of the physiological state of the cells. Proc Natl Acad Sci USA 74: 1811–1815
Nieva-Gomez D, Konisky J, Gennis RB (1976) Membrane changes in *Escherichia coli* induced by colicin Ia and agents known to disrupt energy transduction. Biochemistry 15:2747–2753
Nishimura T, Furusawa M, Yamaizumi M, Okada Y (1976) Method for intercellular injection by cell fusion using erythrocyte ghosts. Cell Struct Funct 1:197–200
Nishiyama Y, Ito Y, Shimokata K, Kimura Y, Nagata I (1976) Polykaryocyte formation induced by VSV in mouse L cells. J Gen Virol 32:85–96
Nomura M (1963) Mode of action of colicins. Cold Spring Harbor Symp Quant Biol 28:315–324
Nomura M (1964) Mechanism of action of colicins. Proc Natl Acad Sci USA 52:1514–1521
Nomura M, Nakamura M (1962) Reversibility of inhibition of nucleic acids and protein synthesis by colicin K. Biochem Biophys Res Commun 7:306–309
Obe G, Natarajan AT (1980) Comparison between inactivated Sendai virus, polyethylene glycol and Tween 80 as permeabilizing agents for introduction of Neurospora endonuclease into X-irradiated cells. Mutat Res 71:133–138
Ohki K, Nakama S, Asano A (1975) Stimulation of virus induced fusion of Ehrlich ascites tumor cells by 3′, 5′-cyclic AMP. Biochem Biophys Res Commun 67:331–337
Ohki K, Asano A, Okada Y (1978) Effects of cyclic AMP and calcium ions on virus induced fusion of Ehrlich ascites tumor cells. Biochim Biophys Acta 507:507–516
Ohno S, Ohno-Iwashita Y, Suzuki K, Imahori K (1977) Purification and characterization of active component and active fragment of colicin E3. Biochem J 82:1045–1053
Okada Y (1958) The fusion of Ehrlich's tumor cells caused by HVJ virus in vitro. Biken J 1:103–110
Okada Y (1969) Factors in fusion of cells by HVJ. Curr Top Microbiol Immunol 48:102–128
Okada Y, Murayama F (1966) Requirement of calcium ions for the cell fusion reaction of animal cells by HVJ. Exp Cell Res 44:527–551
Okada Y, Hosaka Y, Shimizu K, Matsui K (1975a) Somatic hybrid cell formation by viral genome-free envelopes reassembled from solubilized HVJ (Sendai virus). Cell Struct Funct 1:129–132
Okada Y, Koseki I, Kim J, Maeda Y, Hashimoto T, Kanno Y, Matsui Y (1975b) Modification of cell membranes with viral envelopes during fusion of cells with HVJ (Sendai virus). I. Interaction between cell membranes and virus in the early stage. Exp Cell Res 93:368–378
Okada Y, Hashimoto T, Maeda Y (1975c) Modification of cell membranes with viral envelopes during fusion of cells with HVJ (Sendai virus). II. Effects of pretreatment with a small number of HVJ. Exp Cell Res 93:379–387
Oldstone MBA, Fujinami RS, Lampert PW (1980) Membrane and cytoplasmic changes in virus-infected cells induced by interactions of antiviral antibody with surface viral antigen. Prog Med Virol 26:45–93
Olson AC, Billen D (1978) DNA replication in X-irradiated Chinese hamster cells made permeable to deoxyribonucleoside triphosphates. Radiat Res 74:199–204
Olson LC (1976) Methods of cell fusion with Germiston virus. Methods Cell Biol 14:11–22
Orly J, Schramm M (1975) Fatty acid as modulators of membrane functions of catecholamine activated adenylate cyclase of the turkey erythrocyte. Proc Natl Acad Sci USA 72:3433–3437
Papahadjopoulos D, Mayhew E, Poste G, Smith S (1974a) Incorporation of lipid vesicles by mammalian cells provides a potential method for modifying cell behaviour. Nature 252:163–166
Papahadjopoulos D, Poste G, Mayhew E (1974b) Cellular uptake of cyclic AMP captured within phospholipid vesicles and effect on cell-growth behaviour. Biochim Biophys Acta 363:404–418
Papahadjopoulos D, Poste G, Scheffer BE, Vail SI (1974c) Membrane fusion and molecular segregation in phospholipid vesicles. Biochim Biophys Acta 352:10–28
Park JC, Snow RL (1972) Influence of external ATP on permeability and metabolism of dog red blood cells. Am J Physiol 223:888–893
Pasternak CA (1980) Virally mediated changes in cellular permeability. Biochem Soc Trans 8:700–702
Pasternak CA, Micklem KJ (1973) Permeability changes during cell fusion. J Membr Biol 14: 293–303
Pasternak CA, Micklem KJ (1974a) The biochemistry of virus induced cell fusion. Changes in membrane integrity. Biochem J 140:405–411
Pasternak CA, Micklem KJ (1974b) Virally mediated membrane changes: inverse effects on transport and diffusion. Biochem J 144:593–595

Patzer EJ, Moore NF, Barenholz Y, Shaw JM, Wagner RR (1978) Lipid organization of the membrane of vesicular stomatitis virus. J Biol Chem 253:4544–4550
Paul M, Randal N (1976) Ca^{++} influx following fertilization of Urechis caupo eggs. J Cell Biol 70 (2 pt 2): 222a
Perdue JP (1971) The isolation and characterization of plasma membranes from cultured cells. III. The adenosine triphosphate dependent accumulation of Ca^{++} by chick embryo fibroblasts. J Biol Chem 246:6750–6759
Phillips SK, Cramer WA (1973) Properties of the fluorescence probe response associated with the transmission mechanism of colicin E1. Biochemistry 12:1170–1176
Pitts JD (1975) How do animal cells communicate? Nature 255:371–372
Plate CA (1973) Effects of temperature and/or fatty acid substitutions on colicin K action. Antimicrob Agents Chemother 4:16–24
Plate CA (1976) Mutant of *Escherichia coli* defective in response to colicin K and in active transport. J Bacteriol 125:467–474
Plate CA (1979) Requirement for membrane potential in active transport of glutamine by *Escherichia coli*. J Bacteriol 137:221–225
Plate CA, Luria SE (1972) Stages in colicin K action, as revealed by the action of trypsin. Proc Natl Acad Sci USA 69:2030–2034
Plate CA, Sutt JL, Jettin AM, Luria SF (1974) Effects of colicin K on a mutant of *Escherichia coli* deficient in Ca^{++} Mg^{++} activated adenosine triphosphatase. J Biol Chem 249:6138–6143
Ponta H, Altendorf KH, Schweiger M, Hirsch-Kaufmann M, Pfennig-Yeh ML, Herrlich P (1976) *E. coli* membranes become permeable to ions following T7 virus infection. MGG 149:145–150
Poste G (1970) Virus induced polykaryocytosis and the mechanism of cell fusion. Adv Virus Res 16:125–140
Poste G (1972) Mechanisms of virus-induced cell fusion. Int Rev Cytol 33:157–251
Poste G, Allison AC (1971) Membrane fusion reaction: a theory. J Theor Biol 132:165–184
Poste G, Pasternak CA (1978) Virus induced cell-fusion. Cell Surf Rev 5:306–367
Poste G, Reeve P (1972) Inhibition of virus induced cell fusion by local anaesthetics and phenothiazine tranquillizers. J Gen Virol 16:21–28
Prescott DM, Myerson D, Wallace J (1972) Enucleation of mammalian cells with cytochalasin B. Exp Cell Res 71:480–485
Pressman BC (1976) Biological applications of ionophores. Annu Rev Biochem 45:501–530
Puck TT, Lee HH (1954) Mechanism of cell wall penetration by viruses. I. An increase in host cell permeability induced by bacteriophage infection. J Exp Med 9a:481–494
Quastel MR, Kaplan JG (1970) Early stimulation of potassium uptake in lymphocytes treated with PHA. Exp Cell Res 63:230–233
Quastel MR, Kaplan JG (1975) Ouabain binding to intact lymphocytes. Enhancement by phytohemagglutinin and leucoagglutinin. Exp Cell Res 94:351–362
Quastel MR, Dow DS, Kaplan IG (1970) Stimulation of ^{42}K uptake into lymphocytes by phytohemagglutinins and role of intra-cellular K^+ in lymphocyte transformation. In: Harris JE (ed) Proc 5th Leucocyte Culture Conference. Academic, New York, pp 99–123
Ramos S, Kaback HR (1977) The electrochemical proton gradient in *Escherichia coli* membrane vesicles. Biochemistry 16:848–854
Reed RW (1976) Effects of the divalent cation ionophore A23187 on potassium permeability of rat erythrocytes. J Biol Chem 251:3489–3494
Rechsteiner M (1975) Uptake of proteins by red blood cells. Exp Cell Res 93:487–492
Robinson JM, Roos DS, Davidson RL, Karnovsky MJ (1979) Membrane alterations and other morphological features associated with polyethylene glycol-induced cell fusion. J Cell Sci 40:63–75
Roizman B (1962a) Polykaryocytosis induced by viruses. Proc Natl Acad Sci USA 48:228–234
Roizman B (1962b) Polykaryocytosis. Cold Spring Harbor Symp Quant Biol 27:327–340
Rose B, Loewenstein WR (1975) Permeability of cell junction depends on local cytoplasmic calcium activity. Nature 254:250–252
Rozengurt E, Heppel LA (1975) A specific effect of external ATP on the permeability of transformed 3T3 cells. Biochem Biophys Res Commun 67:1581–1589
Rozengurt E, Heppel LA, Friedberg I (1977) Effect of exogenous ATP on the permeability of transformed cultures of mouse cell lines. J Biol Chem 252:4584–4590

Sabet SF (1978) Colicins and bacterial membranes: structures and functions. J Biol Chem 253: 982–995

Scheid A, Choppin PW (1974) Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology 57:475–490

Schein SJ, Kagan BL, Finkelstein A (1978) Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes. Nature 276:159–163

Schlegel RA, Rechsteiner MC (1975) Microinjection of thymidine kinase and bovine serum albumin into mammalian cells with red blood cells. Cell 5:371–379

Schwartz SA, Helinski DR (1971) Purification and characterization of colicin E1. J Biol Chem 246:6318–6327

Seeman P (1967) Transient holes in the erythrocyte membrane during hypotonic hemolysis and stable holes in the membrane after lysis by saponin and lysolecithin. J Cell Biol 32:55–70

Seeman P (1974) Ultrastructure of membrane lesions in immune lysis, osmotic lysis and drug-induced lysis. Fed Proc 33:2116–2124

Sefton BM, Gaffney BJ (1974) Effects of viral proteins on the fluidity of membrane lipids in Sindbis virus. J Mol Biol 90:343–358

Segel GB, Lichtman MA (1976) Potassium transport in human blood lymphocytes treated with phytohemagglutinin. J Clin Invest 58:1358–1369

Segel GB, Gordon BR, Lichtman MA, Hollander MM, Klemperer MR (1976) Exodus of $^{42}K^+$ and $^{86}Rb^+$ from rat thymic and human blood lymphocytes exposed to phytohemagglutinin. J Cell Physiol 87:337–344

Sekiguchi M (1966) Studies on the physiological defect in rII mutants of bacteriophage T4. J Mol Biol 16:503–522

Sekiguchi M, Asano A (1978) Participation of spectrin in Sendai virus-induced fusion of human erythrocyte ghosts. Proc Natl Acad Sci USA 75:1740–1744

Shahar A, Gitelman JP, Mordechai R, Kohn A (1976) Physical changes in HeLa cell membrane adsorbing Sendai virus under conditions of fusion. Sixth Eur Congr Elect Micros, Jerusalem, pp 398–400

Shainberg A, Yagil G, Yaffe D (1969) Control of myogenesis in vitro by Ca^{++} concentration in nutritional medium. Exp Cell Res 58:163–197

Shapira A, Giberman E, Kohn A (1974) Recoverable potassium fluxes variations following adsorption of T4 phages and their ghosts on *Escherichia coli* B. J Gen Virol 23:159–171

Shimizu K, Hosaka Y, Shimizu YK (1972) Solubilization of envelopes of HVJ (Sendai virus) with alkali-emasol treatment and reassembly of envelope particles with removal of the detergent. J Virol 9:842–850

Shimizu YK, Shimizu K, Ishida N, Homma M (1976) On the study of Sendai virus hemolysis. II. Morphological study of envelope fusion and hemolysis. Virology 71:48–60

Shinitzky M (1982) Membrane fluidity and receptor functions. Biomembranes 13 (in press)

Shinitzky M, Barenholz Y (1974) Dynamics of the hydrocarbon layer in liposomes of lecithin and sphingomyelin containing dicetylphosphate. J Biol Chem 249:2652–2658

Shinitzky M, Henkart P (1979) Fluidity of cell membranes – current concepts and trends. Int Rev Cytol 60:121–147

Shinitzky M, Inbar M (1974) Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. J Mol Biol 85:603–615

Shinitzky M, Inbar M (1976) Microviscosity parameters and protein mobility in biological membranes. Biochim Biophys Acta 433:133–149

Silverstein SC, Dales S (1968) The penetration of reovirus RNA and initiation of its genetic function in L-strain fibroblasts. J Cell Biol 36:197–230

Silverstein SC, Steinman RM, Cohn ZA (1977) Endocytosis. Annu Rev Biochem 46:669–722

Smith H (1977) Host and tissue specificities in virus infection of animals. Cell Surf Rev 2:1–46

Smith JD, De Harven E (1974) Herpes Simplex virus and human cytomegalovirus replication in WI-38 cells. II. An ultrastructural study of viral penetration. J Virol 14:945–956

Spiegelstein M, Fuchs P, Haimsohn M, Gittelman J, Kohn A (1976) Membranal changes in HeLa cells adsorbing infective or inactivated Sendai virus. J Cell Biol 70: (2 pt 2) 414a

Steinman RM, Brodie SE, Cohn ZA (1976) Membrane flow during pinocytosis. A serologic analysis. J Cell Biol 68:665–687

Stephenson JR, Dimmock NJ (1975) Early events in influenza virus multiplication. I. Location and fate of the input RNA. Virology 65:77–86

Stephenson JR, Hudson JB, Dimmock NJ (1978) Early events in influenza virus multiplication. II. Penetration of virus into cells at 4 °C. Virology 86:264–271

Stock JB, Rauch B, Roseman S (1977) Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. J Biol Chem 252:1850–1861

Stoffel W, Bister K, Schreiber C, Tunggal B (1976) ^{13}C-NMR studies of the membrane structure of enveloped virions (Vesicular stomatitis virus). Hoppe Seylers Z Physiol Chem 377:905–915

Strittmatter WJ, Hirata F, Axelrod J (1979) Increased Ca^{2+} ATPase activity associated with methylation of phospholipids in human erythrocytes. Biochem Biophys Res Commun 88:147–153

Sugiyama K (1977) Histamine release from rat mast cells induced by Sendai virus. Nature 270: 614–615

Takagaki Y, Kunugita K, Matsuhashi M (1973) Evidence for the direct action of colicin K on aerobic ^{32}P uptake in *Escherichia coli* in vivo and in vitro. J Bacteriol 113:42–50

Takats BJ, Rosenbush JP (1975) Modification of *E. coli* membranes in prereplicative phase of phage T4 infection. Specificity of association and quantitation of bound phage proteins. J Biol Chem 250:2339–2350

Tanaka K, Ohnishi SI (1976) Heterogeneity in the fluidity of intact erythrocyte membrane and its homogenization upon hemolysis. Biochim Biophys Acta 426:218–230

Tanaka K, Sekiguchi M, Okada Y (1975) Restoration of ultraviolet induced unscheduled DNA synthesis of xeroderma pigmentosum cells by the concomitant treatment with bacteriophage T4 endonuclease V and HVJ (Sendai virus). Proc Natl Acad Sci USA 72:4071–4075

Tiffany JM (1977) The interaction of viruses with model membranes. Cell Surf Rev 2:157–194

Tokuda H, Konisky J (1978a) Mode of action of colicin Ia: effect of colicin on the *Escherichia coli* proton electrochemical gradient. Proc Natl Acad Sci USA 75:2579–2583

Tokuda H, Konisky J (1978b) In vitro depolarization of *Escherichia coli* membrane vesicles by colicin Ia. J Biol Chem 253:7731–7739

Tokuda H, Konisky J (1979) Effect of colicins Ia and Ib on ion permeability of liposomes. Proc Natl Acad Sci USA 76:6167–6171

Trams EG (1974) Evidence for ATP action on cell surface. Nature 252:480–482

Uchida T, Kim J, Yamaizumi M, Okada Y (1977) Reassembled HVJ (Sendai virus) envelopes containing non-toxic mutant proteins of diphtheria toxin show toxicity to L-cells. Nature 266:839–840

Uchida T, Kim J, Yamaizumi M, Miyake Y, Okada Y (1979a) Reconstitution of lipid vesicles associated with HVJ (Sendai virus) spikes. Purification and some properties of vesicles containing non-toxic fragment A of diphtheria toxin. J Cell Biol 80:10–20

Uchida T, Kim J, Miyaka Y, Yamaizumi M, Mekada E, Okada Y (1979b) Improved methods using HVJ (Sendai virus) for introducing substances into cells. Biochem Biophys Res Commun 87:371–379

Uratani Y, Cramer WA (1981) Reconstitution of colicin E1 into di-myristoyl-phosphatidylcholine membrane vesicles. J Biol Chem 256:4017–4023

Van Den Berg KJ, Betel I (1972) Increased transport of 2 aminoisobutyric acid in rat lymphocytes stimulated with concanavalin A. Exp Cell Res 76:63–72

Veomett G, Prescott DM, Shay J, Porter KR (1974) Reconstruction of mammalian cells from nuclear and cytoplasmic components separated by treatment with cytochalasin B. Proc Natl Sci USA 71:1999–2002

Volsky DJ, Loyter A (1977) Rearrangement of intramembraneous particles and fusion promoted in chicken erythrocytes by intracellular Ca^{++}. Biochim Biophys Acta 471:243–249

Volsky DJ, Loyter A (1978a) An efficient method for reassembly of fusogenic Sendai virus envelopes after solubilization of intact virions with Triton X-100. FEBS Lett 92:190–194

Volsky DJ, Loyter A (1978b) Role of Ca^{++} in virus-induced membrane fusion. Ca^{++} accumulation and ultrastructural changes induced by Sendai virus in chicken erythrocytes. J Cell Biol 78:465–479

Volsky DJ, Cabantchik ZI, Beigel M, Loyter A (1979) Implantation of the isolated human erythrocyte anion channel into plasma membranes of Friend erythroleukemic cells by use of Sendai virus envelopes. Proc Natl Acad Sci USA 76:5440–5444

Vonka V, Anisimova E, Macek M (1976) Replication of cytomegalovirus in human epitheloid diploid cell line. Arch Virol 52:283–296

Warren GB, Toon PA, Birdsall NJM, Lee AG, Metcalf JC (1974) Reversible lipid titration of the activity of pure ATPase-lipid complexes. Biochemistry 13:5501–5507

Watson DH (1980) Common inner membrane-specific domains of colicins E_1, K, Ia and A. Biochim Biophys Acta 622:287–296

Watson DH, Sherratt DJ (1979) In vivo proteolytic cleavage of colicins requires specific receptor binding. Nature 278:362–364

Weaver CA, Kagan BL, Finkelstein A, Konisky J (1981) Mode of action of colicin Ib. Formation of ion-permeable membrane channels. Biochim Biophys Acta 645:137–142

Weiss MJ, Luria SE (1978) Reduction of membrane potential and immediate effect of colicin K. Proc Natl Acad Sci USA 75:2483–2487

Welsh MJ, Dedmar JR, Brinkley SR, Means AR (1978) Calcium-dependent regulator protein: Localization in mitotic apparatus of eukaryotic cells. Proc Natl Acad Sci USA 75:1867–1871

Wendt L (1970) Mechanism of colicin action. J Bacteriol 104:1236–1241

Wenner C, Hackney J (1976) Inhibition of $^{86}Rb^+$ and $^{22}Na^+$ efflux of Ehrlich Lettrée tumor cells by Ca^{2+}. Arch Biochem Biophys 176:37–42

Williams RJP (1978) The multifarions couplings of energy transduction. Biochim Biophys Acta 505:1–44

Wyke AM, Impraim CC, Knutton S, Pasternak CA (1980) Components involved in virally mediated membrane fusion. Biochem J 190:625–638

Yamaizumi M, Mekad E, Uchida T, Okada Y (1978a) One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. Cell 15:245–250

Yamaizumi M, Uchida I, Okada Y, Furusawa M (1978b) Neutralization of diphtheria toxin in living cells by microinjection of antifragment A contained within released erythrocyte ghosts. Cell 13:227–232

Yamaizumi M, Uchida I, Okada Y (1979) Macromolecules can penetrate the host membrane during the early period of incubation with HVJ (Sendai virus). Virology 95:218–221

Yamamoto H, Nishida K, Beppu T, Arima K (1978) Tryptic digestion of colicin E2 and its active fragment. Biochem J 83:827–834

Zakai N, Kulka RG, Loyter A (1974a) Virus induced fusion as a means of introducing macromolecules and small particles into animal cells. Isr J Med Sci 10:1576

Zakai N, Loyter A, Kulka RG (1974b) Prevention of hemolysis by bivalent metal ions during virus induced fusion of erythrocytes with Ehrlich ascites tumor cells. FEBS Lett 40:331–334

Zakai N, Kulka RG, Loyter A (1976) Fusion of human erythrocyte ghosts promoted by the combined action of calcium and phosphate ions. Nature 263:696–699

Zakai N, Kulka RG, Loyter A (1977) Membrane ultrastructural changes during calcium phosphate-induced fusion of human erythrocyte ghosts. Proc Natl Acad Sci USA 74:2417–2421

Fatty Acid Binding: A New Kind of Posttranslational Modification of Membrane Proteins

MICHAEL F.G. SCHMIDT*

1 Introduction

Many cellular functions are carried out by proteins which are in close association with lipid bilayers. The key structure for regulating many of the cell's activities as a function of the various environmental stimuli is the cell surface membrane. In this regulation, membrane proteins of the cell surface are of utmost importance for receiving extracellular signals as, for instance, through the binding of antigens, hormones, neurotransmitters, lectins, antibodies, neighboring cells, or viruses. The receptors themselves, or other membrane proteins, then transduce information to the appropriate intracellular sites where specific biochemical responses are induced, often including the participation of internal membranes.

* Institut für Virologie, Justus-Liebig-Universität Giessen, Frankfurter Str. 107, D-6300 Giessen Federal Republic of West-Germany

While many ligands, such as antigens, hormones, or viruses, which in a given cell induce specific biochemical reactions have been identified and characterized on a molecular level, their counterparts in the cell surface, the receptors, are usually undefined entities. However, the available data indicate that in many cases surface receptors are structurally represented by glycoproteins (*Kathan* and *Winzler* 1963; *Cuatrecasas* 1974; *Ashwell* and *Morell* 1974; *Cuatrecasas* et al. 1975; *Heidmann* and *Changeux* 1978; *Morgan* 1981; *Lonberg-Holm* and *Philipson* 1981).

To gain more insight into the biosynthesis and properties of membrane glycoproteins, enveloped viruses are increasingly used as model systems. Since these viruses, in contrast to cells, contain only a small number of membrane proteins, it is easy to isolate specific membrane glycoproteins from virus particles in pure form (for review see *Schmidt* 1982c). As will be evident below, the virus system has been widely applied to the study of the biosynthesis and function of membrane glycoproteins in general. The switch-off of host-specific protein synthesis after infection with certain enveloped viruses allows one to observe the biosynthesis of specific viral glycoproteins. It is thus possible to evaluate the various states of modification of these proteins during their maturation to the functional end product (for reviews see *Klenk* and *Rott* 1980; *Gibson* et al. 1980; *Schwarz* and *Schmidt* 1982; *Schlesinger* and *Kääriäinen* 1980; *Simons* and *Garoff* 1980).

In the course of studying the biosynthesis of Sindbis-virus-specific glycoproteins, a new type of posttranslational modification was discovered (*Bracha* et al. 1977b; *Schmidt* et al. 1979). This modification involves the covalent attachment of fatty acids to the polypeptide backbone of the glycoprotein. Since acyl chains are transferred onto the polypeptide, this event is termed "acylation" (*Schmidt* et al. 1979).

It is the purpose of this article to summarize all data available up to July 1982 on fatty acid acylation of membrane proteins. This includes information on the occurrence of fatty acid binding among viral and cellular membrane proteins, the chemical nature of the bond between fatty acid and polypeptide, and finally, the biosynthetic process of fatty acid attachment in relation to other well-characterized co- and posttranslational modifications of these macromolecules. In the discussion, an attempt will be made to explain the implications of fatty acid binding to proteins with respect to the interactions with the lipid bilayer, and to the possible biological functions of the protein-bound acyl chains.

1.1 Modification of Membrane Proteins During Their Biosynthesis

1.1.1 Types of Modifications

During or after their translation on ribosomes many membrane proteins are modified. The main types of modifications are (a) the covalent addition of molecules other than amino acids to the polypeptide, and (b) the proteolytic cleavage of the polypeptide chain at specific sites. The prominent feature of the first type of modification of membrane proteins is the presence of carbohydrate in such proteins, which are therefore, termed glycoproteins. Although known for about 100 years (see *Gottschalk* 1966; *Hughes* 1976), information on the biosynthesis of glycoproteins and the biological functions of their carbohydrate is only recently accumulating (for reviews see *Parodi* and *Leloir* 1979; *Kornfeld* and *Kornfeld* 1980: *Klenk* and *Rott* 1980; *Hubbard* and *Ivatt* 1981; *Schwarz* and *Datema*

1982). Other molecules found covalently linked to amino acids of the polypeptide backbone of proteins are, for instance, acetyl, formyl, and phosphate groups (see *Wold* 1981 for review). However, these kinds of modifications are much less common with membrane proteins than with cytoplasmic or nuclear proteins and by orders of magnitude less frequent than covalently bound carbohydrate. In contrast, the modification described in this article, acylation through the covalent binding of fatty acids to proteins, seems to be very frequent with membrane proteins. The biological significance of acylation, therefore, may well be compared to glycosylation.

The second type of modification of membrane proteins is the proteolytical cleavage of the polypeptide backbone at specific sites. This proteolytical maturation is very widespread and represents a feature quite common with proteins in general (*Reich* et al. 1975; *Holzer* and *Heinrich* 1980; *Wold* 1981).

Proteolytic cleavages occur at different times and sites during or after the translation of a polypeptide. The *cotranslational cleavage* of a common signal peptide has been proven to occur with many membrane and secreted proteins of plant, animal, bacterial, and viral origin (*Wickner* 1979; *Blobel* 1979; *Bonatti* and *Blobel* 1979; *Bar-Nun* et al. 1980; *Nelson* and *Ryan* 1980; *Movva* et al. 1980; *Suchanek* et al. 1980; *Sabatini* et al. 1982; *Lingappa* et al. 1978; *Wirth* et al. 1977; *Bonatti* et al. 1979). Whereas this cotranslational proteolysis seems confined to the rough endoplasmic reticulum, the *posttranslational cleavages* may occur at the endoplasmic reticulum, the Golgi complex, the plasma membrane, or even extracellularly (*Klenk* et al. 1974, 1975; *Klemenz* and *Diggelmann* 1979; *Shapiro* and *August* 1976; *Smith* and *Brown* 1977; *Schlesinger* and *Schlesinger* 1972; *Bracha* and *Schlesinger* 1976; *Lazarowitz* and *Choppin* 1975).

1.1.2 Biosynthesis of Viral Glycoproteins

The use of enveloped animal viruses has proved extremely helpful in correlating biosynthetic modifications of membrane-bound glycoproteins with specific biological functions (*Gibson* et al. 1980; *Rott* 1977, 1979; *Bosch* et al. 1979; *Klenk* et al. 1975, 1978; *Nagai* et al. 1976; *Kääriäinen* and *Söderlund* 1978; *Scheid* and *Choppin* 1976; *Kaluza* et al. 1980; *Schlesinger* and *Kääriäinen* 1980; *Schwarz* and *Schmidt* 1982; *Nakamura* and *Compans* 1978; *Simons* and *Garoff* 1980; *Gething* and *Sambrook* 1981). Likewise, viral systems are indispensable for the study of structural features of membrane proteins with respect to the sites of modification of the polypeptide backbone, i.e. the cleavage and glycosylation sites (*Ward* 1981; *Keil* et al. 1979; *Robertson* et al. 1976; *Bosch* et al. 1981; *Garten* et al. 1981; *Wilson* et al. 1981; *Ward* and *Dopheide* 1979).

Viral glycoproteins are translated like membrane glycoproteins on membrane-bound ribosomes from a specific messenger RNA. In addition to the code for the mature proteins, this mRNA also contains information for a short, hydrophobic amino acid sequence, which in most cases is located at the aminoterminus of the respective protein (*Blobel* and *Dobberstein* 1975; *Rothman* and *Lodish* 1977; *Porter* et al. 1979; *Blobel* and *Sabatini* 1970). This hydrophobic stretch of amino acids (a "signal sequence") aids the insertion of the nascent polypeptide chain into the membrane of the endoplasmic reticulum (*Blobel* and *Dobberstein* 1975; *Wickner* 1979; *Engelman* and *Steitz* 1981; *Sabatini* et al. 1982). During translation the polypeptide is translocated deeper into the lumen of the endoplasmic reticulum and primary glycosylation commences with the transfer of high-mannose-type oligosaccharides from a dolichol-lipid intermediate (*Hemming* 1977;

Parodi and *Leloir* 1979; *Waechter* and *Lennarz* 1976; *Schwarz* et al. 1978) onto specific sites within the primary sequence of the polypeptide (*Nakamura* et al. 1980; *Keil* et al. 1979; *Ward* 1981). Transfer of the nascent polypeptide into the cisternae of the endoplasmic reticulum then proceeds until a hydrophobic region close to the carboxyterminus becomes wedged in the lipid bilayer, thereby anchoring the polypeptide and exposing a few, partially basic amino acids and the carboxyterminus on the cytoplasmic side (*Porter* et al. 1979; *Ward* 1981; *Garoff* et al. 1978; *Simons* and *Garoff* 1980). By an unknown mechanism, the glycoproteins are then transported through the smooth endoplasmic reticulum towards the Golgi complex. During this vectorial intracellular transport the high-mannose oligosaccharides may be "trimmed" to yield complex sugar side chains (*Robbins* et al. 1977; *Hunt* et al. 1978; *Tabas* et al. 1978; *Kornfeld* et al. 1978). From the Golgi complex viral glycoproteins usually move to the plasma membrane where they perform their functions in the assembly of progeny virus particles.

While the intracellular transport is proceeding, many virus-specific glycoproteins are proteolytically cleaved, yielding two or more fragments which may still be linked through disulfide bonds. Although at least with togavirus, influenza, and Rous sarcoma viral glycoproteins, the intracellular and the protein substrate's cleavage sites are defined to some extent, near to nothing is known about the proteolytical enzymes involved in this type of modification (for review see *Klenk* and *Rott* 1980; *Simons* and *Garoff* 1980; *Shapiro* and *August* 1976; *Schlesinger* and *Kääriäinen* 1980).

This route of biosynthesis seems to hold true not only for membrane-bound glycoproteins, but is also principally valid for secreted and lysosomal glycoproteins, which in their mature state function extracellular or in lysosomes. The difference in the final destination of these glycoproteins requires specific recognition mechanisms to provide the proper targeting. Although the Golgi complex is believed to be the cellular organelle in which the "addressing" of glycoproteins occurs, the molecular basis for this process remains to be elucidated (*Palade* 1975; *Tartakoff* 1980; *Bergman* et al. 1981; *Jokinen* et al. 1979; *Hasilik* 1980; *Rothman* 1981).

1.2 Some Properties of Membrane Proteins

One aspect of research on cell surfaces concerns the interaction between proteins and lipids, both of which represent the main chemical components of cellular membranes. To introduce an important distinction, a few words on some properties of membrane proteins seem necessary since interactions between these membrane components may be of an entirely different nature.

Proteins are sometimes associated with lipid bilayers relatively loosely, in which case they are termed peripheral membrane proteins. They can also be inserted deeply or even span the lipid bilayer and are then termed integral membrane proteins. These latter proteins are often closely associated with certain lipids called boundary lipids. In many instances boundary lipids seem essential for the expression of specific biological activities. An example is provided by the mitochondrial cytochrome *c* oxidase, which requires closely associated diphosphatidyl glycerol (cardiolipin) for maximal enzymatic activity (*Awasthi* et al. 1971; *Vik* and *Capaldi* 1977; *Downer* et al. 1976; *Robinson* and *Capaldi* 1977). Similarly, β-hydroxybutyrate dehydrogenase expresses highest enzymatic activity only in the presence of lecithin (*Grover* et al. 1975; *Bock* and *Fleischer* 1975; *Gazotti* et al. 1975).

It is important to note that the interaction between boundary lipid and membrane proteins is of a *noncovalent* nature. Therefore it must be distinguished from the biosynthetic modification of proteins through acylation, which represents the topic of the present article. This novel form of modification results in a *covalent* attachment of fatty acids to the respective polypeptide. As will become apparent below, most protein substrates of acylation that have been characterized to date represent glycosylated membrane proteins.

2 Acylated Proteins

2.1 Viral Glycoproteins with Covalently Linked Fatty Acids

In the course of studying the action of p-nitrophenyl-guanidinium benzoate (NPGB) on viral multiplication (*Bracha* et al. 1977a), the question arose whether lipids might be bound to viral spike glycoprotein. To answer this question, Sindbis virus was grown in chick embryo fibroblasts that were labeled with ^{3}H-palmitic acid. While it had been expected that the viral envelope would be heavily labeled with fatty acids, some ^{3}H-radioactivity was also found to comigrate with viral glycoprotein during the centrifugation of disrupted particles through a detergent-containing sucrose gradient (*Bracha* et al. 1977b). This observation was followed up by the analysis of fatty-acid-labeled Sindbis virus on polyacrylamide gels. The resulting fluorograms revealed that mainly the E2 glycoprotein contained labeled lipid, whereas the E1 glycoprotein, which in virions is present in equimolar amounts, showed only about one-fourth of the radioactivity found in E2 (*Schmidt* et al. 1979).

In order to ascertain and identify the lipid nature of the protein-bound radioactivity, the individual viral glycoproteins were isolated from fatty-acid-labeled Sindbis virus by a variety of methods, including detergent extraction, immunoprecipitation, and preparative polyacrylamide-gel electrophoresis. It was not possible to extract the protein-bound radioactivity with organic solvents in any combination, from either the labeled glycoprotein preparations or from whole virus particles indicating that the lipid was very tightly associated with Sindbis virus glycoproteins. Also pretreatment of virus particles or purified glycoprotein with various detergents failed to render the bound radioactivity susceptible to organic extraction (*Schmidt* et al. 1979). Only through the application of mild alkaline solutions could the tritiated material be cleaved, to be subsequently identified as fatty acid by gas-liquid chromatography (GLC). To establish the molar content of fatty acid in Sindbis virus E1 and E2, these two glycoproteins were isolated from batches of 10–20 mg of *nonlabeled* purified Sindbis virus.

After a sequence of organic extractions to eliminate contaminating envelope lipids the remaining protein-bound lipid radioactivity was cleaved by mild alkali (0.1 *M* potassium hydroxide in methanol, 20 min at room temperature) and quantitated by GLC. This analysis revealed that 1–2 mol fatty acids were bound to 1 mol of Sindbis E1 glycoprotein, while the Sindbis E2 spike glycoprotein contained 5–6 mol fatty acids per mole polypeptide.

In addition to stoichiometric information, these experiments demonstrated that palmitic acid represented the main species of fatty acid bound to these glycoproteins. However, significant amounts of stearic acid and oleic acid were also detected by GLC

(*Schmidt* et al. 1979). Accordingly, viral glycoprotein could be labeled with radioactive oleic and stearic acids, although 9,10-^{3}H-(*N*)-palmitic acid gave the most efficient and most specific labeling (*Schmidt* and *Schlesinger,* unpublished). The fatty acids appear to be bound directly to the polypeptide, since no specific labeling of the acylated glycoproteins could be achieved through the incorporation of ^{32}P-phosphate or 2-^{3}H-glycerol.

From these and other data given below a number of characteristic chemical features can be summarized, which leads to the conclusion that the acyl chains must be covalently linked to the respective protein:

1. Fatty acids are associated with the protein in a linkage which is resistant to detergents and organic extractions with chloroform, methanol, acetone, hexane, ether, and other solvents. The linkage is also resistant to boiling in SDS and during SDS-polyacrylamide-gel electrophoresis (*Schmidt* et al. 1979; *Rice* et al. 1982; *Capone* et al. 1982; *Marinetti* and *Cattieu* 1982).
2. Proteolytic or chemical cleavage of the acyl protein does not liberate the bound fatty acids but instead yields acylated peptides (*Schmidt* and *Schlesinger* 1979; *Petri* and *Wagner* 1980; *Schmidt* 1982a; *Schlesinger* et al. 1981; *Rice* et al. 1982; *Capone* et al. 1982).
3. Fatty acids are released from acyl proteins through treatment with mild alkali in an aqueous or methanolic environment. The latter alkaline hydrolysis yields fatty acid methyl esters via transesterification. These methylesters were identified through GLC (*Schmidt* et al. 1979; *Roßmann* and *Schmidt,* unpublished).
4. Fatty acids are cleaved from acylated proteins during incubation with hydroxylamin at neutral pH. In this case the cleavage products were identified as the respective hydroxamate esters (*Schlesinger* et al. 1980; *Omary* and *Trowbridge* 1981b; *Lambrecht* and *Schmidt,* unpublished).

Meanwhile a series of other enveloped RNA viruses have been analyzed for fatty acid binding in their glycoproteins. These studies revealed a differential distribution of fatty acids among the different species of spike glycoproteins in a given virus (Table 1). In various strains of human and avian influenza viruses labeled with tritiated fatty acid only the hemagglutinin (HA) carries fatty acid, whereas no label could be detected in their second spike glycoprotein, the neuraminidase (NA) (Fig. 1; *Schmidt* 1982b). Very similar results have been obtained with paramyxoviruses. Sendai virus and three different strains of Newcastle disease virus (NDV) which differ in their pathogenicity afford fatty acid binding to their fusion proteins (designated F protein). The other spike glycoprotein of NDV the hemagglutinin-neuraminidase (HN), is virtually free of fatty acid (*Schmidt* 1982b; *Chatis* and *Morrison* 1982; *Schmidt,* unpublished). Vesicular stomatitis virus (VSV), a rhabdovirus, contains 1–2 mol fatty acid per mole of its only glycoprotein, which is designated G protein (*Schmidt* and *Schlesinger* 1979). Also the large glycoproteins (G1 and G2) of LaCrosse virus, a bunyavirus, have recently been reported to be acylated (*Madoff* and *Lenard* 1982). Furthermore, two members of the coronavirus family were found to contain fatty acid in E2, which probably represents the fusogenic one of its two spike glycoproteins (*Schmidt* 1982b; *Niemann* and *Klenk* 1981; *Sturman* et al. 1980). Studying acylation of the glycoproteins of Semliki Forest virus give results almost identical to those with Sindbis virus (both are togaviruses, see above). As seen in Table 1 both Semliki Forest virus glycoproteins contain fatty acid, with E2 being more strongly acylated than E1.

To answer the question whether acylation is host dependent, Semliki Forest virus,

Table 1. Acylated and fatty-acid-free glycoproteins of enveloped viruses

Virus	Cells	Spike glycoproteins		Stoichiometry (mol fatty acid per mol protein)	Reference
		Nonacylated	Acylated		
Rhabdovirus:					
Vesicular stomatitis virus	CEF, CHO	–	G protein	1–2	*Schmidt* and *Schlesinger* (1979)
Togavirus:					
Semliki Forest virus	CEF, BHK, Eveline cells, human lymphoma, insect cells (*Aedes albopictus*)	–	E1, E2	n. d.	*Schmidt* (1982b)
Sindbis virus	CEF, BHK, L cells, insect cells (*Aedes albopictus*)	–	E1, E2	1–2, 5–6	*Schmidt* et al. (1979)
Myxovirus:					
Influenza virus (several strains)	CEF, MDBK, Eveline cells, human lymphoma	NA	HA, HA_2	n. d.	*Schmidt* (1982b)
Newcastle disease virus (several strains)	CEF, MDBK, CAM, CHO	HN	F protein	n. d.	*Schmidt* (1982b) *Chatis* and *Morrison* (1982)
Sendai virus	CEF, CAM	HN (?)	F protein	n. d.	*Roßmann* and *Schmidt* (unpublished)
Coronavirus:					
Bovine coronavirus L9	Bovine fetal thyroid cells	E1	E2	n. d.	*Schmidt* (1982b)
Murine coronavirus (mouse hepatitis virus A59)	Balb C3T3 cells	E1	E2	n. d.	*Niemann* and *Klenk* (1981)
Bunyavirus:					
LaCrosse virus	BHK	–	G1, G2	n. d.	*Madoff* and *Lenard* (1982)

Abbreviations:
CEF – chick embryo fibroblasts; CHO – Chinese hamster ovary cells; BHK – baby hamster kidney cells; MDBK – Madin Darby bovine kidney cells; CAM – chorio allantoic membrane; n. d. – not determined

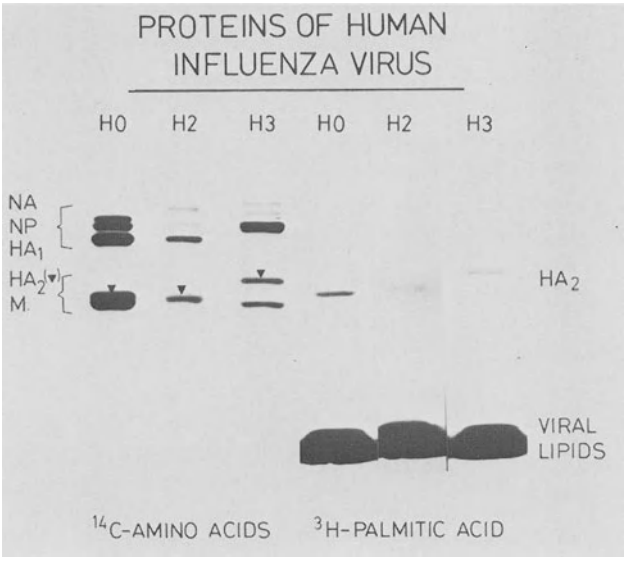

Fig. 1. Acylated polypeptides of human influenza viruses with different hemagglutinin subtypes (*HO, H2,* and *H3*). Influenza virus was grown in chick embryo cells labeled with ^{14}C-protein hydrolysate or ^{3}H-palmitic acid in the presence of 0.001% trypsin in the medium (*Klenk* et al. 1975; *Lazarowitz* and *Choppin* 1975). Released virus particles were purified from the culture fluid and run on a 12% acrylamide gel containing 6 *M* urea. The depicted fluorogram shows viral polypeptides labeled with ^{14}C-amino acids (*left panel*) and with ^{3}H-palmitic acid (*right panel*). Only the smaller cleavage product of the hemagglutinin, designated HA_2, is acylated with fatty acid. The heavily ^{3}H-labeled lipids of the viral envelope are seen as thick black ovals at the bottom of each lane

which has a wide host range, was grown in a number of different cell types including insect, avian, and mammalian cells. In all host cells utilized, the acylation pattern of the virus-specific glycoproteins was found to be the same (*Schmidt* 1982b). In addition to the viruses listed above, preliminary results show fatty acid binding also with glycoproteins coded by murine sarcoma virus (*Schmidt, Schneider, Hunsmann,* unpublished) and with herpesvirus (*Magee* and *Schlesinger*, to be published). Likewise, recent reports indicate that the transforming proteins p60^{src}, p21, and P120 of Rous sarcoma, Harvey sarcoma, and Abelson virus, respectively, are acylated with fatty acids (*Magee* and *Schlesinger*, to be published).

Although not all enveloped viruses have been analyzed for acylation, it seems justified to draw a few conclusions. Firstly, fatty acid binding occurs with enveloped viruses of totally different taxonomic groups including RNA and DNA viruses, whether they are oncogenic or not. Secondly, acylation does not depend on the use of specific host cells, since different cell types were used to propagate the different viruses under study. Thirdly, it is obvious that acylation is restricted to certain species of the specific proteins of a given virus, which may indicate specialized structural or functional features.

In the past many features of membrane glycoproteins have been first detected with viral glycoproteins (see above). It is therefore reasonable to suspect that acylation may also be a structural component of membrane glycoproteins of nonviral origin. And this is indeed the case, as will become apparent in the following section.

2.2 Acylated Membrane Proteins from Noninfected Cells

2.2.1 Classical Proteolipids

Although there is no precedent for covalently bound fatty acids in typical membrane glycoproteins of eukaryotic cells (*Hughes* 1976), tightly bound fatty acids have been reported for a few nonglycosylated bacterial proteins over several years (Table 2). The only protein which by rigorous chemical analysis has been shown to contain covalently bound lipid is the lipoprotein of *E. coli*, which ties the bacterial outer membrane to the peptidoglycan layer (*Braun* and *Rehn* 1969; *Braun* and *Bosch* 1972; *Hantke* and *Braun* 1973; *Chattopad-*

Table 2. Acylated and fatty-acid-free cellular membrane proteins

Membrane protein	Cell type	Acylated	Reference
Lipoprotein	*E. coli*	+	*Hantke* and *Braun* (1973)
Penicillinase	Bacillus species, *S. aureus*	+	*Nielsen* and *Lampen* (1982); *Lai* et al. (1981); *Smith* et al. (1981)
P20	CEF, BHK, KB cells, mouse myeloma cells	+	*Schlesinger* et al. (1980) *Berger* and *Schmidt* (unpublished)
Numerous undefined	CEF, BHK, KB cells, mouse myeloma cells	+	*Schlesinger* et al. (1980) *Berger* and *Schmidt* (unpublished)
Myelin proteolipoprotein	Rat brain	+	*Folch-Pi* and *Lees* (1951); *Schmidt, Schlesinger* and *Agrawal* (unpublished); *Agrawal* et al. (1982)
DM 20	Rat brain	+	*Agrawal* et al. (1982)
Butyrophilin	Milk fat globule membrane	+	*Keenan* et al. (1982)
Xanthine oxidase	Milk fat globule membrane	+	*Keenan* et al. (1982)
F_c receptor	Cells infected with herpesvirus	+	*Magee* and *Schlesinger* (to be published)
$p60^{src}$, p21, p120 transforming proteins	Cells infected with Rous sarcoma, Harvey sarcoma, and Abelson virus	+	*Magee* and *Schlesinger* (to be published)
Ca^{2+}-ATPase	Sarcoplasmic reticulum	+	*MacLennan* et al. (1972)
Transferrin receptor	Human leukemic T-cell lines CCRF-CEM and RPMI 8402	+	*Omary* and *Trowbridge* (1981b)
Histocompatibility antigen HLA	Human leukemic T-cell lines CCRF-CEM and RPMI 8402	−	*Omary* and *Trowbridge* (1981b)
Human T200	Human leukemic T-cell lines CCRF-CEM and RPMI 8402	−	*Omary* and *Trowbridge* (1981b)
Membrane-bound IgM	Human leukemic T-cell lines Ramos and Daudi	−	*Schmidt* and *Fleischer* (unpublished)

hyay and *Wu* 1977). More recent reports describe covalent lipid binding also with membrane penicillinase of *Bacillus licheniformis* and related bacteria (*Sawai* and *Lampen* 1974; *Yamamoto* and *Lampen* 1976a, b; *Smith* et al. 1981; *Lai* et al. 1981; *Nielsen* et al. 1981; *Nielsen* and *Lampen* 1982).

For eukaryotic cells two classes of highly specialized membrane-bound nonglycosylated protein complexes have been reported to contain peptide components with tightly linked fatty acids: the myelin and the sarcoplasmic proteolipids. The first fatty-acid-carrying protein ever described is the protein complex of the myelin membrane (*Folch-Pi* and *Lees* 1951; *Stoffyn* and *Folch-Pi* 1971). In the course of developing the now widely applied procedures for the extraction of lipids from tissue (*Folch* et al. 1951), these investigators discovered a protein complex in lipid extracts from brain tissue. This protein was found to be soluble in chloroform/methanol (2:1, v/v), but insoluble in water. To indicate its lipidlike nature this type of protein was termed "proteolipid" (*Folch-Pi* and *Lees* 1951). Structural analysis of the protein components isolated from the myelin membrane revealed that myelin proteolipid contains 2 mol esterified fatty acid per mole of polypeptide (*Gagnon* et al. 1971; *Moscarello* et al. 1973; *Jolles* et al. 1977; *Lees* et al. 1979). This finding was recently confirmed by in vivo labeling experiments during which tritiated fatty acid coupled to bovine serum albumin was injected into the brains of rats. It was found that radioactivity was specifically incorporated into proteolipid of the myelin membrane (*Schmidt, Schlesinger* and *Agrawal,* unpublished; *Agrawal* et al. 1982). During the course of these studies a second myelin protein, designated DM 20 (*Agrawal* et al. 1972), was also detected in fatty-acid-labeled form (*Agrawal* et al. 1982).

The second example of a proteolipid with covalently linked fatty acids is provided by the Ca^{2+}-dependent ATPase of the sacroplasmic reticulum. This enzyme complex contains a protein component which carries 1–2 fatty acid moieties in covalent linkage (*MacLennan* et al. 1972; *MacLennan* 1975).

Studies following *Folch-Pi* and *Lees'* original reports on myelin proteins revealed that proteolipids were also present in a variety of other tissues of plant or animal origin and were frequently located in mitochondria of the respective cells (*Folch-Pi* and *Stoffyn* 1972; *Zill* and *Harmon* 1962; *Lapetina* et al. 1968; *Murakami* et al. 1962; *Folch-Pi* and *Sakura* 1976; *Lees* et al. 1979). More recently hydrophobic proteins have been shown to be functional constituents of the mitochondrial ATPase complex. Since these proteins bind dicyclohexylcarbodiimide (DCCD), they are often referred to as DCCD-binding proteins (*Tzagloff* and *Meagher* 1972; *Nelson* et al. 1977; *Criddle* et al. 1977; *Sebald* et al. 1979). Although soluble in organic solvents, which qualifies DCCD-binding proteins as proteolipids, no evidence for covalent attachment of any lipids or fatty acids in DCCD-binding proteins has as yet been reported.

In a recent review article *Schlesinger* (1981) suggested altering the original operational definition for the term "proteolipid" (*Folch-Pi* and *Lees* 1951). He suggested the term be used for proteins that contain a lipid moiety as part of their primary structure. Thus proteolipids defined by this new criterion would be analogous to glycoproteins, phosphoproteins, etc., which are terms based on structural features of the respective entities. From my point of view this alteration might lead to the confusion of old and new definitions of the term proteolipid in the literature. Therefore I suggest that we retain the definition originally introduced by *Folch-Pi* and *Lees* (1951) for proteins soluble in organic solvents, and to introduce the new term *acylprotein* for all proteins whose primary structure is modified through covalently bound fatty acids whether they are soluble in organic

solvents or not. Thus, for example, myelin proteolipid would be an acylprotein whereas DCCD-binding proteins would not belong to this new category.

2.2.2 Acylproteins from Cells in Tissue Culture

Since membrane proteins of noninfected tissue culture cells usually represent only a small fraction of total cellular protein, it has been more difficult to study their biosynthesis and properties compared with viral membrane proteins. However, the limitation due to the relative scarcity of specific membrane proteins has been overcome by the development of powerful isolation techniques such as cell fractionation, affinity chromatography, immunological procedures, or their combinations (see *Azzi* et al. 1981). A number of cellular acylproteins have been identified using these techniques (see Table 2).

Accordingly, acylation with *nonviral* proteins was first detected when membrane fractions from cells labeled with ^{3}H-palmitic acid were analyzed (*Schlesinger* et al. 1980). Approximately 20 different protein bands with the ^{3}H-label were detected after polyacrylamide-gel electrophoresis of chloroform/methanol-extracted fractions. Since the proteins could not be labeled in the presence of cycloheximide, fatty acid incorporation must be dependent on protein biosynthesis and is not due to any kind of strong lipid affinity to certain proteins as, for instance, described for bovine serum albumin (*Spector* 1975). Furthermore, no ^{32}P-phosphate was incorporated into the proteins which could be labeled with ^{3}H-palmitic acid and, therefore, the presence of phospholipid, as in the boundary lipids of certain membrane proteins (see above), is most unlikely (*Schlesinger* et al. 1980; *Schmidt*, unpublished). It is noteworthy that acylproteins of about 20 000 daltons molecular weight were found in a number of different cell types (KB cells, chick embryo fibroblasts, mouse myeloma cells, and babyhamster kidney cells) (*Schlesinger* et al. 1980; *Berger* and *Schmidt*, unpublished). Unfortunately no information is yet available on the precise intracellular origin or the structural and functional characteristics of this common acylprotein.

By applying monoclonal antibodies for immunoprecipitation of cell lysates, *Omary* and *Trowbridge* (1981b) demonstrated fatty acid binding with a defined surface glycoprotein, the transferrin receptor of the plasma membrane of human lymphoma cells. In the same report the authors described the lack of fatty acids in two other cell surface glycoproteins, the T200 glycoprotein and the major histocompatibility antigen (HLA). Our own results are compatible with these findings and lend support to the hypothesis that only certain membrane glycoproteins are acylated (*Schmidt* and *Fleischer*, unpublished). As mentioned above, this feature had already become apparent from the study of acylproteins of viral origin (*Schmidt* 1982a, b).

Very recently fatty acid binding has been detected in a number of membrane proteins in rat tissues, human red cells, and in polymorphonuclear cells (*Marinetti* and *Cattieu* 1982). Furthermore, specific labeling with ^{3}H-fatty acids was also achieved with rat erythrocyte membrane proteins and with the two major proteins of the milk fat globule membrane butyrophilin and xanthine oxidase (*Keenan* et al. 1982).

3 Structure of the Linkage Between Fatty Acids and the Polypeptide

3.1 Linkage in Acylproteins of Bacterial Origin

Of all proteins which have been reported to contain tightly bound lipid (for recent reviews see *Schlesinger* 1981 and *Lees* et al. 1979), the murein lipoprotein of the outer membrane of *E. coli* is the only one for which the precise chemical structure of the lipid-protein linkage has been characterized (*Braun* and *Radin* 1969). By analyzing peptides of the lipoprotein, *Hantke* and *Braun* (1973) proved the presence of diglyceride linked to the aminoterminal cysteine residue through a thioether linkage. The same amino acid, in addition to this lipid moiety, also contained one acyl chain in amide linkage, thereby blocking the aminoterminus.

Contrasting results have been reported on the structure of lipid bound to membrane penicillinase of *Bacillus licheniformis*. From a series of investigations *Yamamoto* and *Lampen* (1975, 1976a, b) concluded that the protein was covalently modified through the binding of phosphatidic acid to serine. These results were later disputed by *Simons* et al. (1978), who failed in their attempt to prepare peptides from penicillinase from the same organism with covalently linked lipid. However, the case does not seem to be closed yet, because researchers from three different laboratories have since reported that membrane penicillinase can be biosynthetically labeled with ^{32}P-phosphate, 2-^{3}H-glycerol, and ^{3}H-palmitic acid. The label can be released from membrane penicillinase when the aminoterminus is cleaved through proteolytic digestion (*Lai* et al. 1981; *Nielsen* et al. 1981; *Smith* et al. 1981). Both findings support the original reports by *Yamamoto* and *Lampen* (1975, 1976a, b), but they do not add information with regard to the type of linkage between lipid and polypeptide. However, in their most recent report, *Nielsen* and *Lampen* (1982) demonstrate that covalent lipid binding also applies to β-lactamases of other gram-positive organisms, e.g., *Bacillus cereus* and *Staphylococcus aureus*. After performic acid oxidation and hydrolysis of the membrane forms of penicillinases from *Bacillus licheniformis* and from the above-mentioned species, these authors isolated glyceryl cysteine sulfone (*Nielsen* and *Lampen* 1982). Their results suggested that membrane penicillinases are covalently modified through lipids in a manner very similar to the above-mentioned well-known lipoprotein of the outer membrane of *E. coli* (*Hantke* and *Braun* 1973).

3.2 Linkage in Acylproteins Generated in Tissue Culture Cells

Although the data summarized in Sect. 2 on the properties of protein-bound fatty acids are regarded as very strong evidence for an ester linkage between fatty acids and hydroxy-amino acids of the polypeptide, more definitive chemical analysis of the acylation site has been sought by a number of laboratories. The isolation of small fatty-acid-containing peptides through proteolytical degradation of purified viral or cellular acylproteins was attempted. Acylated peptides were initially obtained through pronase digestion of the VSV G protein and subsequently isolated through extraction with organic solvents (*Schmidt* and *Schlesinger* 1979). In the course of purifying fatty-acid-carrying peptides for amino acid and sequence analyses, unfavorable properties of this material became apparent. Extreme stickiness, tendency to aggregate, and undefined losses of material continue to make it extremely difficult to generate conclusive sequence data on the

attachment site. However, despite these problems in handling acylpeptides, initial amino acid data on fragments of the VSV G protein revealed a relative enrichment of serine residues which, through their hydroxyl groups, could represent the potential binding partners for fatty acid in an ester linkage (*Schmidt* and *Schlesinger* 1979). Similar results were also obtained for acylpeptides derived from the influenza hemagglutinin, and the glycoproteins of Semliki Forest virus (*Schmidt,* unpublished).

While the exact binding sites for fatty acids in acylproteins have not yet been determined, the attempts to localize their topographical position have been more successful. By making use of the protective function of the lipid bilayer during controlled proteolytical digestion of the external portions of membrane-inserted glycoproteins, particles containing membrane-bound spike fragments were generated (Fig. 2). Through such types of experiments it was established that the protein-bound fatty acids are located in polypeptide regions which are not accessible to proteolytic enzymes (*Schlesinger* et al. 1981; *Petri* and *Wagner* 1980; *Rice* et al. 1982; *Schmidt* 1982b; *Capone* et al. 1982; *Omary* and *Trowbridge* 1981b).

Although these data strongly suggested that fatty acids were bound in the carboxy-terminal region of the respective acylprotein, direct proof for such a topographical position was only recently provided through the analysis of large acylated protein fragments.

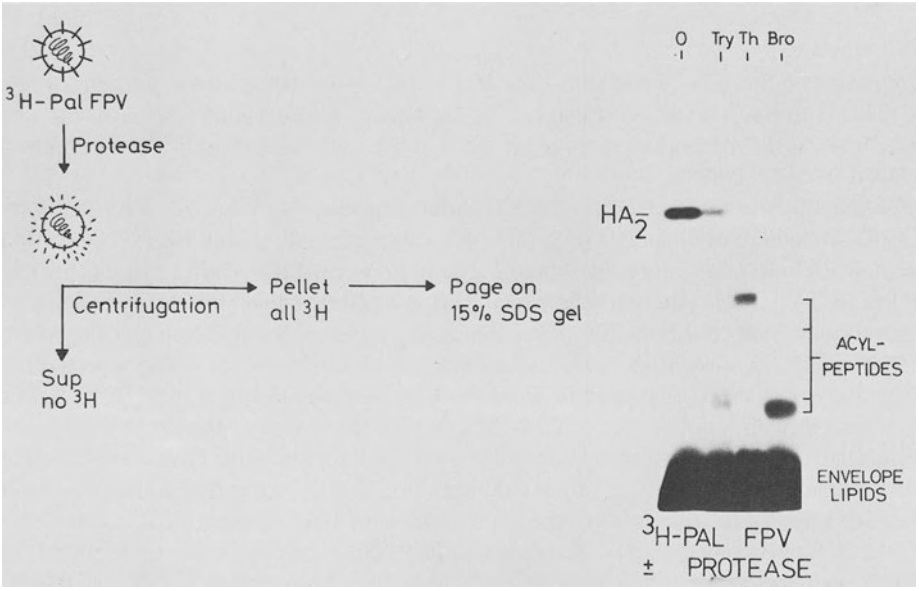

Fig. 2. Analysis on polyacrylamide-gel electrophoresis of membrane-bound acylpeptides after controlled proteolysis of ^{3}H-palmitate-labeled virus particles. The schematic illustration on the *left half* of the figure depicts the experimental procedure. On the *right* a fluorogram of influenza viral acylproteins before and after treatment of virus particles with proteases is shown (*O*, control; *Try,* trypsin; *Th*, thermolysin; *Bro,* bromelain). The enzymes were present at a concentration of 2 µg/ml. Incubations were for 3 min at 37 °C (*Schmidt* 1982b). Application of this experimental setup yielded similar results with a number of other RNA-envelope viruses, for instance vesicular stomatitis virus (*Schlesinger* et al. 1981; *Petri* and *Wagner* 1980; *Capone* et al. 1982), Sindbis virus (*Schmidt,* unpublished; *Rice* et al. 1982), and Semliki Forest virus (*Schmidt,* unpublished)

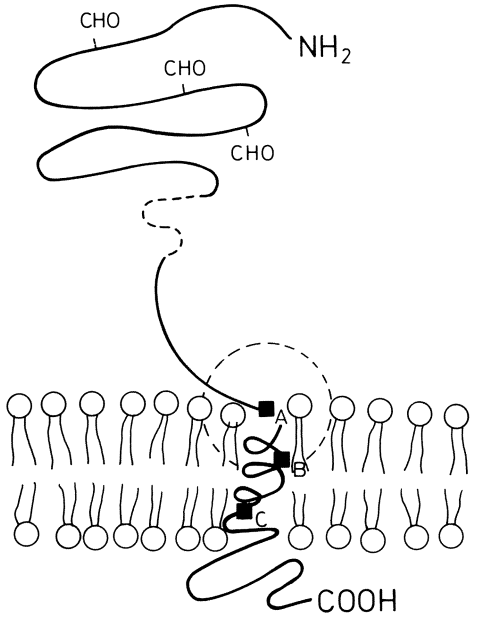

Fig. 3. Model of membrane glycoprotein with potential acylation sites. Fatty acids of fusogenic viral glycoproteins are probably bound to hydroxyamino acids inside or close to the membrane-spanning fragment of the respective polypeptide. If the acylation site is located in the outer leaflet of the lipid bilayer (designated *A*), the fatty acids could potentially be involved in the induction of fusion with heterologous membranes (*Schmidt* 1982a)

By cyanogen bromide (CNBr) cleavage of the purified influenza hemagglutinin a single CNBr peptide was obtained which carried fatty acids. This acylated fragment could be identified as the membrane-spanning peptide of the small subunit of the hemagglutinin (HA_2) (*Schmidt* 1982b).

Recently two research groups have reported amino acid sequence data on the membrane-embedded acylpeptides of Sindbis virus glycoproteins E1 and E2, and of VSV G protein. In both cases fatty-acid-containing peptides were obtained after external proteolytic attack on whole virus particles (see Fig. 2). It was shown that fatty acids were quantitatively confined to the membrane-spanning segments of the respective glycoprotein (*Rice* et al. 1982; *Capone* et al. 1982). Furthermore, *Schlesinger* et al. (1981) reported the isolation of a short oligopeptide from VSV G protein through treatment of this acylprotein with iodobenzoic acid. Although only low yields of the peptide were obtained, after purification it could be confined to an extracytoplasmic position of the spike just "outside" the lipid bilayer of the viral envelope (Fig. 3). Amino acid analysis of this peptide revealed the presence of nine amino acids, five of which were identified as serine residues (*Schlesinger* et al. 1981; *Rose* and *Gallione* 1981). This finding lends support to the hypothesis that serine might serve as the binding partner for fatty acids in acylproteins (*Schmidt* and *Schlesinger* 1979).

4 The Biosynthetic Event of Acylation

While a large body of information has accumulated on the biosynthetic modification of proteins through glycosylation and proteolytic cleavages (*Wold* 1981) our knowledge of the biosynthetic process of fatty acid addition to proteins is very limited. The available data have been derived mainly from pulse-chase experiments with virus-infected tissue

culture cells. Very few data have been reported for acylation in noninfected cells. Some useful information has been obtained through the use of viral glycoprotein mutants and through the application of various metabolic inhibitors.

4.1 Acylation in Virus-Infected Cells

Experimental data on the process of covalent fatty acid addition to membrane proteins were initially obtained through the study of the biosynthesis of VSV and Sindbis virus glycoproteins in infected chick embryo fibroblasts. The incorporation of ^{3}H-palmitic acid into virus-specific glycoproteins during short pulses was related to other well-documented modifications of these polypeptides. It was revealed that in cells infected with Sindbis virus, not the mature glycoprotein E2, but its immediate precursor, PE2, represented the acceptor for fatty acids (Fig. 4). Short-pulse-labeled PE2 was subse-

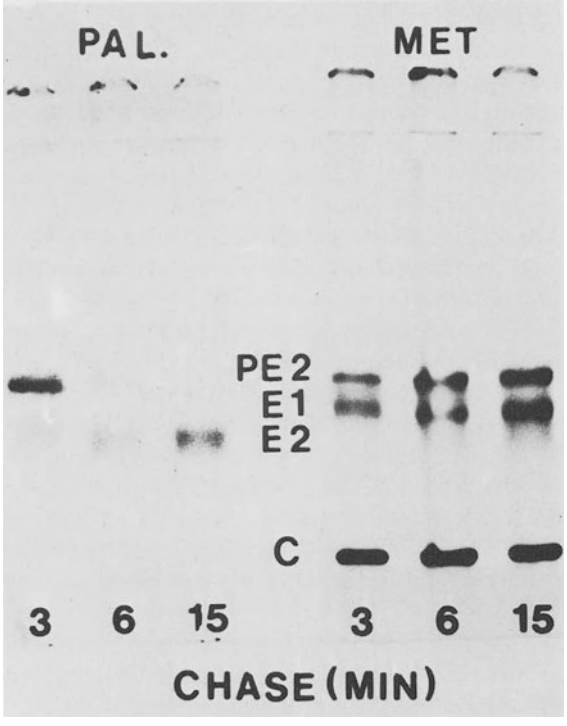

Fig. 4. Cleavage of short-pulse-labeled acylated Sindbis virus precursor glycoprotein *PE2* during a chase. A fluorogram of radiolabeled proteins from Sindbis virus-infected chick embryo cells is shown. The infected cells were labeled at 4.5 h postinfection for 3 min with ^{3}H-palmitic acid (*PAL*) and ^{35}S-methionine (*MET*). Pulse media were replaced by media containing nonlabeled palmitic acid or methionine, and cell lysates were prepared for polyacrylamide-gel electrophoresis after chase periods of 3, 6, and 15 min. While fatty-acid-labeled *PE2* is cleaved into *E2* after 6 min of chase, cleavage of the ^{35}S-methionine-labeled species of *PE2* only starts after 15 min of chase. This indicates that newly synthesized *PE2* takes significantly longer to reach the intracellular cleavage site than freshly acylated *PE2* (*Schmidt* and *Schlesinger* 1980). Almost identical results were obtained with BHK cells infected with Semliki Forest virus (*Berger* and *Schmidt,* unpublished)

quently cleaved very rapidly into E2 during a chase period of only 3–6 min. Since newly synthesized Sindbis PE2 pulse labeled with ^{35}S-methionine took at least 20–30 min to be cleaved, it was concluded that fatty acid addition occurs after the translation of PE2 and just prior to the cleavage of this precursor glycoprotein (*Schmidt* and *Schlesinger* 1980). Acylation must thus be regarded as a posttranslational event (see Fig. 4). The same type of results were obtained with BHK cells infected with Semliki Forest virus (SFV). Again it was the precursor glycoprotein p62 which was acylated first and which then became cleaved into E2 and E3 (*Berger* and *Schmidt,* unpublished).

In addition to defining acylation as a posttranslational process, this type of experiment provides another important piece of information. At the time of pulse labeling (4 h postinfection), precursors and the mature forms of the respective virus-specific proteins are abundant in the infected cells (*Klenk* and *Rott* 1980; *Schlesinger* and *Kääriäinen* 1980; *Schmidt,* unpublished). If acylation represented a nonspecific adhesion of fatty acids to the glycoproteins, one would expect all the different forms of this protein to be labeled during the short pulse of the infected cells with ^{3}H-fatty acid. Since only the precursor glycoproteins are labeled under such conditions acylation must be regarded as a highly specific event which depends on a defined conformation and/or a specific intracellular location of the respective acceptor polypeptide.

The posttranslational character of acylation is also emphasized by data from experiments using cycloheximide and tunicamycin, or virus mutants with defects in their glycoprotein. It was shown, for instance, that cycloheximide inhibits acylation of G protein and of Sindbis virus glycoproteins only after a lag phase of about 15 min (*Schmidt* and *Schlesinger* 1980), which can be taken as direct proof that fatty acid addition does not occur during translation of the polypeptide. Likewise, a block of intracellular transport of G protein by tunicamycin (*Gibson* et al. 1979) also prevents acylation, suggesting that the newly synthesized glycoproteins must be transported to the intracellular site of acylation. Furthermore, when virus-infected cells were pulse labeled with both ^{35}S-methionine and ^{3}H-palmitic acid and the released virus particles were analyzed after increasing chase periods, ^{3}H-labeled glycoproteins preceded the ^{35}S-labeled species in the virions by 15–20 min (*Schmidt* and *Schlesinger* 1980). The studies with ts-mutants of Sindbis virus, VSV, and influenza viruses revealed that acylation was severely inhibited at nonpermissive temperature while allowing for unrestricted protein synthesis (*Schmidt* and *Schlesinger* 1980; *Zilberstein* et al. 1980; *Schmidt* and *Klenk,* unpublished). This finding again stresses the posttranslational nature of fatty acid attachment to the respective viral glycoproteins.

Extending the information on acylation with regard to the intracellular location of the acylating enzymes, *Schmidt* and *Schlesinger* (1980) demonstrated that in VSV-infected chick embryo cells acylation occurs only 3–5 min prior to the completion of oligosaccharide trimming of the G protein. Since it is known that the last steps of this trimming process are conducted by mannosidases present in the Golgi apparatus (*Kornfeld* and *Kornfeld* 1980; *Hubbard* and *Ivatt* 1981; *Tabas* and *Kornfeld* 1979; *Grinna* and *Robbins* 1979, 1980; *Rothman* 1981), it was concluded that acylation occurs in the immediate vicinity of the Golgi apparatus, possibly during late stages of the transit between the endoplasmic reticulum and the Golgi apparatus (*Schmidt* and *Schlesinger* 1980). This hypothesis was recently confirmed through experimental data reported by *Dunphy* et al. (1981). Through cell fractionation studies with Chinese hamster ovary cells (CHO cells) infected with VSV, these authors found that G protein labeled with ^{3}H-palmitic acid in a

short pulse sedimented with the same membrane fraction that also carried α-1,2-mannosidase activity. From their knowledge of the precise location of this glucosidase they concluded that acylation of the G protein must have occurred very close to the cis-portion of the Golgi apparatus (*Dunphy* et al. 1981).

That acylation does not function at a location between the Golgi apparatus and the plasma membrane has been established, at least for viral acylproteins. By growing Sindbis virus and VSV in the presence of the ionophore monensin, *Johnson* and *Schlesinger* (1980) found that intracellular transport of viral glycoproteins functions normally between the endoplasmic reticulum and the Golgi apparatus, but that any further transport towards the plasma membrane of the cells was inhibited. When the glycoproteins were labeled with ^{3}H-palmitic acid in the presence of monensin no effect at all was recorded with regard to acylation of Sindbis virus and VSV glycoproteins (*Johnson* and *Schlesinger* 1980).

Few attempts have yet been made to block acylation of viral glycoproteins by cerulenin (*Omura* 1976) and nafenopin (*Schwarz,* personal communication), drugs which interfere with lipid metabolism. It was found in our laboratory that during in vivo experiments with Semliki Forest virus in BHK cells, both of these drugs severely inhibited both ^{14}C-amino *and* ^{3}H-palmitic acid incorporation into viral glycoprotein, indicating a generalized toxic effect on the cell metabolism. These observations are at variance with a recent report by *Schlesinger* and *Malfer* (1982), who under certain conditions observed a more selective inhibitory effect of cerulenin. These authors found that less tritiated fatty acid was incorporated into VSV and Sindbis virus glycoproteins when virus-infected chick cells had been treated with cerulenin prior to pulse labeling with ^{3}H-palmitic acid. Since cerulenin inhibits the de novo synthesis of fatty acids (*Omura* 1976), it is presently not clear how it influences the incorporation of exogenously applied ^{3}H-palmitic acid into glycoproteins. Although suggested by the above data, a direct inhibitory effect on the acyltransferase is unlikely since cerulenin, even at extremely high concentrations (up to 150 µg/ml), had no effect on the transfer of acyl chains onto lipid acceptors in vitro (*Berger* and *Schmidt,* unpublished). However, different enzymes may catalyze the transfer of fatty acids onto the polypeptide and lipid acceptors.

4.2 Acylation in Noninfected Cells

Information about acylation in noninfected cells is scarce since for eukaryotic cells acylation of nonviral proteins has been described for only a few membrane proteins, most extensively for the transferrin receptor. However, since viral glycoproteins are synthesized by cellular enzymes, it is to be expected that the acylation of viral and nonviral membrane proteins is catalyzed by the same cellular enzymes.

Schlesinger et al. (1980) reported that acylation of cellular membrane proteins, as with viral species, can be inhibited by inhibitors of protein biosynthesis. This demonstrates the specific nature of the event, or at least excludes the possibility of acylating proteins through adsorptive effects. In a more recent study of the biosynthesis of the transferrin receptor, *Omary* and *Trowbridge* (1981a) reported that acylation continued to function even after glycosylation had been blocked through tunicamycin. This result is in accordance with the findings of *Schmidt* and *Schlesinger* (1979), who had shown that the G protein of VSV strain Orsay (*Gibson* et al. 1978, 1979), grown at 30 °C, can be acylated even if

no carbohydrates are bound to the polypeptide backbone of this protein. Both these results represent evidence that oligosaccharides are neither involved as acceptors for fatty acids, nor are they required for allowing the acyltransferases to operate. The only prerequisite for acylation seems to be that the acceptor polypeptide reach a certain intracellular location, probably that of the protein-acyltransferases.

While showing the same chemical binding characteristics, the transferrin receptor seems to differ from viral acylproteins in a few respects. In their recent communication, *Omary* and *Trowbridge* (1981a) demonstrate that this surface protein becomes acylated after oligosaccharide processing is completed. This conclusion is based on the finding that transferrin receptor labeled with ^{3}H-fatty acid during a short pulse is completely resistant to β-endo-glucosaminidase H. Furthermore, by comparing the half-lives of receptor labeled with either ^{35}S-methionine or with ^{3}H-palmitic acid, the above authors found that tritiated fatty acids were lost from the protein at a faster rate than explainable by a mere protein turnover. It was concluded from these data that the transferrin receptor may be subject to deacylation, thereby making this protein available for the addition of new fatty acids during its internalization cycle (*Omary* and *Trowbridge* 1981a; *Morré* et al. 1979; *Karin* and *Mintz* 1981; *Octave* et al. 1981). It was also shown that in this system acylation continued after protein synthesis inhibition by emetine; this emphasizes the authors' hypothesis of de- and reacylation reactions (*Omary* and *Trowbridge* 1981a). The reasons for the discrepancy between the findings with the receptor glycoprotein and the data available for the acylation of viral glycoproteins are presently not understood. However, the transferrin receptor must afford some special features because, in order to function in iron transport, it is subject to internalization (*Morré* et al. 1979; *Karin* and *Mintz* 1981; *Octave* et al. 1981). This process is unlikely to occur with viral glycoproteins, because once at the plasma membrane the mature glycoproteins are subject to homo- and heterologous interactions with viral structural components. This leads to the budding of progeny virus particles (*Simons* and *Garoff* 1980). Also, it is noteworthy that glycosylation of the transferrin differs from that of viral glycoproteins. While the oligosaccharide processing of VSV G protein takes about 15–30 min, the same process with the receptor protein requires a period of at least 4 h (*Omary* and *Trowbridge* 1981a; *Kornfeld* and *Kornfeld* 1980).

Presently, no protein-acyltransferases have been isolated, or even localized, intracellularly. Also the lipid donor for the acyl moities transferred onto the protein has not yet been identified. Since palmitoyl-CoA functions as the acylation precursor during the biosynthesis of phospholipids, its involvement in the transfer of acyl chains onto protein is to be expected (*Bell* and *Coleman* 1980). On the other hand, phospholipids or neutral lipids cannot be excluded as potential acyl donors. Circumstantial evidence for the donor function of phospholipids in the acylation of the bacterial murein lipoprotein has been obtained by *Lai* et al. (1980) and *Chattopadhyay* and *Wu* (1977). Our own in vivo experiments with eukaryotic membrane glycoproteins also indicate the potential for general cellular lipid to act as donor in the acylation of protein. After a long period of labeling of BHK cells with ^{3}H-palmitic acid the pulse medium was replaced with nonlabeled culture fluid and cells were infected with Semliki Forest virus. Four to six hours later cell lysates were prepared and analyzed for protein-bound tritiated fatty acids. Although after long-term labeling no labeled palmitoyl-CoA could be detected in extracts of the cells, acylation of glycoprotein had occurred quite effectively (*Berger* and *Schmidt,* unpublished). However, these experiments do not exclude palmitoyl-CoA as the acyl donor. Because of the very rapid turnover of the intracellular pool of this metabolite, the amount present

may have been too small to be detected by thin-layer chromatography and subsequent radiochromatogram scanning. It thus becomes obvious that an in vitro system of acylation is required to test the suitability of different lipid species for the transfer of acyl chains into polypeptides.

5 Functional Role of Protein-Bound Fatty Acids

Myelin proteolipid was the first acylated protein described more than 30 years ago (*Folch-Pi* and *Lees* 1951). However, the wide occurrence of acylation as a posttranslational modification of membrane proteins has been revealed only during the last 3 years (*Schmidt* et al. 1979; *Schmidt* 1982a, b; *Magee* and *Schlesinger* to be published; *Schlesinger* 1981; *Omary* and *Trowbridge* 1981b; *Madoff* and *Lenard* 1982; *Marinetti* and *Cattieu* 1982; *Keenan* et al. 1982; *Agrawal* et al. 1982; *Nielsen* et al. 1981). It seems natural that to date little experimental evidence for the functional role of this new phenomenon of acylation in cell biology is available, given our limited knowledge about the biological significance of glycosylation, a long-known posttranslational modification of proteins. However, it is not too early to attempt to compare various speculative models that have been discussed in this field.

5.1 The Anchor Hypothesis

Adding hydrophobic fatty acid residues to a protein will certainly increase the hydrophobic affinity of the respective stretch of the polypeptide. It therefore is logical to assume that acylation will contribute to the anchorage of membrane proteins to the lipid bilayer of biological membranes (*Schmidt* et al. 1979), a hypothesis which was followed by other authors (*Omary* and *Trowbridge* 1981a; *Marinetti* and *Cattieu* 1982; *Keenan* et al. 1982; *Agrawal* et al. 1982; *Petri* and *Wagner* 1980). Experimental support for this theoretically based hypothesis has been provided mainly by experiments by *Huang* et al. (1980), who chemically acylated soluble immunoglobulins with fatty acids. Only after such chemical modifications were those glycoproteins suitable for incorporation into liposomes. Furthermore, the possibility was discussed that deacylation of the transferrin receptor at the cell surface may be related to its internalization (*Bretscher* 1977; *Morré* et al. 1979; *Omary* and *Trowbridge* 1981a). This seems an attractive idea, although no data were given that would explain the molecular mechanism of this trigger for receptor recycling. It is known that receptor glycoproteins remain membrane-bound during their internalization. Triggering this process through deacylation, therefore, certainly does not lead to the release of the respective protein from the bilayer of the plasma membrane or from internalized membrane vesicles.

The only data that more directly indicate an anchor function for protein-bound fatty acids come from recent reports on the acylation of transforming proteins and on the fluorescence anisotropy of fatty acids in VSV G protein (*Magee* and *Schlesinger*, to be published; *Petri* et al. 1981). Transforming proteins coded for by certain tumor viruses are synthesized on cytoplasmic ribosomes before they are inserted into membranes (*Levison* et al. 1981). This posttranslational membrane insertion, or anchoring process, is possibly facilitated through the covalent attachment of fatty acids. In a more direct approach, *Petri* et al. (1981) specifically labeled VSV G protein with 16-(9-anthroyloxy)-palmitate and reconstituted it into dipalmitoylphosphatidylcholine vesicles. With such liposomes the mobility of protein-bound fatty acids as a function of temperature could be determined

by fluorescence measurements. The authors found a strong interaction between acyl chains of the G protein and lipids of the artificial vesicles which led to the removal of bilayer lipid from the phase transition during temperature shift experiments (*Petri* et al. 1981). This experiment is important in several respects. It shows that fatty acids bound to VSV G protein are accessible to the environment and thus are not hidden in any clefts or pockets which may be present in the native configuration of the glycoprotein. The data furthermore confirm the biochemical evidence for a membrane location of the acylation site (see Sect. 3.2) through physicochemical methods.

The fact that quite a few fatty-acid-free membrane proteins do exist (*Omary* and *Trowbridge* 1981b; *Schmidt* 1982a, b) does not necessarily exclude the validity of the anchor hypothesis. Similar to glycosylation, where mutations in the polypeptide chain of VSV G protein render the glycoprotein completely functional even without any oligosaccharide chains (*Gibson* et al. 1978, 1979, 1980, 1981), conformational features of nonacylated membrane proteins may provide the required membrane affinity. This may apply, for instance, to the influenza neuraminidase, to the HN glycoprotein of paramyxoviruses, to coronavirus glycoprotein, E1 (*Schmidt* 1982a), to human and murine T200 glycoprotein, and to the histocompatibility antigen, HLA (*Omary* and *Trowbridge* 1981b; *Schmidt* and *Fleischer,* unpublished). One of these nonacylated species deserves special attention, the μ chain of the membrane form of human immunoglobulin (IgM) (*Schmidt* and *Fleischer,* unpublished). This membrane glycoprotein has a surprisingly high proportion of hydrophilic hydroxyamino acids (10 out of a total of 26) within the membrane-associated segment (*Rogers* et al. 1980). Thus acylation, which potentially would convert these hydroxyl-containing residues into hydrophobic moieties thereby fixing the macromolecule into the bilayer, does not seem to be necessary to hold the μ chain in the membrane. Since the hydrophobicity index of the membrane-spanning fragment of IgM is fairly low, one must assume a three-dimensional configuration of this molecule which prevents the exposure of too many hydroxyamino acids to the lipid environment. With acylated membrane proteins, more "configurational freedom" of the membrane-attached region would be provided since "unwanted" hydroxyl groups would be hydrophobically masked.

At first glance the above hypothesis is seriously challenged by certain features of the biosynthesis of membrane proteins. It is widely accepted that initial membrane insertion and subsequent anchorage are processes operating during the translation of the respective molecules (see Sect. 1.1.2), but the attachment of fatty acids, at least with membrane glycoproteins, occurs in the "early" Golgi complex (*Schmidt* and *Schlesinger* 1980; *Dunphy* et al. 1981; *Rothman* 1981), or possibly even later (*Omary* and *Trowbridge* 1981a). A reasonable explanation for this discrepancy is based on the assumption that different stabilities of membrane anchorage may be required during biosynthesis. At early stages membrane affinity of the nascent polypeptide may of necessity be relatively weak, allowing for some flexibility to facilitate the action of contranslationally modifying enzymes. Reaching a higher degree of maturity, the glycoprotein may become anchored more tightly in the lipid bilayer when it becomes modified through fatty acid addition at the site of acylation.

5.2 The Transport Hypothesis

Schmidt and *Schlesinger* (1979) and *Zilberstein* et al. (1980) investigated a number of ts mutants of VSV which carried defects in the G protein. In both reports the lack of acy-

lation at the restrictive temperature is described. With most of the mutants of VSV, the G protein failed to reach the Golgi complex during its biosynthesis, and thus lacked fatty acids. However, *Zilberstein* et al. (1980) detected one mutant, VSV ts L 511 (V), which in Vero cells leads to the synthesis of almost fully glycosylated, but still nonacylated, G protein. Since this species of G protein did not appear on the cell surface, the authors claimed that acylation may be required for the transport of membrane glycoproteins from the Golgi complex to the plasma membrane. However, they did not provide any information on the possible molecular mechanism for this transport function of protein-bound acyl chains.

Meanwhile, more virus mutants of VSV and influenza virus with defects in their glycoproteins have been studied (*Scholtissek* and *Bowles* 1975; *Lohmeyer* and *Klenk* 1979). With these mutants the above hypothesis could not be substantiated, because with none of them was it possible to exclude defects other than in acylation (*Schmidt,* unpublished). With the data available it therefore seems impossible to directly correlate intracellular transport with the presence of fatty acids in the glycoprotein under study at the present time. However, it certainly would be of great interest if a viral ts mutant could be identified with a defect strictly limited to the acylation site of the respective glycoprotein. Obviously, such mutants would facilitate research on the functional relevance of protein-bound fatty acids.

5.3 The Fusion Trigger Hypothesis

Since membrane proteins are modified through fatty acid addition, it seems reasonable to look for common features of the acylated species. It is striking that almost all viral membrane glycoproteins which are acylated with fatty acids are also believed to be involved in fusion induction (*Schmidt* 1982a). The best-documented examples for this property are represented by the F protein of paramyxoviruses (e.g., Newcastle disease virus, Sendai virus); the hemagglutinin, HA_2, of human and avian influenza viruses; and the E2 protein of murine and bovine coronaviruses (*Klenk* and *Rott* 1980; *Schmidt* 1982b; *Scheid* and *Choppin* 1974; *Huang* et al. 1980a, b; *Sturman* et al. 1980). Although no direct experimental evidence has yet been provided for the involvement of protein-bound acyl chains in membrane fusion, a number of findings indicate their potential participation. Oleic acid has long been known to be fusogenic (*Ahkong* et al. 1973) and, among others, this fatty acid has been shown to be a component of acylproteins (*Schmidt* et al. 1979; *Keenan* et al. 1982). Furthermore, using an in vitro system of fusion between artificial membranes and erythrocytes it was recently found that specific lipids inhibit the fusion process (*Huang*, to be published). Similar results had been obtained by *Sands* et al. (1979), who reported the inhibition of penetration of bacteriophage PR4 into *E. coli* through certain fatty acid derivatives. Although neither report offered a molecular mechanism for the observed specific inhibition of fusion, it has been proposed that certain lipid structures in the cellular plasma membranes may function as "fusion receptors." Through an excess of exogenous lipids, these "receptors" could be rendered nonfunctional for their interaction with fusogenic acylproteins which may lead to the observed inhibition of fusion and penetration. The potential function of protein-bound acyl chains in fusion induction is also supported by our knowledge of the topographical location of fatty acids within the polypeptide backbone of viral glycoproteins. Structural analysis of the acylation site in the G

protein of VSV strongly indicates that the acyl chains are bound to amino acids located at the external boundary of the viral lipid bilayer (position A in Fig. 3; *Schlesinger* et al. 1981) where they could function best in triggering fusion with a closely associated membrane.

Clearly, this fusion trigger hypothesis is derived exclusively from data on acylated viral spike glycoproteins, and certainly more structural and functional data are required to substantiate it. Whether this hypothesis has any relevance to acylated membrane proteins in general remains to be seen, since the nonviral proteins found thus far to be acylated serve receptor or enzymatic functions (see Table 2). However, since fusion is a high-frequency process during membrane traffic in any cell (*Morré* et al. 1979; *Bretscher* 1977; *Singer* and *Nicolson* 1975), it must be expected that the cells have some means of controlling these processes. Analogous to the properties of viral glycoproteins, it is anticipated that membrane proteins found to be acylated in noninfected cells may be fusogenic, in addition to their enzymatic or receptor functions. Certainly more experimental data will be necessary to substantiate this hypothesis.

6 Conclusions

Acylproteins must be regarded as a class of proteins involved with many cellular activities operating in a membrane environment. The fact that proteins modified through fatty acids have been found in viral, bacterial, plant, and animal membranes emphasizes their potential importance in cell biology. Obviously, at a time in which the crucial role of cell membranes in cellular function becomes more and more apparent, information on the functional role of this novel kind of polypeptide substituent is of major interest.

Despite the lack of direct evidence for fatty acid function in acylproteins, various models have been discussed based on distribution and structure. However, it will not be surprising if none of the given hypotheses alone suffices to describe the function of acyl chains in proteins. It is more likely that combinations of the individual theories operate with a given acylprotein. Furthermore, as with the oligosaccharides in glycoproteins, the acyl chains in different acylproteins may well serve different functions. Once it is possible to generate deacylated forms of a given acylprotein in the native conformation, meaningful experiments can then be conducted to assess the contribution of acyl chains to the biological activity of these membrane proteins.

In addition to the determination of the functional significance of acylation, there are two more major questions, the answers to which would strongly contribute to an understanding of the function of acylproteins. First of all, the precise chemical structure of the acylation site needs to be elucidated. Secondly, the intracellular location and general identification of the polypeptide acyltransferases need to be determined with certainty.

Acylproteins, through their classical form of "proteolipids," have been known for some 30 years (*Folch-Pi* and *Lees* 1951), but to date neither with these - except for bacterial lipoprotein (*Hantke* and *Braun* 1973) - nor with the recently detected acylproteins (*Schmidt* et al. 1979) has it yet been possible to identify the chemical linkage between fatty acid and polypeptide. However, with all the data on acylation summarized in this article, any initial doubt about the covalent nature of this linkage should be dissipated. Nevertheless, protein chemical analysis of short acylated peptides from various acylproteins (*Jolles* et al. 1981; *Schmidt* 1982a; *Gorski* and *Schmidt*, unpublished) needs to be taken further despite the unfavorable properties of fatty-acid-containing hydrophobic peptides.

This is necessary in order to verify our hypothesis that in most acylproteins fatty acids are linked to hydroxyl-containing amino acids. With regard to the intracellular location of the acylation event, the evidence presented points to the cis-Golgi complex as the acylating organelle. Clearly this conclusion is based on knowledge about the intracellular location of certain other enzymes that operate during glycosylation. Therefore, cell fractionation studies are necessary to prove this hypothesis in a more direct way. Once a precise localization of the cellular acylation site has been achieved, the modificational step of fatty acid addition would be suitable to serve as a marker for specific intracellular membranes during cell biology experiments. Through the establishment of an in vitro system of acylation additional questions could be addressed which eventually would lead to the biochemical characterization of protein-acyltransferases.

Acknowledgement. I thank *Rudi Rott* and *Milton J. Schlesinger* for many stimulating discussions and for encouragement in our research on acylation. The valuable assistance of *Deborah Gorski, Eva Kröll,* and *Margot Seitz* in editorial work and for typing the manuscript is thankfully acknowledged. Work of the author cited in this review was supported by Sonderforschungsbereich 47, a grant from Stiftung Volkswagenwerk, research fellowships of the Deutsche Forschungsgemeinschaft to the author and through research grants to *M.J. Schlesinger* of the National Science Foundation and the National Cancer Institute.

References

Agrawal HC, Burton RM, Fishman MA, Mitchell RF, Prensky AL (1972) Partial characterization of a new myelin protein component. J Neurochem 19:2083–2089

Agrawal HC, Randle CL, Agrawal D (1982) In vivo acylation of rat brain myelin proteolipid protein. J Biol Chem 257:4588–4592

Ahkong QF, Fisher D, Tampion W, Lucy JA (1973) The fusion of erythrocytes by fatty acids, esters, retinal, and α-tocopherol. Biochem J 136:147–155

Ashwell G, Morell AG (1974) The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. Adv Enzymol 41:99–128

Awasthi YC, Chuang TF, Keenan TW, Crane FL (1971) Tightly bound cardiolipin in cytochrome oxidase. Biochem Biophys Acta 226:42–52

Azzi A, Brodbeck V, Zahler P (eds) (1981) Membrane proteins – a laboratory manual. Springer, Berlin Heidelberg New York

Bar-Nun S, Kreibich G, Adesnik M, Alterman L, Negishi M, Sabatini DD (1980) Synthesis and insertion of cytochrome p-450 into endoplasmic reticulum membranes. Proc Natl Acad Sci USA 77:965–969

Bell RM, Coleman RA (1980) Enyzmes of glycerolipid synthesis in eukaryotes. Annu Rev Biochem 49:459–487

Bergmann JE, Tokuyasu KT, Singer SJ (1981) Passage of an integral membrane protein the vesicular stomatitis virus glycoprotein through the Golgi apparatus en route to the plasma membrane. Proc Natl Acad Sci USA 78:1746–1750

Blobel G (1979) From gene to protein. In: Russel TR, Brew K, Faber H, Schultz J (eds) Information transfer in normal and abnormal cells. Academic Press, New York, pp 347–358

Blobel G, Dobberstein B (1975) Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J Cell Biol 67:835–851

Blobel G, Sabatini DD (1970) Controlled proteolysis of nascent polypeptides in rat liver cell fractions. J Cell Biol 45:130–145

Bock H, Fleischer S (1975) Preparation of a homogeneous soluble D-β-hydroxybutyrate apodehydrogenase from mitochondria. J Biol Chem 250:5774–5781

Bonatti S, Blobel G (1979) Absence of cleavable signal sequence in Sindbis virus glycoprotein PE2. J Biol Chem 254:12261–12264

Bonatti S, Cancedda R, Blobel G (1979) Membrane biogenesis, in vitro cleavage, core glycosy-

lation, and integration into microsomal membranes of Sindbis virus glycoproteins. J Cell Biol 80:219–224
Bosch FX, Orlich M, Klenk HD, Rott R (1979) The structure of the hemagglutinin a determinant for the pathogenicity of influenza viruses. Virology 95:197–207
Bosch FX, Garten W, Klenk HD, Rott R (1981) Proteolytic cleavage of influenza hemagglutinins: Primary structure of the connecting peptide between HA_1 and HA_2 determines proteolytic cleavability and pathogenicity of avian influenza viruses. Virology 113:725–735
Bracha M, Schlesinger MJ (1976) Defects in RNA^+ temperature-sensitive mutants of Sindbis virus and evidence for a complex of PE2-E1 viral glycoproteins. Virology 74:441–449
Bracha M, Sagher D, Brown A, Schlesinger MJ (1977a) The protease inhibitor p-nitrophenyl-p-guanidinobenzoate inactivates Sindbis and other enveloped viruses. Virology 77:45–55
Bracha M, Sagher D, Schlesinger MJ (1977b) Reaction of the protease inhibitor p-nitrophenyl-p-guanidinobenzoate with Sindbis virus. Virology 83:246–253
Braun V, Bosch V (1972) Sequence of the murein lipoprotein and the attachment site of the lipid. Eur J Biochem 28:51–69
Braun PE, Radin NS (1969) Interactions of lipids with a membrane structural protein from myelin. Biochemistry 8:4310–4318
Braun V, Rehn K (1969) Chemical characterization, spatial distribution and function of a lipoprotein (mureinlipoprotein) of the *E. coli* cell wall. The specific effect of trypsin on the membrane structure. Eur J Biochem 10:426–438
Bretscher MA (1977) Membrane structure: Some general principles. Science 181:622–629
Capone J, Toneguzzo F, Ghosh HP (1982) Synthesis and assembly of membrane glycoproteins: Membrane anchoring COOH-terminal domain of vesicular stomatitis virus envelope glycoprotein G contains fatty acids. J Biol Chem 257:16–19
Chatis PA, Morrison TG (1982) Fatty acid modification of Newcastle disease virus glycoproteins. J Virol 43:342–347
Chattopadhyay PK, Wu HC (1977) Biosynthesis of the covalently linked diglyceride in murein lipoprotein of *Escherichia coli*. Proc Natl Acad Sci USA 74:5318–5322
Criddle RS, Packer L, Shich P (1977) Oligomycin-dependent ionophoric protein subunit of mitochondrial adenosine-triphosphatase. Proc Natl Acad Sci USA 74:4806–4810
Cuatrecasas P (1974) Membrane receptors. Annu Rev Biochem 43:169–214
Cuatrecasas P, Hollenberg MD, Chang KJ, Bennett V (1975) Hormone receptor complexes and their modulation of membrane function. Recent Prog Horm Res 31:37–78
Downer NW, Robinson NC, Capaldi RA (1976) Characterization of a seventh different subunit of beef heart cytochrome *c* oxidase. Similarities between the beef heart enzyme and that from other species. Biochemistry 15:2930–2936
Dunphy WG, Fries E, Urbani LJ, Rothman JE (1981) Early and late functions associated with the Golgi apparatus reside in distinct compartments. Proc Natl Acad Sci USA 78:7453–7457
Engelman DM, Steitz TA (1981) The spontaneous insertion of proteins into and across membranes: The helical hairpin hypothesis. Cell 23:411–422
Folch J, Ascoli J, Lees M, Meath JA, LeBaron FN (1951) Preparation of lipid extract from brain tissue. J Biol Chem 191:833–841
Folch-Pi J, Lees M (1951) Proteolipids a new type of tissue lipoproteins. Their isolation from brain. J Biol Chem 191:807–817
Folch-Pi J, Sakura JA (1976) Preparation of the proteolipid apoprotein from bovine heart, liver, and kidney. Biochim Biophys Acta 427:410–427
Folch-Pi J, Stoffyn PJ (1972) Proteolipids from membrane systems. Ann NY Acad Sci 195:86–107
Gagnon J, Finch PR, Wood DD, Moscarello MA (1971) Isolation of a highly purified myelin protein. Biochemistry 10:4756–4763
Garoff H, Schwarz RT (1978) Glycosylation is not necessary for membrane insertion and cleavage of Semliki Forest virus membrane proteins. Nature 274:487–490
Garoff H, Simons K, Dobberstein B (1978) Assembly of the Semliki Forest virus membrane glycoproteins in the membrane of the endoplasmic reticulum in vitro. J Mol Biol 124:587–600
Garten W, Bosch FX, Rott R, Klenk HD (1981) Proteolytic activation of the influenza virus hemagglutinin. The structure of the cleavage site. Virology 115:361–374
Gazotti P, Bock H, Fleischer S (1975) Interaction of D-β-hydroxybutyrate apodehydrogenase with phospholipids. J Biol Chem 250:5782–5790

Gething MJ, Sambrook J (1981) Cell-surface expression of influenza hemagglutinin from a cloned DNA copy of the RNA gene. Nature 293:620–625
Gibson R, Leavitt R, Kornfeld S, Schlesinger S (1978) Synthesis and infectivity of vesicular stomatitis virus containing nonglycosylated G protein. Cell 18:671–679
Gibson R, Schlesinger S, Kornfeld S (1979) The nonglycosylated glycoprotein of vesicular stomatitis virus is temperature-sensitive and undergoes intracellular aggregation at elevated temperatures. J Biol Chem 254:3500–3607
Gibson R, Kornfeld S, Schlesinger S (1980) A role for oligosaccharides in glycoprotein biosynthesis. Trends Biochem Sci 11:290–293
Gibson R, Kornfeld S, Schlesinger S (1981) The effect of oligosaccharide chains of different sizes on the maturation and physical properties of the G protein of vesicular stomatitis virus. J Biol Chem 256:456–462
Gottschalk A (1966) Glycoproteins - their composition, structure and function. Elsevier, Amsterdam, pp 1–28
Grinna LS, Robbins PW (1979) Glycoprotein biosynthesis. Rat liver microsomal glucosidases which process oligosaccharides. J Biol Chem 254:8814–8818
Grinna LS, Robbins PW (1980) Substrate specificities of rat liver microsomal glycosidases which process glycoproteins. J Biol Chem 255:2255–2258
Grover AK, Slotboom AJ, De Haas GH, Hammes GG (1975) Lipid specificity of β-hydroxybutyrate dehydrogenase activation. J Biol Chem 250:31–38
Hantke K, Braun V (1973) Covalent binding of lipid to protein. Diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the *Escherichia coli* outer membrane. Eur J Biochem 34:284–296
Hasilik A (1980) Biosynthesis of lysosomal enzymes. Trends Biochem Sci 5:237–240
Heidmann T, Changeux JP (1978) Structural and functional properties of the acetylcholine receptor protein in its purified and membrane-bound states. Annu Rev Biochem 47:317–357
Hemming FW (1977) Dolichol phosphate, a coenzyme in the glycosylation of animal membrane-bound glycoproteins. Biochem Soc Trans 5:1223–1331
Holzer H, Heinrich PC (1980) Control of proteolysis. Annu Rev Biochem 49:63–91
Huang A, Huang L, Kennel SJ (1980) Monoclonal antibody covalently coupled with fatty acid. J Biol Chem 255:8014–8018
Huang RTC, Rott R, Wahn K, Klenk HD, Kohama T (1980a) The function of neuraminidase in membrane fusion induced by myxoviruses. Virology 107:313–319
Huang RTC, Wahn K, Klenk HD, Rott R (1980b) Fusion between cell membrane and liposomes containing the glycoproteins of influenza virus. Virology 104:294–302
Huang RTC (to be published) Involvement of glycolipids in membrane fusion induced by myxoviruses. J Gen Virol
Hubbard SC, Ivatt RJ (1981) Synthesis and processing of asparagine-linked oligosaccharides. Annu Rev Biochem 50:555–583
Hughes RC (1976) Membrane glycoproteins - a review of structure and function. Butterworths, London, pp 1–60
Hunt LA, Etchison JR, Summers DF (1978) Oligosaccharide chains are trimmed during synthesis of the envelope glycoprotein of vesicular stomatitis virus. Proc Natl Acad Sci USA 75:754–758
Johnson DC, Schlesinger MJ (1980) Vesicular stomatitis and Sindbis virus glycoprotein transport to the cell surface is inhibited by ionophores. Virology 103:407–424
Jokinen M, Gahmberg CG, Andersson LC (1979) Biosynthesis of the major human red cell sialoglycoprotein, glycophorin A, in a continuous cell line. Nature 279:604–607
Jolles J, Nussbaum JL, Schoentgen F, Mandel P, Jolles P (1977) Structural data concerning the major rat brain myelin proteolipid P7 apoprotein. FEBS Lett 74:190–194
Kääriäinen L, Söderlund H (1978) Structure and replication of alphaviruses. Curr Top Microbiol Immunol 82:15–69
Kaluza G, Rott R, Schwarz RT (1980) Carbohydrate-induced conformational changes of Semliki Forest virus glycoproteins determine antigenicity. Virology 102:286–299
Karin M, Mintz B (1981) Receptor-mediated endocytosis of transferrin in developmentally totipotent mouse teratocarcinoma stem cells. J Biol Chem 256:3245–3252
Kathan RH, Winzler RJ (1963) Structure studies on the myxovirus hemagglutination inhibitor of human erythrocytes. J Biol Chem 238:21–25

Keenan TW, Heid HW, Stadler J, Jarasch ED, Franke WW (1982) Tight attachment of fatty acids to proteins associated with milk lipid globular membrane. Eur J Cell Biol 26:270–276
Keil W, Klenk HD, Schwarz RT (1979) Carbohydrates of influenza virus III. Nature of linkage of oligosaccharide side-chains to protein in influenza virus glycoproteins. J Virol 31:253–256
Klemenz R, Diggelmann H (1979) Extracellular cleavage of the glycoprotein precursor of Rous sarcoma virus. J Virol 29:285–292
Klenk HD, Rott R (1980) Cotranslational and posttranslational processing of viral glycoproteins. Curr Top Microbiol Immunol 90:19–47
Klenk HD, Wöllert W, Rott R, Scholtissek C (1974) Association of influenza virus proteins with cytoplasmic fractions. Virology 57:28–41
Klenk HD, Rott R, Orlich M, Blödorn J (1975) Activation of influenza A viruses by trypsin treatment. Virology 68:426–439
Klenk HD, Schwarz RT, Schmidt MFG, Wöllert W (1978) The structure and biosynthesis of the carbohydrate moiety of the influenza virus hemagglutinin. Top Infect Dis 3:83–99
Kornfeld R, Kornfeld S (1980) Structure of glycoproteins and their oligosaccharide units. In: Lennarz WJ (ed) The biochemistry of glycoproteins and proteoglycans. Plenum, New York, pp 1–34
Kornfeld S, Li E, Tabas I (1978) The synthesis of complextype oligosaccharides. J Biol Chem 253:7771–7778
Lai JS, Philbrick WM, Wu HC (1980) Acyl moieties in phosholipids are the precursors for the fatty acids in murein lipoprotein of *Escherichia coli.* J Biol Chem 255:5384–5387
Lai JS, Sarvas M, Brammar WJ, Neugebauer K, Wu HC (1981) *Bacillus licheniformis* penicillinase synthesized in *Escherichia coli* contains covalently linked fatty acid and glyceride. Proc Natl Acad Sci USA 78:3506–3510
Lapetina EG, Soto EF, De Robertis E (1968) Lipids and proteolipids in isolated subcellular membranes of rat brain cortex. J Neurochem 15:437–442
Lazarowitz SG, Choppin PW (1975) Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the hemagglutinin polypeptide. Virology 48:440–454
Lees MB, Sakura JD, Sapirstein VS, Curatolo W (1979) Structure and function of proteolipids in myelin and non-myelin membranes. Biochim Biophys Acta 559:209–230
Levinson AD, Courtneidge SA, Bishop JM (1981) Structural and functional domains of the Rous sarcoma virus transforming protein ($p60^{src}$). Proc Natl Acad Sci USA 78:1624–1628
Lingappa VR, Katz FN, Lodish HF, Blobel G (1978) A signal sequence for the insertion of a transmembrane glycoprotein. J Biol Chem 253:8667–8670
Lohmeyer J, Klenk HD (1979) A mutant of influenza virus with a temperature-sensitive defect in the posttranslational processing of the hemagglutinin. Virology 93:134–145
Lonberg-Holm K, Philipson L (1981) Virus Receptors, part 2, Chapman and Hall, Hampshire
MacLennan DH (1975) Resolution of the calcium transport system of sarcoplasmic reticulum. Can J Biochem 53:251–261
MacLennan DH, Yip CC, Iles GH, Seeman P (1972) Isolation of sarcoplasmic reticulum protein. Cold Spring Harbor Symp Quant Biol 37:469–477
Madoff DH, Lenard J (1982) A membrane glycoprotein that accumulates intracellularly: Cellular processing of the large glycoprotein of LaCrosse virus. Cell 28:821–829
Magee AI, Schlesinger MJ (to be published) Fatty acid acylation of eukaryotic cell membrane proteins. Biochim Biophys Acta
Marinetti GV, Cattieu K (1982) Tightly (covalently) bound fatty acids in cell membrane proteins. Biochim Biophys Acta 685:109–116
Morré DJ, Kartenbeck J, Franke WW (1979) Membrane flow and interconversions among endomembranes. Biochim Biophys Acta 559:71–152
Morgan EH (1981) Inhibition of reticulocyte iron uptake by NH_4Cl and CH_3HN_2. Biochim Biophys Acta 642:119–134
Moscarello MA, Gagnon J, Wood DD, Anthony J, Epand RM (1973) Conformational flexibility of a myelin protein. Biochemistry 12:3402–3406
Movva NR, Nakamura K, Inouye M (1980) Amino acid sequence of the signal peptide of omp A protein, a major outer membrane protein of *Escherichia coli.* J Biol Chem 255:27–29
Murakami M, Sekine H, Funahashi S (1962) Proteolipid from beef heart muscle. Application of organic dialysis to preparation of proteolipid. J Biochem (Tokyo) 51:431–435

Nagai Y, Klenk HD, Rott R (1976) Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology 72:494–508
Nakamura K, Compans RW (1978) Effects of glucosamine, 2-deoxy-D-glucose, and tunicamycin on glycosylation, sulfation, and assembly of influenza virus glycoproteins. Virology 84:303–319
Nakamura K, Bhown AS, Compans RW (1980) Glycosylation sites on influenza viral glycoproteins. Virology 107:208–221
Nelson CE, Ryan CA (1980) In vitro synthesis of preproteins of vacuolar compartmented proteinase inhibitors that accumulate in leaves of wounded tomato plants. Proc Natl Acad Sci USA 77:1975–1979
Nelson N, Eytan E, Notsani B, Sigrist H, Sigrist-Nelson K, Gitler C (1977) Isolation of chloroplast N,N′-dicyclo-hexylcarbodiimide-binding proteolipid, active in proton translocation. Proc Natl Acad Sci USA 74:2375–2378
Nielsen JBK, Lampen JO (1982) Membrane-bound penicillinases in gram-positive bacteria. J Biol Chem 257:4490–4495
Nielsen JBK, Caulfield MP, Lampen JO (1981) Lipoprotein nature of *bacillus licheniformis* membrane penicillinase. Proc Natl Acad Sci USA 78:3511–3515
Niemann H, Klenk HD (1981) Coronavirus glycoprotein E1: A new type of viral glycoprotein. J Mol Biol 153:993–1010
Octave JN, Schneider YJ, Crichton RR, Trouet A (1981) Transferrin uptake by cultured rat embryo fibroblasts. The influence of temperature and incubation time, subcellular distribution, and short-term kinetic studies. Eur J Biochem 115:611–618
Omary MB, Trowbridge IS (1981a) Biosynthesis of the human transferrin receptor in culture cells. J Biol Chem 256:12888–12892
Omary MB, Trowbridge IS (1981b) Covalent binding of fatty acid to the transferrin receptor in cultured cells. J Biol Chem 256:4715–4718
Omura S (1976) The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. Bacterial Rev 40:681–697
Palade G (1975) Intracellular aspects of the process of protein synthesis. Science 189:347–358
Parodi AJ, Leloir LF (1979) The role of lipid intermediated in the glycosylation of proteins in the eucaryotic cell. Biochim Biophys Acta 559:1–37
Petri WA JR, Wagner RR (1980) Glycoprotein micelles isolated from vesicular stomatitis virus spontaneously partition into sonicated phosphatidylcholine vesicles. Virology 107:543–547
Petri WA JR, Pal A, Barenholz Y, Wagner RR (1981) Fluorescence anisotropy of a fatty acid covalently linked in vivo to the glycoprotein of vesicular stomatitis virus. J Biol Chem 256: 2625–2627
Porter AG, Barleer C, Carvey NH, Hallewell RA, Threlfall G, Emtage JS (1979) Complete nucleotide sequence of an influenza virus hemagglutinin gene from cloned DNA. Nature 282:471–477
Reich E, Rifkin DB, Shaw E (eds) (1975) Protease and biological control. Cold Spring Harbor Laboratory, New York
Rice CM, Bell JR, Hunkapillar MW, Strauss EG, Strauss JH (1982) Isolation and characterization of the hydrophobic COOH-terminal domains of the Sindbis virion glycoproteins. J Mol Biol 154:355–378
Robbins PW, Hubbard SC, Turco SJ, Wirth DF (1977) Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. Cell 12:893–900
Robertson JS, Etchison JR, Summers DF (1976) Glycosylation sites of vesicular stomatitis virus glycoprotein. J Virol 19:871–878
Robinson NC, Capaldi RA (1977) Interaction of detergents with cytochrome oxidase. Biochemistry 16:375–380
Rogers J, Early P, Carter C, Calame K, Bond M, Hood L, Wall R (1980) Two mRNAs with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin μ chain. Cell 20:303–312
Rose JK, Gallione CJ (1981) Nucleotide sequences of the mRNAs encoding the vesicular stomatitis virus G and M proteins determined from cDNA clones containing the complete coding regions. J Virol 39:519–528
Rothman JE (1981) The Golgi apparatus: Two organelles in tandem. Science 213:1212–1219
Rothman JE, Lodish HF (1977) Synchronised transmembrane insertion and glycosylation of a nascent membrane protein. Nature 269:775–780

Rott R (1977) The structural basis of the function of influenza virus glycoproteins. Med Microbiol Immunol 164:23–33
Rott R (1979) Molecular basis of infectivity and pathogenicity of myxoviruses. Arch Virol 59: 285–298
Sabatini DD, Kreibich G, Morimoto T, Adesnik M (1982) Mechanisms for the incorporation of proteins in membranes and organelles. J Cell Biol 92:1–22
Sands JA, Reinhardt A, Auperin D, Landin P (1979) Inhibition of entry of the lipid-containing bacteriophage PR 4 by fatty acid derivatives. J Virol 29:413–416
Sawai T, Lampen JO (1974) Purification and characteristics of plasma membrane penicillinase from *Bacillus licheniformis* 749/C. J Biol Chem 249:6288–6294
Scheid A, Choppin PW (1974) Identification and biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an intact precursor protein of Sendai virus. Virology 57:475–490
Scheid A, Choppin PW (1976) Protease activation mutants of Sendai virus. Activation of biological properties by specific proteases. Virology 69:265–277
Schlesinger MJ (1981) Proteolipids. Annu Rev Biochem 50:193–206
Schlesinger MJ, Kääriäinen L (1980) Translation and processing of alphavirus proteins. In: Schlesinger WR (ed) Togaviruses. Academic Press, New York, pp 371–392
Schlesinger MJ, Malfer C (1982) Blocking fatty acid acylation of glycoproteins inhibits vesicular stomatitis and Sindbis virus particle formation. J Biol Chem 257:9887–9890
Schlesinger S, Schlesinger MJ (1972) Formation of Sindbis virus proteins: Identification of a precursor for one of the envelope proteins. J Virol 10:925–932
Schlesinger MJ, Magee AI, Schmidt MFG (1980) Fatty acid acylation of proteins in cultured cells. J Biol Chem 255:10021–10024
Schlesinger MJ, Magee AI, Schmidt MFG (1981) Fatty acid acylation of VSV glycoprotein. In: Bishop DHL, Compans RW (eds) The replication of negative strand viruses. Elsevier North-Holland, Amsterdam, pp 673–678
Schmidt MFG (1982a) Acylation of proteins – a new type of modification of membrane glycoproteins. Trends Biochem Sci 7:322–324
Schmidt MFG (1982b) Acylation of viral spike glycoproteins – A feature of enveloped RNA-viruses. Virology 116:327–338
Schmidt MFG (1982c) Preparation of viral envelope components. In: Kornberg HL, Metcalf J, Hesketh TR, Northcote DL, Pogson C, Tipton KF (eds) Techniques in lipid and membrane biochemistry. Elsevier North-Holland, Amsterdam vol B419, pp 1–15
Schmidt MFG, Schlesinger MJ (1979) Fatty acid binding to vesicular stomatitis virus glycoprotein – a new type of posttranslational modification of the viral glycoprotein. Cell 17:813–819
Schmidt MFG, Schlesinger MJ (1980) Relation of fatty acid attachment to the translation and maturation of vesicular stomatitis and Sindbis virus membrane glycoproteins. J Biol Chem 255:3334–3339
Schmidt MFG, Bracha M, Schlesinger MJ (1979) Evidence for covalent attachment of fatty acids to Sindbis virus glycoproteins. Proc Natl Acad Sci USA 76:1678–1691
Scholtissek C, Bowles AL (1975) Isolation and characterization of temperature-sensitive mutants of fowl plague virus. Virology 67:567–587
Schwarz RT, Datema R (1982) The lipid pathway of protein glycosylation, its inhibitors, and the biological significance of protein-bound carbohydrates. Adv Carbohyd Chem Biochem 40: 287–379
Schwarz RT, Schmidt MFG (1982) Tunicamycin in virology. In: Tamura G (ed) Tunicamycin. Japan Scientific Societies, Tokyo, pp 99–116
Schwarz RT, Rohrschneider JM, Schmidt MFG (1976) Suppression of glycoprotein formation of Semliki Forest, influenza, and avian sarcoma virus by tunicamycin. J Virol 19:782–791
Schwarz RT, Schmidt MFG, Lehle L (1978) Glycosylation in vitro of Semliki Forest virus and influenza virus glycoproteins and its suppression by nucleotide-2-deoxyhexose. Eur J Biochem 85:163–172
Sebald W, Graf T, Lukins AB (1979) The dicyclohexylcarbodiimide-binding protein of the mitochondrial ATPase complex from *Neurospora crassa* and *Saccharomyces cerevisiae*. Eur J Biochem 93:587–599

Shapiro SZ, August JA (1976) Proteolytic cleavage events in oncornavirus protein synthesis. Biochim Biophys Acta 458:375–396
Simons K, Garoff H (1980) The budding mechanism of enveloped animal viruses. J Gen Virol 50:1–21
Simons K, Sarvas M, Garoff H, Helenius A (1978) Membrane-bound and secreted forms of penicillinase from *Bacillus licheniformis*. J Mol Biol 126:673–690
Singer SJ, Nicolson GL (1975) The fluid mosaic model of the structure of cell membranes. Science 175:720–731
Smith JF, Brown DT (1977) Envelopment of Sindbis virus: Synthesis and organization of proteins in cells infected with wild type and maturation defective mutants. J Virol 22:662–678
Smith WP, Tai PC, Davis BD (1981) *Bacillus licheniformis* penicillinase: Cleavage and attachment of lipid during cotranslational secretion. Proc Natl Acad Sci USA 78:3501–3505
Spector AA (1975) Fatty acid binding to plasma albumin. J Lip Res 16:165–179
Stoffyn P, Folch-Pi J (1971) On the type of linkage binding fatty acids present in brain white matter proteolipid apoprotein. Biochem Biophys Res Commun 44:157–161
Sturman LS, Holmes KV, Behnke J (1980) Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. J Virol 33:449–462
Suchanek G, Kreil G, Hermodson MA (1980) Amino acid sequence of honeybee prepromellitin synthesized in vitro. Proc Natl Acad Sci USA 75:701–704
Tabas I, Kornfeld S (1979) Purification and characterization of rat liver Golgi-mannosidase capable of processing asparagine-linked oligosaccharides. J Biol Chem 254:11655–11663
Tabas I, Schlesinger S, Kornfeld S (1978) The processing of high mannose oligosaccharides to form complex type oligosaccharides on the newly synthesized polypeptides of the vesicular stomatitis G protein and the IgG-heavy chain. J Biol Chem 253:716–722
Tartakoff AM (1980) The Golgi complex: crossroads for vesicular traffic. Int Rev Exp Pathol 22:227–252
Tzagoloff A, Meagher P (1972) Assembly of the mitochondrial membrane system. J Biol Chem 247:594–603
Vik SB, Capaldi RA (1977) Lipid requirements for cytochrome *c* oxidase activity. Biochemistry 16:5755–5759
Waechter CJ, Lennarz WJ (1976) The role of polyprenol-linked sugars in glycoprotein synthesis. Annu Rev Biochem 45:95–112
Ward CW (1981) Structure of influenza virus hemagglutinin. Curr Top Microbiol Immunol 94:1–71
Ward CW, Dopheide TAA (1979) Primary structure of the Hongkong (H3) hemagglutinin. Br Med Bull 35:51–56
Wickner W (1979) The assembly of proteins into biological membranes: The membrane trigger hypothesis. Annu Rev Biochem 48:23–45
Wilson IA, Skehel JJ, Wiley DC (1981) Structure of the hemagglutinin membrane glycoprotein of influenza virus at 3 Å resolution. Nature 289:366–373
Wirth DF, Katz F, Small B, Lodish HF (1977) How a single-stranded Sindbis virus mRNA directs the synthesis of one soluble protein and two integral membrane glycoproteins. Cell 10:253–263
Wold F (1981) In vivo chemical modification of proteins (posttranslational modification). Annu Rev Biochem 50:783–814
Yamamoto S, Lampen JO (1975) Membrane penicillinase of *Bacillus licheniformis* 749/C, a phospholipoprotein. J Biol Chem 250:3212–3213
Yamamoto S, Lampen JO (1976a) Membrane penicillinase of *Bacillus licheniformis* 749/C: Sequence and possible repeated tetrapeptide structure of the phospholipopeptide region. J Biol Chem 251:1457–1461
Yamamoto S, Lampen JO (1976b) The hydrophobic membrane penicillinase of *Bacillus licheniformis* 749/C. J Biol Chem 251:4102–4110
Zilberstein A, Snider MD, Porter M, Lodish HF (1980) Mutants of vesicular stomatitis virus blocked at different stages in maturation of the viral glycoprotein. Cell 21:417–427
Zill LP, Harmon EA (1962) Chloroplast proteolipid. Biochim Biophys Acta 53:579–581

Escherichia coli Virus T1: Genetic Controls During Virus Infection

ERWIN F. WAGNER*,**, BERNHARD AUER*, AND MANFRED SCHWEIGER*

1 Introduction

The study of bacterial viruses has contributed important information for the development of modern biology. The theoretical genetical considerations of *Timofeev-Ressovsky*, *Zimmer*, and *Delbrück* (*Timofeev-Ressovsky* et al. 1935) in the 1930s and the use of phages as experimental objects (for a review see *Cairns* et al. 1966) were the basis for many breakthroughs in modern biology. Advances in knowledge of recombination, replication, transcription, and translation and their controls depended essentially on bacterial virus systems. Although there is some apparent discrimination against "lower systems," the exciting recent progress in genetic engineering results from studies on bacterial viruses. Another illustration of the importance of research on bacterial viruses is the elucidation of the role of a protein kinase in oncogenic viruses, a development

* Institut für Biochemie der Universität, A-6020 Innsbruck
** Present address: The Institute for Cancer Research, Fox Chase, Philadelphia, Pa. 19111, USA

which was triggered by the discovery of the first viral protein kinase in T7-infected *E. coli* (*Rahmsdorf* et al. 1973).

Although the most popular bacterial viruses such as T7, T4 and λ, are the subjects of several reviews, T1 is very much neglected. However, this virus is of special interest. Besides reviewing the present knowledge about T1, we shall discuss in particular the special features of the T1 system.

2 Characteristics of the Virus Particle

2.1 Physical and Chemical Properties

Virus T1, the least-studied host/viral system among the (-odd bacterial viruses, is well-known and respected for its wide range of host specificity. The virus is therefore called the "killer phage" and particular care is essential in dealing with T1.

T1 and T5 belong to the same morphological group, namely group B of *Bradley* (1967) and group IV of *Tikhonenko* (1970). Both viruses have a polyhedral head and a long non-contractile tail. T1 is indistinguishable in size from λ, but smaller than T5. The head measures about 60 nm × 60 nm, whereas the tail is about 150 nm long and 7 nm wide without fibers at its end (*Tikhonenko* 1970). It is closely related, genetically and physiologically, to D20, which grows preferentially on *Shigella* (*Trautner* 1960).

Each T1 particle contains a single, linear, unnicked, double-stranded DNA molecule with a molecular weight of 31×10^6 daltons (97 000 base pairs), and has a sedimentation coefficient of 33.5 S (*Bresler* et al. 1967, 1972). T1 DNA is terminally redundant to the extent of 6.5% of the genome or 2800 nucleotide pairs, with a standard deviation of 1.2% or 530 base pairs (*MacHattie* et al. 1972; *Lee* et al. 1976). This terminal repetition of T1 is about ten times the length of that found in T3 or T7 DNA. The terminal repetitive sequence at the ends of T1 DNA gives rise to an increase in the recombination frequency for terminal markers (*Michalke* 1967). The DNA of T1 contains the four common bases with a guanine-cytosine (GC) content of 48% (*Creaser* and *Taussig* 1957; *Brody* et al. 1967). It has limited permutations in the nucleotide sequence (*Gill* and *MacHattie* 1976).

Virus T1 has been used to some extent to study DNA damage in ultraviolet (UV)-irradiated, thermally treated particles (*Bohne* et al. 1968, 1970; *Hotz* and *Mauser* 1969, 1970; *Hotz* et al. 1971, *Stephan* et al. 1970), and in γ-irradiated particles (*Coquerell* and *Hagen* 1972). The effects of UV irradiation and aerosol and surface inactivation on T1 survival (*Mosin* et al. 1972, 1974; *Mosin* 1977a, b; *Trouwborst* 1972a, b, c, 1974a, b) as well as damage caused by iodine-125 decay have been investigated (*Krisch* 1972; *Schmidt* and *Hotz* 1979, *Krisch* et al. 1978). T1 has also been used to study the transfer of UV-induced thymine dimers from parental to progeny DNA (*Sauerbier* and *Hirsch-Kauffmann* 1968).

Heat-stable mutants of T1, designated "*st*", can be isolated which have a buoyant density less than wild-type virus particles (*Ritchie* and *Malcolm* 1970). These "*st*" mutants probably have a small amount of DNA deleted, as do similar mutants of T3, T5 and T7, but differences between the molecular weights of T1 *st* and T1 st^+ have not been detected. Besides heat stability, the high dry resistance of T1 particles is an additional feature of this virus system.

2.2 Gene Map of T1

Twenty-five T1 genes have been identified by standard means, and a large number of amber mutants, comprising 25 complementation groups, have been collected (*Michalke* 1967). Considering that the molecular weight of T1 DNA is 31×10^6 daltons, which could

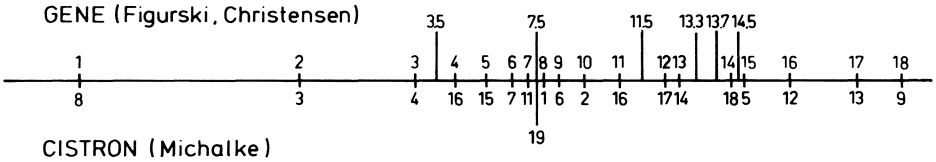

Fig. 1. Genetic map of T1. The original nomenclature of cistrons (*Michalke* 1967) is correlated with the new nomenclature (*Figurski* and *Christensen* 1974)

Table 1. Genetic map of T1

Gene[a]	Mutant	Gene products[b] (molecular weight)	Function[c]
1	*am16*	38 000	DO
2	*am5*	60 000	DO
3	*am6*	73 000	T
3.5	*am201*	-	DA, Grn
4	*am23*	38 000	DA, H, Grn
5	*am15*	150 000	T
6	*am18*	-	T
7	*am35*	-	T
7–8	*am208*	-	-
8	*am32*	-	T
9	*am13*	62 000	T
10	*am2*	120 000; 45 000	T
11	*am9*	20 000	T
11.5	*am304*	-	-
12	*am37*	-	H
13	*am10*	36 000	H
13.3	*am283*	-	-
13.7	*am216*	-	-
14	*am45*	36 000	H
14.5	*am246*	-	-
15	*am11*	-	H
16	*am4*	-	H
17	*am7*	-	H
18	*am30*	-	H
-	*am280*	-	-

[a] Current genetic map of T1 based on that published by *Michalke* (1967); *Figurski* and *Christensen* (1974); *Ritchie* and *Joicey* (1980)

[b] Molecular weights of T1 gene products obtained by amber mutant infections of nonpermissive hosts (*Wagner* et al. 1977)

[c] Functions of T1 genes are specified as described by *Figurski* and *Christensen* (1974). *DO*, deficient in DNA synthesis; *DA*, premature arrest of DNA synthesis; *H*, defective in head formation; *T*, defective in tail formation; *Grn*, general recombination system for T1, as described by *Ritchie* et al. (1980b)

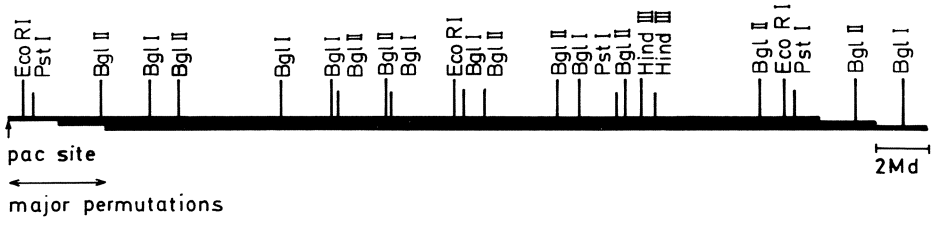

Fig. 2. Restriction cleavage map of T1 according to *Ramsay* and *Ritchie* (1980)

encode about 31 average-sized proteins (*Wagner* et al. 1977a), it is evident that several T1 genes have not been mapped. Thus, any genetic map of T1 is at the moment incomplete. Figure 1 shows an adapted map generated originally by *Michalke* (1967), renumbered and extended by *Figurski* and *Christensen* (1974), *McCorquodale* (1975), and *Ritchie* and *Joicey* (1980). The map is linear, but recombination values are much higher at both ends of the map than they are in the central position (*Michalke* 1967). Since T1 DNA contains a terminally redundant region sufficient to code for two or three average-sized proteins (6.5% of the genome), it may be that *Michalke's* terminal markers (T1 *am16* and T1 *Sc* or *AK*) are within this region. Such a location would explain not only the high recombination frequencies at the ends of the T1 gene map, but also the high frequency of heterozygous progeny produced in crosses involving these terminal genes. Table 1 lists the functions and some of the assigned proteins of certain T1 genes. It is apparent that certain gene functions are clustered within the T1 genome: genes involved in T1 DNA synthesis are located between genes 1 and 4, whereas genes involved in tail or head formation span positions 5 to 11 and 12 to 18, respectively. No functional correlation of specific T1 gene products has yet been described.

Recently, *Ramsay* and *Ritchie* (1980) described a restriction map for T1 DNA. Five widely used restriction enzymes were applied to locate the cleavage sites on the viral DNA (Fig. 2). It was found that *Eco*RI, *Hind*III, and *Pst*I cut the DNA twice, whereas *Bgl*I and *Bgl*II had multiple cleavage sites on T1 DNA (6 and 8). The use of recombinant DNA techniques will certainly help to elucidate T1 gene structure and function.

2.3 T1 Structural Proteins

T1 viral particles contain 15 principal polypeptides which can be separated by SDS-polyacrylamide gel electrophoresis (*Martin* et al. 1976; *Toni* et al. 1976; *Wagner* et al. 1977a). These polypeptides range in molecular weight from about 180 000 for P1 to about 10 000 for P15 (Fig. 3). Four proteins, P8, P10, P11, and P13, make up about 80% of the total mass and must therefore be regarded as the major structural proteins of the virion. *Toni* et al. (1976) and *Martin* et al. (1976) have shown that two of the major structural proteins (P8 and P10) are synthesized in the form of a larger precursor with a molecular weight of about 40 000, which is cleaved to give the final products of about 35 000 daltons for P8 and 33 000 daltons for P10. About 60% of the coding capacity of the viral DNA is taken up by the structural proteins of the virus (*Wagner* et al. 1977a).

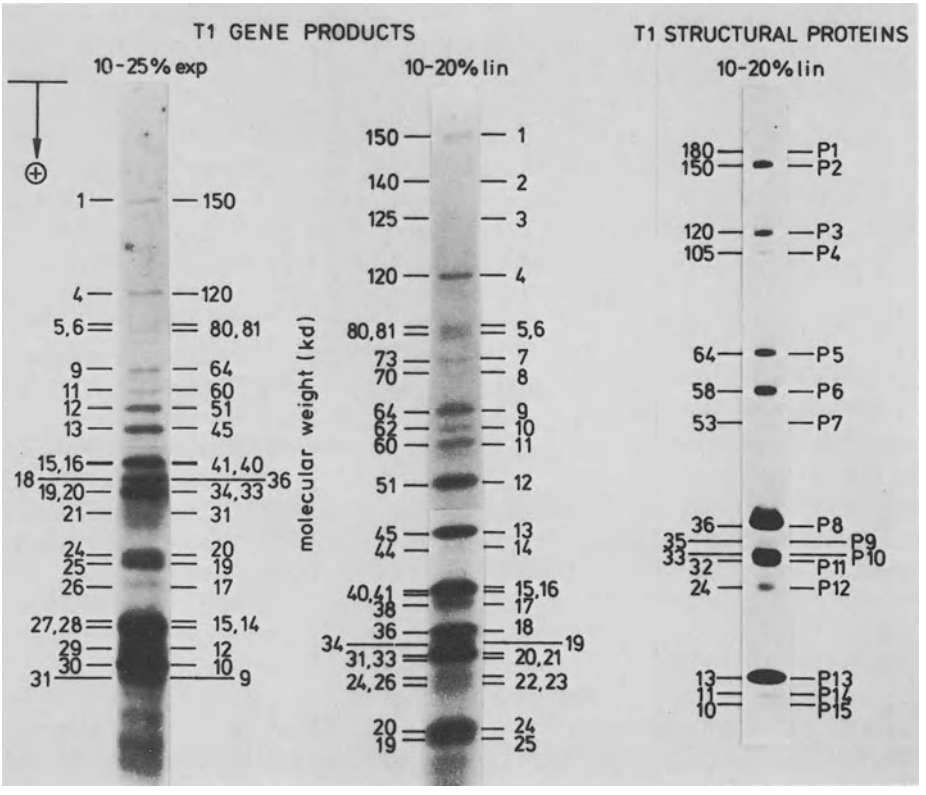

Fig. 3. SDS-polyacrylamide gel electrophoresis of T1 proteins

3 The Process of Infection

3.1 Exclusion

Simultaneous infection by different bacterial viruses results, in most cases, in the growth of one virus species only. Thus, coinfection of *E. coli* by T2 and T1 results in propagation of T2 only; T1 does not develop (*Delbrueck* and *Bailey* 1946; *French* et al. 1952; for review on exclusion see *Schweiger* and *Hirsch-Kauffmann* 1982). The mechanism of exclusion in this case is unknown. However, for other viruses such as T7 and ε15 the mechanism of exclusion has been elucidated. For T7 it was shown that only one phage genome can enter the cell, since only one genome is available for transcription, replication, and UV-reactivation (*Hirsch-Kauffmann* et al. 1976). The cell wall apparently is altered in a way that precludes injection of genomes of later absorbing viruses. Furthermore, for virus ε15 it has been shown that exclusion is coupled to alterations of carbohydrate structures on the host surface (for review see *Lindberg* 1973).

Of special interest regarding T1 is the λ-dependent exclusion of T4 *r* mutants. The product of the "*rex*" gene of λ alters the cell membrane in such a way that T4 mutants, defective in the *rI*, *rII*, or *rIII* genes, are unable to develop in hosts which are lysogenic for

λ. This λ *rex* locus is also responsible for abortive infection by other viruses such as T5 *lr-* (*Howard* 1967; *Jacquemin-Sablon* and *Lanni* 1973), T7 *rbl-* (*Pao* and *Speyer* 1975), lambdoid phage (*Toothman* and *Herskowitz* 1980), and T1 (*Christensen* and *Geiman* 1973; *Geimann* et al. 1974). Infection of *E. coli* lysogenic for λ with T1 results in small burst sizes with a significantly reduced latent period, whereas in *rex*⁻ mutants T1 development is normal. In recent more detailed studies on the exclusion of T1 by λ, *Christensen* and co-workers (*Christensen* et al. 1978; *Gawron* et al. 1980) have defined two specific exclusion mechanisms. The "early" exclusion is due to the *N* gene product, which is adjacent to the *rex* locus; it is independent of λ *Q* and it is at least partically ineffective against a gene 4 mutant of T1 (T1 *am23*). The "late" exclusion is reported to be dependent on late λ gene expression (*Q* dependent). Host mutations (*grow, nusA*, and *nusB*), known to interfere with replication by affecting *N* gene expression, also interfere with the ability of λ to exclude T1 (*Christensen* et al. 1978). The mechanism of suppression of the T1 "DNA arrest" mutant (T1 *am23*) is not yet clear. When T1 macromolecular synthesis under *N*-mediated excluding conditions was investigated, no substantial alteration in T1 mRNA production was found in the excluding cultures (*Gawron* et al. 1980). However, T1-specific protein synthesis was severely reduced. This suggests that a translational control mechanism is responsible for the impaired T1 development under those excluding conditions.

3.2 Transduction by T1

Virus T1 is a generalized transducing phage (*Drexler* 1970), although the efficiency of transduction (EOT) of various bacterial markers differs considerably (*Drexler* and *Kylberg* 1975; *Kylberg* et al. 1975). Besides genetic loci of the host, T1 also transduces complete *Mu* genomes from *E.coli* lysogenic for *Mu*. The efficiency of *Mu* transduction by T1 depends on the location of *Mu* on the *E. coli* chromosome (*Bending* and *Drexler* 1977). However, these efficiencies are different from those of the bacterial genes in which *Mu* is integrated (*Drexler* and *Kylberg* 1975). The reasons for this are unknown.

T1 can also transduce λ genomes (*Drexler* 1972a, b, 1973; *Drexler* and *Christensen* 1979). The phenotypically mixed particles which result from T1 growth on *E. coli* lysogenic for λ have the host range and serum sensitivity of T1, but produce λ progeny. This phenomenon is facilitated by the similar sizes of the DNAs of T1 and λ. Thus, T1 cuts and packages λ DNA in pieces as big as T1 DNA. The efficiency of T1 transduction depends on several viral genes (*Borchert* and *Drexler* 1980). Amber mutants in 19 genes were grown on three different amber-suppressor hosts and the EOT of host markers determined. Phage mutants with defects in DNA metabolism (DO, DA; see Table 1) exerted significant increases in the EOT compared with mutants in the early genes which control phage DNA synthesis. The efficiency of transduction seems to depend on the availability of viral DNA versus host DNA and on the packaging of DNA. If less T1 DNA is available, or alternatively more host DNA, the EOT is increased (*Borchert* and *Drexler* 1980).

Roberts and *Drexler* (1981a) isolated T1 mutants with elevated rates of transduction, the *tar* mutants (T1 transducing with altered rates). These T1 *tar* mutants transduce defective λ with 50–27 000-fold efficiency. The mutations are located between, but not in, genes 2 and 3 (*am5* and *am6*). This region of the T1 genome is responsible for DNA synthesis and DNA maturation. Gene 2 mutants (*am5*) are defective in DNA synthesis

(*Figurski* and *Christensen* 1974) and mutants in gene 3.5 (*am20l*) and 4 (*am23*) are required for the cutting of newly synthesized T1 DNA into short concatemers (*Ritchie* and *Joicey* 1980). The T1 *tar* mutants seem to be impaired in breakdown of host DNA (*Roberts* and *Drexler* 1981b). It would be reasonable to assume that the presence of high-molecular-weight host DNA permits the packaging of this DNA into T1 coats.

3.3 T1 and Host Restriction

Bacterial viruses have developed various methods to overcome the restriction barriers of the hosts. Viral DNA can be masked by the modification system of the host (e.g., λ; *Arber* 1974) or the DNA can be modified by uncommon bases such as hydroxymethylcytosine or by glucosylated hydroxymethylcytosine nucleotides (e.g., T-even phages; *Lehman* and *Pratt* 1960). Alternatively, the virus can actively inhibit the restriction endonucleases of the host (e.g., T3, T7; *Schweiger* et al. 1978; *Spoerel* et al. 1979).

Virus T1 has developed its own way to overcome the host restriction system by "supermodification" (*Wagner* et al. 1979). T1 plates with full efficiency on most *E. coli* strains whether it was grown on modification-negative or positive hosts (*Klein* and *Sauerbier* 1965; *Wagner* et al. 1979). T1 DNA always contains 1.7% N^6-methyladenine and 0.2% 5-methylcytosine, even though the hosts in which the T1 viruses are grown contain 0.7%–2.4% N^6-methyladenine and 0.1%–1% 5-methylcytosine. Thus, methylation of T1 DNA is independent of the host strain (*Wagner* et al. 1979). From this we concluded that T1 induces inhibition of host modification as well as supermodification, which protects the viral DNA from host restriction endonucleases. Restriction enzyme analysis of T1 DNA with the isoschisomeric restriction endonucleases *Sau*3A, *Dpn*I, *Mbo*I (*Roberts* 1981) which distinguish between methylated and unmethylated GATC, showed that all GATC sequences in T1 are methylated; the same is true for T1 DNA isolated from phages grown on a mutant host lacking the ability to methylate the GATC sequence (*Auer* and *Schweiger*, unpublished). This further supports the existence of a T1-coded methyltransferase. An increase of DNA-methyltransferase activity after T1 infection has also been reported (*Hausmann* and *Gold* 1966; *Hirsch-Kauffmann* and *Sauerbier* 1968).

Although T1 is not restricted by most *E. coli* strains, it is restricted by R plasmids of the H incompatibility group (*Taylor* and *Grant* 1976) and by lysogenic P1. T1 grown on nonlysogenic *E. coli* is efficiently restricted by *E. coli* P1 (*Potts* and *Christensen* 1974; *Wagner* et al. 1979). In contrast to more commonly studied restriction systems in the T1/P1 system, a substantial fraction of the infecting T1 DNA is not degraded (*Christensen* 1974). At high multiplicities of infection, unmodified T1 (T1.0) productively infects a large portion of restrictive host cells (P1 lysogens), provided that protein synthesis was uninhibited during the first 5 min of infection (*Kotval* and *Christensen* 1981). T1.0 also complements amber mutants, and genetic markers can be rescued by coinfection or superinfection, even when this superinfection is delayed for up to 1 h. The role of a T1-specific protein, synthesized soon after infection and apparently somehow involved in recombination, is not yet clear. It could antagonize a nuclease, or protect the T1 DNA by binding to the DNA, or it could mediate the binding of T1 DNA to membranes.

3.4 Mechanism of Infection

*T*one *n*oninfecting (*ton*) mutants of *E. coli* have been widely used to study phage development (for review see *Braun* et al. 1976; *Neilands* 1979). It has been found that these *ton* mutants grow only in the presence of high concentrations of iron. Recently, the involvement of *ton* gene functions on iron transport has been elucidated. Genetically, two loci, *ton A* and *ton B*, were found. The *ton A* gene product is located in the outer cell membrane and is part of the ferrichrome uptake system (Fig. 4). It is also necessary for the action of albomycin and colicin M (*Graham* and *Stocker* 1977). *Ton B* functions at the internal membrane and is part of the uptake system for ferric enterobactin, ferrichrome, ferric citrate, and other siderophore iron compounds (*Wookey* and *Rosenberg* 1978; *Neilands* 1979). It, too, is involved in the action of albomycin, colicin M, and also colicin B. (Virus T5 infection requires only a functional *ton B*.)

Functional *ton A* and *ton B* gene products are essential not only for T1 infection but also for Φ 80 development. Originally it was believed that *ton A* and *ton B* form the receptor for T1 (*Braun* et al. 1976). This cannot be the case because *ton B* is on the inner membrane. What then is the role of *ton B* in T1 infection? The very early events of infection, which lead to adsorption of the T1 virus to the cell, take place in two distinguishable steps. The first is the reversible attachment of the virus to the receptor complex. For the second step, the irreversible adsorption, *ton B* and cellular energy are required (*Garen* and *Puck* 1951; *Christensen* and *Tolmach* 1955). This energy is supplied as proton motive force (*Hancock* and *Braun* 1976). It has been shown for certain viruses that this interaction occurs at

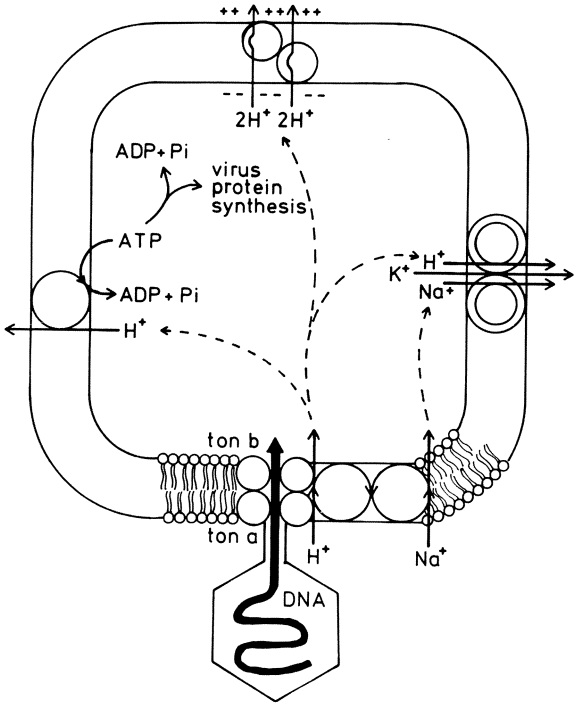

Fig. 4. The early events during T1 infection. DNA invasion dissipates the energy gradients of the membrane. ATP is utilized to extrude protons and the reduced nucleosidetriphosphate content inhibits host RNA translation (*Wagner* et al. 1981, *Wagner* and *Schweiger* 1981)

specific adhesion sites between the cell wall and the membrane (*Bayer* 1968). Accompanying the irreversible adsorption are physical membrane alterations which can be measured by the quenching of fluorescence of pyrene (*Ponta* et al. 1977), or with the help of other fluorescent dyes, e.g. 1-anilino-naphthalene-8-sulfonate (*Schweiger* et al. 1978). These fast responses of bacterial membranes to virus adsorption were carefully studied in various virus infections (*Bayer* and *Bayer* 1981) with a special focus on T4 and T5 (*Labedan* and *Goldberg* 1979; *Labedan* and *Letellier* 1981). The requirement of the proton gradient of the membrane in combination with *ton A* and *ton B*, the constituents of the iron uptake system, suggests the involvement of the proton-driven iron transport system in the uptake of the viral DNA. This view is supported by the fact that the proton gradient is used up early in the course of T1 infection (*Wagner* et al. 1980). The rapid dissipation of the membrane energy is of special interest. Combined with the rapid decrease in the proton gradient is a profound alteration in the physiology of the cell. All active transport systems, except the Phosphoenolpyruvate-dependent phosphotransferase sugar uptakes, are dramatically reduced immediately upon T1 infection, whether the energy for transport comes from ATP, or from the proton motive force (*Wagner* et al. 1980). Detailed analysis with mutants in the proton-driven ATPase (*unc*$^-$ mutants *Schairer* and *Haddock* (1972) revealed that the drop of the proton gradient is primarily responsible for the inhibition of transport. These mutants, which are unable to interconvert the proton gradient into ATP and vice versa, do not reduce the ATP-driven transports following T1 infection. The explanation for this observation is that the *unc*$^-$ mutants cannot use their ATP in an attempt to reestablish the proton gradient. Consequently, the ATP concentration in these cells is not reduced and ATP-driven transports are not affected (*Wagner* et al. 1980). An additional consequence of the prevention of the ATP reduction in the *unc*$^-$ mutants is that host gene expression is not shut off (see Sect. 4.6 on T1-induced host repression), since a lowered nucleoside-triphosphate pool is responsible for host shut off in wild-type *E. coli*.

In the *unc*$^-$ mutants the proton-driven transport systems are strongly inhibited, suggesting that T1 infection affects the proton gradient (*Wagner* et al. 1980). Indeed, the membrane potential drops immediately after the start of infection. The depolarization of the cell membrane indicates strong effects of T1 infection on ion gradients. Potassium is massively released from the infected cells. The potassium gradient is of special interest since it is a form of energy storage and the major intracellular cation (*Kepes* et al. 1977). The massive efflux of the positively charged potassium ions and the depolarization (decrease of internal negative charge) of the cell membrane apparently are contradictions. Negative ions must be released from the cell or cations must enter the cell, or both. Indeed, anions such as sulfate and phosphate are released after T1 infection, and calcium and sodium ions flow into the cells (*Wagner* et al. 1980). The energetic aspects of the early events of T1 infection are of central importance to the development of T1.

Virus T1 grows rapidly, producing as many as 300 progeny per infected cell within 15 min at 37 °C. Since the molecular weight of T1 DNA is 31×10^6, about 10^{10} daltons of DNA must be synthesized during this time. That amount of DNA is approximately four to six times the amount of host DNA present at the beginning of infection. In addition, a huge amount of viral-specific protein and RNA must be synthesized. Energy is obviously required for all of these syntheses. However, oxidative phosphorylation, the major source of energy of the cell, is not stimulated after T1 infection (*Schweiger* et al. 1978), nor is glucose uptake stimulated (*Wagner* et al. 1980). This suggests that the energy supply is not

expanded at the expense of anaerobic glycolysis of external glucose. Thus, the energy can be supplied only from internal cellular resources: either anaerobic glycolysis of stored substrates such as carbohydrates or fats, or from stored energy in the form of concentration gradients. Since anaerobic glycolysis of stored substrates is very inefficient, it is probably not a significant source of energy. However, most of the concentration gradients, such as the proton gradient and cation gradients (such as sodium, potassium, and calcium), are dissipated within the very early phase of T1 infection.

Three questions remain: (a) What is the energy content of the gradients used for, (b) why is it used up within the first few minutes of infection, and (c) how does T1 get enough energy for the later phases of virus development? Within the first minute of T1 infection, DNA invasion is the major event. Does the energy of the gradients supply the energy for the uptake of the viral DNA? The involvement of the iron uptake system in T1 infection suggests that T1 DNA is actively pulled into the cell by this transport system, driven by the proton gradient, as is the iron uptake. The *ton B* protein, which cannot be part of the receptor since it is located in the inner cell membrane, could function to channel the viral DNA, and the dissipation of the proton gradient could supply the energy for invasion of viral DNA. An alternative hypothesis would be that the energized state of the membrane could supply the physical state for the formation of channels through which the viral DNA could enter (*Grinius* 1980). However, the proton gradient is required not only while DNA invasion is initiated but during the whole process of DNA uptake. Uncouplers interrupt DNA invasion at each stage of infection (*Witkiewicz* and *Schweiger*, unpublished). Additionally, we might expect the formation of unspecific channels for DNA penetration to result in the breakdown of the permeability barrier for small molecules. This does not occur: the PEP-dependent phosphotransferase systems for the uptake of sugars are not inhibited, since α-methylglucoside continues to be accumulated (*Wagner* et al. 1980). In contrast, these transports are greatly stimulated (a finding which led to the understanding of phosphotransferase systems control by the proton gradient of the membrane (*Reider* et al. 1979)). o-Nitrophenylgalactoside (oNPG), another small molecule, does not enter cells after T1 infection. Thus, if such channels are formed, they must be of a size which does not permit free passage of oNPG. The obvious dilemma is that the channels must be large enough for the passage of DNA but too small for sugars or oNPG. Although the mode of DNA invasion during infection is not known, the arguments presented above appear to favor the taking over of active transport systems for DNA penetration through the membranes.

4 Gene Expression After T1 Infection

4.1 Pattern of T1 Gene Expression

After infection with T1 a characteristic pattern of gene expression is induced in which host gene expression is turned off and various T1-specific proteins are synthesized on specific time schedules (*Toni* et al. 1976; *Martin* et al. 1976; *Wagner* et al. 1977a, 1977b). Three main classes of T1 proteins can be distinguished: (a) an "early" class of proteins (class I) that appears immediately after infection and whose production is shut off at around 11 min, coincident with the onset of virus DNA replication; (b) an "early-late" class (class II) of proteins synthesized from 3–4 min after infection and at a declining rate

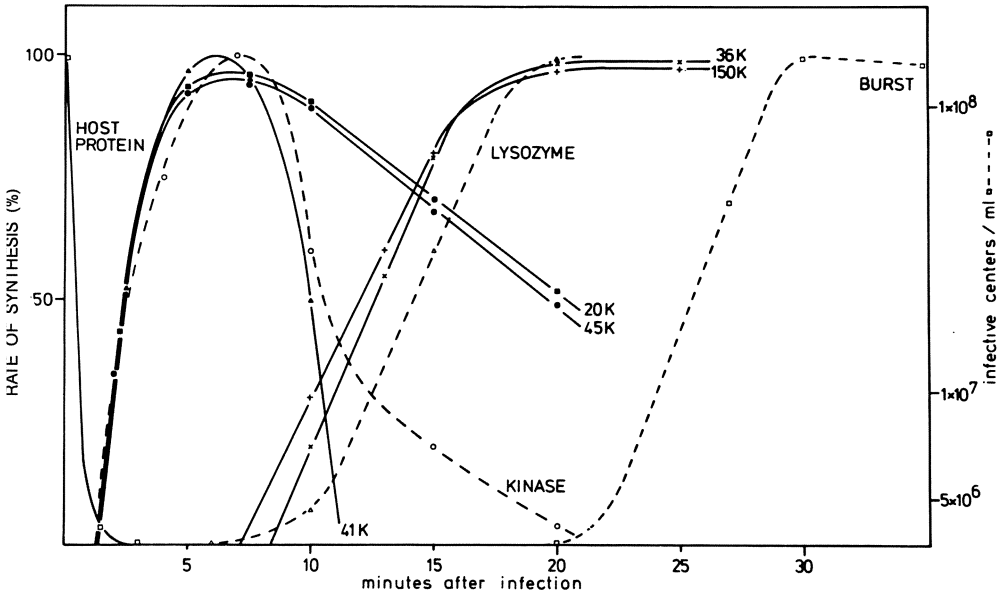

Fig. 5. Protein and enzyme synthesis after T1 infection. Key: —, host; ▲—▲ early; ●—●, ■—■, early-late; x—x, +—+ late

until cell lysis; and (c) a "late" class (class III) whose synthesis starts at 8 min postinfection and continues at an increasing rate until lysis (Fig. 5). *Martin* et al. (1976) have also defined class IV (early-continuous) and class V (late-continuous) T1 proteins, five representatives of each class having been detected. UV irradiation of the host cells before infection does not alter the time course of T1 protein synthesis. The precision of the schedule for T1-induced protein synthesis indicates the existence of extensive gene regulation. The schedule of the early T1 class seems to be dictated, as in T7 (*Schweiger* et al. 1978), by the oriented invasion of T1 DNA during infection (*Gill* and *MacHattie* 1975).

4.2 mRNA Synthesis After Infection

Male and *Christensen* (1970) have studied by solution hybridization the synthesis of host-specific and virus-specific mRNA in bacteria infected with unmodified T1B and modified T1B (P1) virus T1. Synthesis of virus-specific RNA increases after infection and synthesis of host-specific RNA declines. The amount and rate of T1 mRNA synthesis is comparable to that data reported for T4 or λ infection. However, little or no virus-specific mRNA synthesis could be detected in T1B infection of *E. coli* B lysogenic for phage P1 (*E. coli* B (P1)) (*Lederberg* 1957). The synthesis of host-specific mRNA continued, although at a declining rate, for a significant period (10 min) after T1 infection. Nevertheless, cells productively infected with T1 cannot be induced to synthesize the host enzyme β-galactosidase (*Male* and *Christenson* 1970; *Wagner* et al. 1977b; *Jiresova* and *Janecek* 1977), the block to synthesis being at the level of translation. β-Galactosidase activity was detected in *E. coli* B(P1) under restricting conditions for T1 DNA uptake. How-

ever, it has not been shown conclusively that the amount of enzyme synthesized in this case is not due to uninfected host cells. Further studies have led to the conclusion that the synthesis of host mRNA from inducible genes does not occur unless the T1 DNA is inactivated. Methylation of T1 DNA can also influence the rate of T1-specific mRNA synthesis (*Male* and *Christensen* 1970).

4.3 T1-Specific Protein Synthesis

The size of T1 DNA (31×10^6 daltons) provides information for about 31 proteins of average molecular weight of 50 000. Indeed, 31 proteins were detected in T1-infected, UV-irradiated host cells, ranging from 9000 to 150 000 daltons (*Wagner* et al. 1977a; see also *Toni* et al. 1976 and *Martin* et al. 1976). The T1-specific proteins were classified according to the time of their synthesis. There are three early T1 proteins which can be clearly distinguished in UV-irradiated host cells, where no host background activities are detectable. Four to eight early-late and four to six late T1 proteins have been identified. The late proteins include both nonstructural, e.g., protein 7 with a molecular weight of 73 000 and structural, like P2 and P8 T1 polypeptides (see Fig. 3). With the aid of T1 amber mutants, we have assigned distinct gene products to 11 genes out of 18 known T1 genetic loci. In addition, amber peptides were detected, corresponding to genes 5, 10, 13, and 14

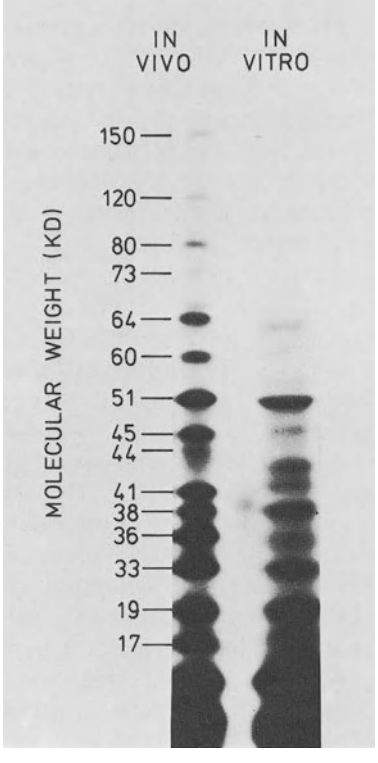

Fig. 6. SDS-polyacrylamide gel electrophporesis of T1 in vitro protein synthesis

(*Wagner* et al. 1977a). The sum of the calculated molecular weight of the 31 T1 proteins identified on SDS gels is 1.59×10^6 daltons, which covers 93% of the total possible information of T1 DNA.

All early and early-late T1 proteins were made in an in vitro T1 DNA-dependent system (*Schweiger* and *Herrlich* 1974) and a good correlation between in vivo and in vitro synthesized T1 proteins was observed (Fig. 6). However, no late T1 proteins were detected in the in vitro system (*Wagner* 1978), what might eventually allow the analysis of the control of late T1 gene expression.

4.4 Positive Control of Late T1 Protein Synthesis

The induction of late T1 protein synthesis is caused by a control mechanism which deserves special attention and which offers many advantages over other positive controls studied in various T virus systems (T3, T4, or T7). At 8 min after infection, this class of T1 proteins (at least six polypeptides synthesized in large quantities) is switched on and is continuously synthesized at increasing rates until lysis (*Schweiger* et al. 1978). The expression of late T1 genes does not require viral DNA replication. Nalidixic acid, an inhibitor of replication, and T1 amber mutants defective in DNA replication (*Figurski* and *Christensen* 1974) did not affect late protein synthesis (*Wagner* et al. 1977a). No viral-specific RNA polymerase has been identified and the host polymerase is required until T1 development is completed. The enzyme remains rifampicin-sensitive throughout the infectious cycle. T1 infection of a rifampicin-resistant host never revealed RNA polymerase activity which was sensitive to this drug. Thus, T1 uses only the host RNA polymerase for its transcription, but the question remains as to whether altered cell conditions or a modified form of the enzyme or both are responsible for the specificity of transcription. It is also interesting that during infection overall RNA polymerase activity declines before virus development is complete.

In order to investigate the arrangement of late T1 genes and the involvement of possible regulatory elements in controlling late T1 protein synthesis, studies were undertaken using the gene and transcription unit mapping technique (*Herrlich* et al. 1974; for a review see *Sauerbier* and *Hercules* 1978). The method is based on the target-size-dependent UV inactivation of transcriptional units (*Ponta* et al. 1979). A unit of 215 000 daltons, covering three to four late T1 genes, was calculated using the UV sensitivities, measured as rates of synthesis of individual late proteins after UV irradiation (*Wagner* 1978; Fig. 7). In addition, a 60 000-dalton protein, which could be a good candidate in the control of late T1 gene expression, was identified since most of the late T1 proteins are commonly inactivated to a larger extent than are other T1 proteins. These experiments also showed that most of the early and early-late T1 genes are arranged in monocistronic transcription units (with single promoters).

Little is known about the functions of the late T1 genes. It is evident that some of them encode T1 structural proteins (*Martin* et al. 1976). Recently, we have found lysozyme activity following T1 infections which increased in parallel with late T1 protein synthesis (see Fig.5; *Wagner* 1978). Experiments are in progress to correlate the lysozyme activity with a distinct gene product and to elucidate further the mechanism of how this particular late T1 gene product is regulated.

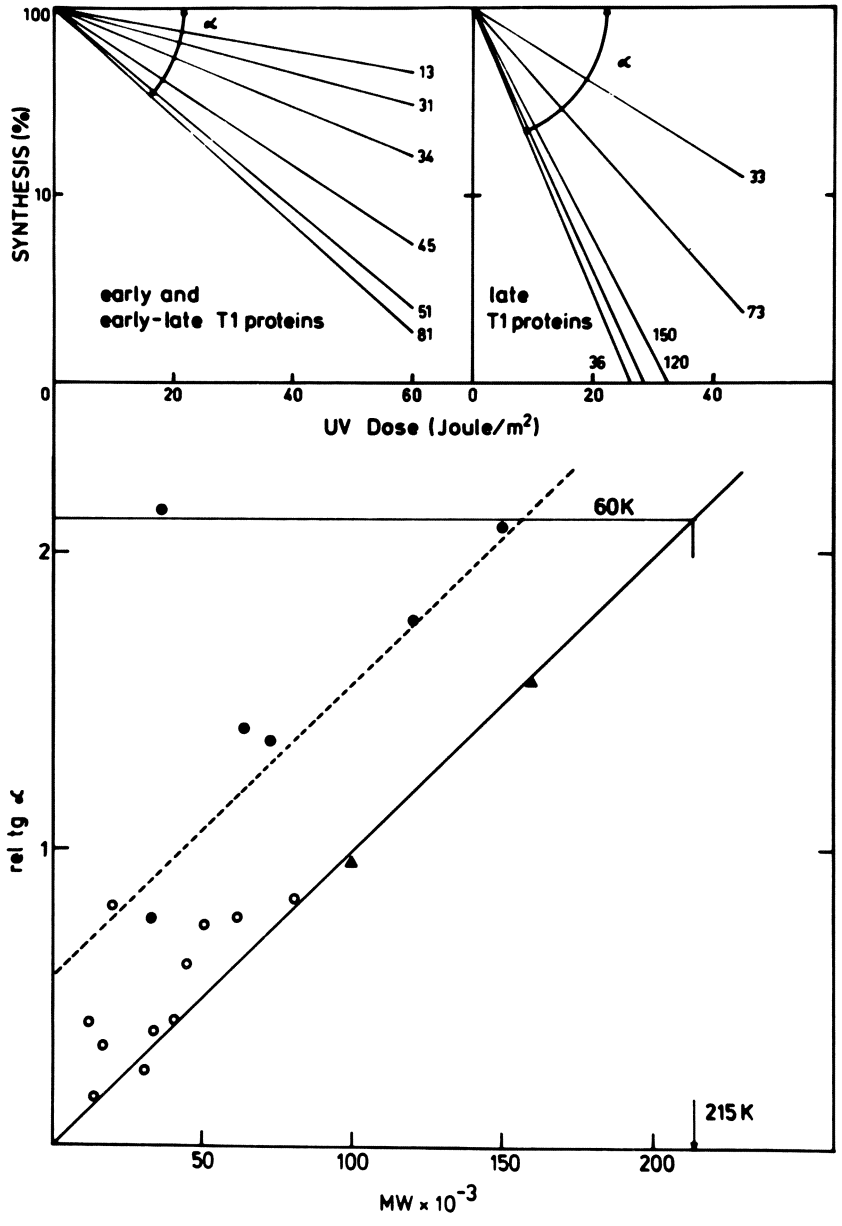

Fig. 7. UV inactivation of T1 protein-synthesis. The target sizes of transcriptional units have been determined as described by *Ponta* et al. (1979). The relative inactivations rates of individual proteins (*early* and *early-late* ○—○, *late* ●—●, *standards* ▲—▲) are plotted as tangents of the inactivation slope against the molecular weights. The curve for the late proteins shows a parallel displacement of a target size corresponding to a protein with a molecular weight of 60000. This protein is presumably a control protein for the turn on of the late T1 proteins

4.5 T1 Genes and Their Functions

An attempt to identify the function of each T1 gene has been made by *Figurski* and *Christensen* (1974). However, since the gene map of T1 is still incomplete, a correlation of the T1 gene products as identified on gels with genes and functions has not yet been completely established. T1 amber mutants representing the 18 known genes (*Michalke* 1967) were examined for their functions in DNA synthesis, head or tail production, and cell lysis. The early genes *1* and *2*, encoding an 38K protein and a 60K protein respectively, are absolutely necessary for synthesis of T1 DNA, although it is not known what enzymatic functions these proteins have (*Wagner* et al. 1977a; Table 1). Mutants in genes *1* and *2* can still shut off host DNA synthesis; however, this shut off is prevented if chloramphenicol is present at the time of infection. Therefore, a T1-induced protein must be responsible for the inhibition, which probably enables the virus to utilize host DNA as a source of nucleotides for its own DNA synthesis. This is also implied by the short latent period of T1 development (*Delbrueck* 1945; *Wagner* et al. 1977a). Furthermore, mutants in genes *1* and *2* affect the lysis kinetics of the infected cells (*Figurski* and *Christensen* 1974).

Gene *3.5 (am201)* and gene *4 (am23)* also affect T1 DNA synthesis (*Ritchie* and *Joicey* 1980), exhibiting a DNA synthesis arrest phenotype when grown under nonpermissive

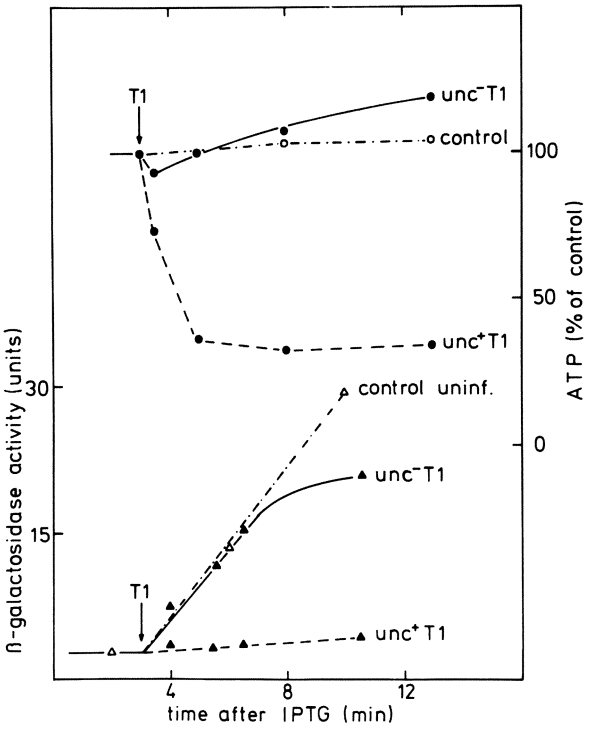

Fig. 8. ATP-mediated T1 host repression. Host repression is depicted by induction of a representative host enyzme, β-galactosidase, which is correlated with the content of ATP in ATPase negative (*unc*⁻) and wild-type (*unc*⁺) cells (*Schweiger* and *Wagner* 1979)

conditions. In addition to their effects on DNA synthesis, mutations in these two genes profoundly depress recombination in T1, suggesting that these two genes encode components of a general recombination system, referred to as T1 *Grn* (*Ritchie* et al. 1980b). The product of gene *4* is also necessary for head assembly. Obviously, this protein could be involved in the coordinated packaging of T1 DNA into T1 heads. By using an in vitro complementation assay and electron microscopy, *Figurski* and *Christensen* (1974) have found that gene *3* and genes *5–11* are involved in tail biosynthesis, whereas gene *4* and genes *12–18* are necessary to produce heads (see also Table 1).

As mentioned above, lysozyme activity was found following T1 infection, although the gene that codes for T1-specific lysozyme has not yet been identified. In addition, a protein kinase activity, which is an early T1 gene function, has been described (*Wagner* 1978). However, the gene responsible for kinase activity and its function in T1 development are not known. The search for other specific enzymes encoded by T1, such as ligase or SAMase, has been unsuccessful so far.

4.6 T1-Induced Host Repression

Following T1 infection virtually all host genes are shut off within 1–3 min. (A detailed description of this gene control mechanism in T1-infected cells was recently reviewed by *Schweiger* and *Wagner* (1979).) The host repression is especially interesting for economical reasons in virus development since it enables T1 to produce exclusively its own gene products without competition from host-directed synthesis. Very early after T1 infection, host protein synthesis, whether measured as amino acid incorporation into proteins or as enzyme synthesis (*Toni* et al. 1976; *Wagner* et al. 1977a, 1977b), is repressed. The analysis of this rapid inhibition revealed that translation of host mRNA is specifically blocked (*Wagner* et al. 1977b). Kinetic experiments showed that T1 affected the initiation step of host translation, whereas translation of T1 mRNA still took place. A discriminating control protein, such as the "translational repressor" reported for T7 (*Schweiger* et al. 1978), could not be found. Host repression could be exhibited in the absence of viral protein synthesis. The shut off did not operate in in vitro systems prepared from T1 infected cells even when crude extracts (gently lysed cells without further purification) were used (*Wagner* et al. 1977b). Therefore, an involvement of intact membrane structures seems likely. Furthermore, host macromolecular synthesis continued in *unc*$^-$ cells (Fig. 8, *Wagner* and *Schweiger* 1980). As outlined in Sect. 2, an immediate effect of T1 infection on the host cell is the reduction of the membrane energy, followed by the inhibition of membrane-associated activities such as the active transport system. As a consequence, intracellular ATP levels decrease drastically in T1-infected *unc*$^+$ cells, whereas ATP levels remain almost unaffected in *unc*$^-$ cells (Fig. 8). Thus, it appears that host mRNA translation probably cannot take place in the presence of low ATP concentrations in *unc*$^+$ cells. However, T1-specific mRNA can still be translated. In *unc*$^-$ cells, where ATP levels remain constant, both types of mRNAs are translated and host repression cannot rapidly be established. It was concluded from these data that ATP, or the concentration of a related high-energy phosphate, is responsible for controlling the specificity of translation and that the functional ATPase together with the T1-induced membrane alterations cause repression of host gene expression.

4.7 DNA Replication

Little work has been published on T1 DNA replication. Like other *E. coli* viruses, T1 forms concatemeric intermediates during replication (*Ritchie* and *Joicey* 1978). These intermediates are of the approximate length of three genome equivalents (*Gill* and *MacHattie* 1976). By "headfull" maturation, these concatemeres are processed for packaging (*MacHattie* 1977). T1 DNA replication requires viral and host functions. Host factors are necessary for elongation but not for the initiation step (*Bourque* and *Christensen* 1980). Only temperature-sensitive cellular mutations of genes involved in DNA elongation affect T1 propagation. Two T1 genes (genes *1, 2*) are necessary for T1 DNA synthesis, and mutations in two other genes (genes *3.5, 4*) cause DNA arrest (*Figurski* and *Christensen* 1974). It is noteworthy that T1 gene *4* complements with the λ-Red system (*Christensen* 1976). In an extensive study, *Ritchie* and *Joicey* (1980) analyzed DNA replication in mutants of 25 known viral genes. Besides the mutants in genes *1* and *2* which were blocked immediately after the temperature shift, two other genes (genes *3.5, 4*), described above as DNA arrest mutants, failed to make concatemeric DNA in nonpermissive hosts. Another group of mutants (genes *3–11*), in particular those involved in head formation, accumulated concatemeric intermediates. The authors suspected that the T1 mutants which did not form the concatemeric intermediate were similar to T7 gene *6* mutants. The T7 *gp 6* was identified as an exonuclease (*Kerr* and *Sadowski* 1972). The group of T1 mutants which accumulated concatemeres could be blocked in head assembly or in headfull sizing of the DNA (*Ritchie* and *Joicey* 1980).

Interestingly, T1 DNA replication and degradation of host DNA appear to be coupled (*Christensen* et al. 1981). Inhibition of T1 DNA synthesis by infection of a nonpermissive host with amber mutants in genes *1–4* with a *ts* mutant, or after addition of nalidixic acid, causes inhibition of digestion of host DNA. Mutants in genes *1* and *2* do not degrade host DNA. Similarly, nalidixic acid prevents T1 DNA synthesis and breakdown of the host genome. Shift to nonpermissive temperatures of temperature-sensitive mutants in these genes causes immediate cessation of T1 DNA synthesis (*Walling* and *Christensen* 1981). Since T1 utilizes the host machinery for DNA elongation (*Bourque* and *Christensen* 1980), an involvement of *gps 1* and *2* in initiation of replication is likely (*Walling* and *Christensen* 1981).

4.8 T1 Morphogenesis

Very little is known about morphogenesis of T1. *Figurski* and *Christensen* (1974) have suggested that morphogenesis of T1 proceeds along two parallel pathways, one for heads and the other for tails. Mutants defective in head assembly will assembly tails, and vice versa. When a lysate with heads is added to a lysate with tails, mature progeny viruses are formed. *Toni* et al. (1976) suggested that a complex pattern of T1 morphogenesis can be anticipated on the basis of the existence of one or more precursor molecules for the structural proteins, which possibly give rise to the final components of the virus particle through proteolytic cleavage.

5 Conclusions

T1 has provided new insights in several areas:

Virus T1 has developed an efficient method of avoiding the restriction system of the host. A T1-specific DNA-methyltransferase supermodifies its DNA and thereby makes the DNA inaccessible to host restriction endonucleases.

The T1 system is useful for the elucidation of a membrane-mediated control of gene expression. Such controls are of special interest, since they appear to be involved in many biological processes such as: the immune response of B-lymphocytes, the activation of maternal mRNA of oocytes after fertilization, and the response of certain hormones. T1 gives a clue for the understanding of a membrane-mediated control of host gene expression.

For infection T1 requires the proton motive force of the host membrane. The analysis of this energy requirement indicates that the process of DNA invasion during infection is energy dependent and therefore an active transport process.

T1 infection is stringently coupled to the iron transport system of the host. The genetics and biochemistry of T1-resistant *E. coli* mutants (*ton A* and *ton B*) have provided a new view of virus-receptor interactions.

In summary, the T1 system has provided us with many clues and it certainly will be very attractive in the future, since many general aspects of viral infection can easily be studied with the virus T1, if one ignores its reputation as a "dangerous" virus.

Acknowledgment. We like to thank Dr. R. Lennox for critical reading of the manuscript and for helpful discussions. The work in the laboratory of the authors was supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (Projektnr. 4241).

References

Arber W (1974) DNA modification and restriction. Prog Nucleic Acid Res Mol Biol 14:1–37

Bayer ME (1968) Adsorption of bacteriophages to adhesions between wall and membrane of *Escherichia coli.* J Virol 2:346–356

Bayer ME, Bayer MH (1981) Fast responses of bacterial membranes to virus adsorption: a fluorescent study. Proc Natl Acad Sci USA 78:5618–5622

Bending MM, Drexler H (1977) Transduction of bacteriophage Mu by bacteriophage T1. J Virol 22:640–645

Bohne L, Coquerelle T, Hagen U (1968) Alteration of the DNA molecule in irradiated T1 phages. Stud Biophys 7:131–136

Bohne L, Coquerelle T, Hagen U (1970) Radiation sensitivity of bacteriophage DNA. II. Breaks and crosslinks after irradiation in vivo. Int J Radiat Biol 17:205–215

Borchert LD, Drexler H (1980) T1 genes which affect transduction. J Virol 33:1122–1128

Bourque LW, Christensen JR (1980) Synthesis of coliphage T1 DNA: requirement for host DNA genes involved in elongation. Virology 102:310–316

Bradley DE (1967) Ultrastructure of bacteriophages and bacteriocins. Bacteriol Rev 31:230–314

Braun V, Hancock REW, Hantke K, Hartmann A (1976) Functional organization of the outer membrane of *E. coli:* phage and colicin receptors as components of iron uptake systems. J Supramol Struct Cell Biochem 5:37–58

Bresler SE, Kiselev NA, Manjakov UF, Mosevitsky MJ, Timkovsky AL (1967) Isolation and physicochemical investigation of T1 bacteriophage DNA. Virology 33:1–9

Bresler SE, Dadivanyan LP, Mosevitskii MJ (1972) Examination of recombinant DNA molecules of bacteriophage T1 using electron microscopic autoradiography. Mol Biol 6:226–230
Brody EN, Mackal RP, Evans EA Jr (1967) Properties of infectious T1 deoxyribonucleic acid. J Virol 1:76–85
Cairns J, Stent GS, Watson JD (eds) (1966) Phage and the origins of molecular biology. Cold Spring Harbor, New York
Christensen JR (1974) Complementation by restricted phage T1. J Virol 14:1411–1418
Christensen JR (1976) Red system of bacteriophage λ complements the growth of a bacteriophage T1 gene *4* mutant. J Virol 17:713–717
Christensen JR, Geiman JM (1973) A new effect of the *rex* gene of phage λ: premature lysis after infection by phage T1. Virology 56:285–290
Christensen JR, Tolmach LDJ (1955) On the early stages of infection of *Escherichia coli* by bacteriophage T1. Arch Biochem Biophys 57:195–207
Christensen JR, Gawron MC, Halpern J (1978) Exlusion of bacteriophage T1 by bacteriophage λ. I. Early exclusion requires λ. *N* gene product and host factors involved in *N* gene expression. J Virol 25:527–534
Christensen JR, Figurski DH, Schreil WH (1981) The synthesis of coliphage T1 DNA: degradation of the host chromosome. Virology 108:373–380
Coquerelle TM, Hagen U (1972) Loss of adsorption and injection abilities in γ-irradiated phage T1. Int J Radiat Biol 21:31–41
Creaser EH, Taussig A (1957) The purification and chromatography of bacteriophages on anion-exchange cellulose. Virology 4:200–208
Delbrueck M (1945) The burst size distribution in the growth of bacterial viruses (bacteriophages). J Bact 50:131–135
Delbrueck M, Bailey WT (1946) Induced mutations in bacterial viruses. Cold Spring Harbor Symp Quant Biol 11:33–37
Drexler H (1970) Transduction by bacteriophage T1. Proc Natl Acad Sci USA 66:1083–1088
Drexler H (1972a) Transduction of *Gal*$^+$ by coliphage T1. Role of hybrids of bacterial and prophage λ deoxyribonucleic acid. J Virol 9:273–279
Drexler H (1972b) Transduction of *Gal*$^+$ by coliphage T1. II. Role of transcription control in the efficiency of transduction. J Virol 9:280–285
Drexler H (1973) Transduction of *Gal*$^+$ by coliphage T1. III. Requirement for transcription and translation in recipient cells. J Virol 12:1072–1077
Drexler H (1977) Specialized transduction of the *Bio*$^+$*d* region by coliphage T1. Mol Gen Genet 152:59–63
Drexler H, Christensen JR (1979) Transduction of bacteriophage by bacteriophage T1. J Virol 30:543–550
Drexler H, Kylberg KJ (1975) Effect of UV irradiation on transduction by coliphage T1. J Virol 16:263–266
Figurski DH, Christensen JR (1974) Functional characterization of the genes of bacteriophage T1. Virology 59:397–407
French RC, Graham AF, Lesley SM, van Rooyen CE (1952) The contribution of phosphorus from *T2r*$^+$ bacteriophage to progeny. J Bact 64:597–607
Garen A, Puck TT (1951) The first two steps of the invasion of host cells by bacterial viruses II. J Exp Med 94:177–189
Gawron MC, Christensen JR, Shoemaker TM (1980) Exclusion of bacteriophage T1 by bacteriophage λ. II. Synthesis of T1-specific macromolecules under *N*-mediated excluding conditions. J Virol 35:93–104
Geiman JM, Christensen JR, Drexler H (1974) Interactions between the vegetative states of phages λ and T1. J Virol 14:1430–1434
Gill GS, MacHattie LA (1975) Oriented extrusion of DNA from coliphage T1 particles. Virology 65: 297:303
Gill GS, MacHattie LA (1976) Limited permutations of the nucleotide sequence in bacteriophage T1 DNA. J Mol Biol 104:505–515
Graham AC, Stocker BAD (1977) Genetics of sensitivity of *Salmonella* species to colicin M and bacteriophages T5, T1 and ES18. J Bact 130:1214–1223

Grinius L (1980) Nucleic acid transport driven by ion gradient across cell membrane. FEBS Lett 113(1):1–10
Hancock REW, Braun V (1976) Nature of the energy requirement for the irreversible adsorption of bacteriophages T1 and Φ 80 to *Escherichia coli*. J Bact 125:409–415
Hausmann R, Gold M (1966) The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. IX. Deoxyribonucleic acid methylase in bacteriophage-infected *Escherichia coli*. J Biol Chem 241:1985–1994
Herrlich P, Rahmsdorf HJ, Pai SH, Schweiger M (1974) Translational control induced by bacteriophage T7. Proc Natl Acad Sci USA 71:1088–1092
Hirsch-Kauffmann M, Sauerbier W (1968) Inhibition of modification and restriction for phages λ and T1 by coinfecting T3. Mol Gen Genet 102:89–94
Hirsch-Kauffmann M, Pfennig-Yeh M, Ponta H, Herrlich P, Schweiger M (1976) A virus-specified mechanism for the prevention of multiple infection – T7 – and T3-mutual and superinfection exclusion. Mol Gen Genet 149:243–249
Hotz G, Mauser R (1969) Infectious DNA from coliphage T1. Some properties of the spheroplast assay system. Mol Gen Genet 104:178–182
Hotz G, Mauser R (1970) Infectious DNA from coliphage T1. II. Host-cell reactivation of UV-irradiated molecules. Mol Gen Genet 108:233–242
Hotz G, Mauser R, Walser R (1971) Infectious DNA from coliphage T1. III. Occurrence of single strand breaks in stored, thermally treated, and UV-irradiated molecules. Int J Radiat Biol 19:519–536
Howard BD (1967) Phage lambda mutants deficient in r_{II} exclusion. Science 158:1588–1589
Jacquemin-Sablon A, Lanni YT (1973) Lambda-repressed mutants of bacteriophage T5. I. Isolation and genetical characterization. Virology 56:230–237
Jiresova M, Janecek J (1977) Inhibition of β-galactosidase synthesis in *E. coli* after infection with different DNA and RNA phages. Folia Microbiol (Praha) 22:173–181
Kepes A, Meury J, Robin A, Jimeno J (1977) Some ion transports in *E. coli* (Transport of potassium and of anionic sugars). In: Semenza G, Carafoli E (eds) Biochemistry of membrane transport. FEBS Symposion No 42. Springer, Berlin Heidelberg New York, pp 633–647
Kerr C, Sadowski PD (1972) Gene *6* exonuclease of bacteriophage T1. I. Purification and properties of the enzyme. J Biol Chem 247:305–310
Klein A, Sauerbier W (1965) Role of methylation in host-controlled modification of phage T1. Biochem Biophys Res Commun 18:440–445
Kotval JS, Christensen JR (1981) Requirement for protein synthesis for survival of unmodified bacteriophage T1 in a restricting host. J Virol 37:931–935
Krisch R (1972) Lethal effects of iodine-125 decay by electron capture in *Escherichia coli* and in bacteriophage T. Int J Radiat Biol 21:167–189
Krisch R, Krasin F, Sauri CJ (1978) DNA breakage, repair, and lethality accompanying iodine-125 decay in microorganisms. Curr Top Radiat Res Q 12:355–368
Kylberg KJ, Bendig MM, Drexler H (1975) Characterization of transduction by bacteriophage T1: time of production and density of transducing particles. J Virol 16:854–858
Labedan B, Goldberg EB (1979) Requirement for membrane potential in injection of phage T4 DNA. Proc Natl Acad Sci USA 76:4669–4673
Labedan B, Letellier L (1981) Membrane potential changes during the first step of coliphage infection. Proc Natl Acad Sci USA 78:215–219
Lederberg S (1957) Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria. Virology 3:496–513
Lee CS, Pyreitz RE, Thomas CA Jr (1976) Kinetic studies on the cyclization of T7 and T1 DNAs. Biopolymers 15:671–686
Lehman JR, Pratt EA (1960) On the structure of the glucosylated hydroxymethyl cytosine nucleotides of coliphages T2, T4, and T6. J Biol Chem 235:3254–3259
Lindberg AA (1973) Bacteriophage receptors. Annu Rev Microbiol 27:101–117
MacHattie LA (1977) DNA maturation by the "headful" mode in bacteriophage T1. J Mol Biol 110: 441–465
MacHattie LA, Rhoades M, Thomas CA JR (1972) Large repetition in the non-permuted nucleotide sequence of bacteriophage T1 DNA. J Mol Biol 72:645–656
McCorquodale DJ (1975) The T-odd bacteriophages. CRC Crit Rev Microbiol: 101–159

Male CJ, Christensen JR (1970) Synthesis of messenger ribonucleic acid after bacteriophage T1 infection. J Virol 6:727–737
Martin DTM, Adair CA, Ritchie DA (1976) Polypeptides specified by bacteriophage T1. J Gen Virol 33:309–319
Michalke WE (1967) Erhöhte Rekombinationshäufigkeit an den Enden des T1-Chromosoms. Mol Gen Genet 99:12–33
Mosin AF, Tsyb TS, Petrovka KM (1972) Decrease in the survival of T1 phage exposed to UV irradiation at early stages of *E. coli* B-cell infection. Radiobiologiya 12:873–878
Mosin AF, Petrovka KM, Tsyb TS (1974) Photoreactivation of T1 bacteriophage irradiated in a complex with *E. coli* B in the early stages of infection. Radiobiologiya 14:58–62
Mosin AF (1977a) Some features of reaction of intracellular bacteriophage-T1 to UV irradiation. Microbios 20:115–123
Mosin AF (1977b) Effect of chloramphenicol and cyanide on increase in UV resistance of intracellular bacteriophage T1. Microbios 20:125–131
Neilands JB (1979) The ironic function of bacteriophage receptors. Trends Biochem Sci 4:115–118
Pao CC, Speyer JF (1975) Mutants of T7 bacteriophage inhibited by lambda prophage. Proc Natl Acad Sci USA 72:3642–3646
Ponta H, Grätzel M, Pfennig-Yeh M, Hirsch-Kauffmann M, Schweiger M (1977) Membrane alteration induced by T7 virus infection. FEBS Lett 73:207–209
Ponta H, Pfennig-Yeh M, Wagner EF, Schweiger M, Herrlich P (1979) Radiation sensitivity of messenger RNA. Mol Gen Genet 175:13–17
Potts TV, Christensen JR (1974) Physiological study of cooperative infection by restricted bacteriophage T1. J Virol 14:1319–1325
Rahmsdorf HJ, Herrlich P, Pai SH, Schweiger M, Wittmann HG (1973) Ribosomes after infection with bacteriophage T4 and T7. Mol Gen Genet 127:259–271
Ramsay N, Ritchie DA (1980) A physical map of the permuted genome of bacteriophage T1. Mol Gen Genet 179:669–675
Reider E, Wagner EF, Schweiger M (1979) Control of phosphoenolpyruvate-dependent phosphotransferase-mediated sugar transport in *Escherichia coli* by energization of the cell membrane. Proc Natl Acad Sci USA 76:5529–5533
Ritchie DA, Malcolm FE (1970) Heat-stable and density mutants of phages T1, T3 and T7. J Gen Virol 9:35–43
Ritchie DA, Joicey DH (1978) Formation of concatemeric DNA as an intermediate in the replication of bacteriophage T1 DNA molecules. J Gen Virol 41:609–622
Ritchie DA, Joicey DH (1980) Identification of some steps in the replication of bacteriophage T1 DNA. Virology 103:191–198
Ritchie DA, Christensen JR, Pergh JC, Bourque LW (1980b) Genes of coliphage T1 whose products promote general recombination. Virology 105:371–378
Roberts JR (1981) Restriction and modification enzymes and their recognition sequences. Nucleic Acids Res 9:r75–r96
Roberts MD, Drexler H (1981a) Isolation and genetic characterization of T1-transducing mutants with increased transducing frequency. Virology 112:662–669
Roberts MD, Drexler H (1981b) T1 mutants with increased transduction frequency are defective in host chromosome degradation. Virology 112:670–677
Sauerbier W, Hirsch-Kauffmann M (1968) Transfer of ultraviolet light-induced thymine dimer from parental to progeny DNA in bacteriophage T1 and T4. Biochem Biophys Res Commun 33:32–37
Sauerbier W, Hercules K (1978) Gene and transcription unit mapping by radiation effects. Annu Rev Genet 12:329–363
Schairer HU, Haddock BA (1972) β-galactoside accumultation in a Mg^{2+}, Ca^{2+}-activated ATPase deficient mutant of *E. coli*. Biochem Biophys Res Commun 48:544–551
Schmidt, A, Hotz G (1973) Occurrence of double-strand breaks in coliphage T1-DNA by iodine-125 decay. Int J Radiat Biol 24:307–313
Schweiger M, Herrlich P (1974) DNA-directed enyzme synthesis in vitro. Curr Top Microbiol Immunol 65:59–132

Schweiger M, Hirsch-Kauffmann M (1982) The role of cell membranes in infection with bacterial viruses and colicins. In: Cohen P, Van Heyningen S (eds) Molecular action of toxins and viruses. Elsevier, Amsterdam, pp 191–217

Schweiger M, Wagner EF (1979) Nucleoside-triphosphate-mediated discrimination of gene expression in T1-infected *E. coli*. In: Koch G, Richter D (eds) Regulation of macromolecular synthesis by low molecular weight mediators. Academic, pp 249–262

Schweiger M, Wagner EF, Hirsch-Kauffmann M, Ponta H, Herrlich P (1978) Biochemistry of development of *E. coli* viruses T7 and T1. FEBS Lett 43:171–186

Spoerel N, Herrlich P, Bickel T (1979) A novel bacteriophage defence mechanism: the anti-restriction protein. Nature 278:30–34

Stephan GM, Miltenburger HG, Hotz G (1970) Ultraviolet-induced strand breaks in 5-bromouracil-substituted DNA of phage T1. Z Naturforsch B 25:1037–1042

Taylor DE, Grant RB (1976) Inhibition of bacteriophage λ, T1 and T7 development by R plasmids of H incompatibility group. Antimicrob Agents Chemother 10:762–764

Tikhonenko AS (1970) Ultrastructure of bacterial viruses. Plenum, New York. Translated from Russian by Haigh B

Timofeev-Ressovsky NW, Zimmer KG, Delbrück M (1935) Über die Natur der Genmutation und der Genstruktur 13. Nachr d Akad Wissensch Göttingen, Math-Phys Kl Fachgruppe VI, 1

Toni M, Conti G, Schito GC (1976) Viral protein synthesis during replication of bacteriophage T1. Biochem Biophys Res Commun 68:545–552

Toothman P, Herskowitz I (1980) *Rex*-dependent exclusion of lambdoid phages. Virology 102: 133–146

Trautner TA (1960) Genetische und physiologische Beziehungen zwischen den Bacteriophagen T1 und D20. Z Vererbungsl 91:317–324

Trouwborst T, Winkler KC (1972a) Protection against aerosol-inactivation of bacteriophage T1 by peptides and amino acids. J Gen Virol 17:1–11

Trouwborst T, De Jong JC (1972b) Mechanism of the inactivation of the bacteriophage T1 in aerosols. Appl Microbiol 23:938–941

Trouwborst T, De Jong JC, Winkler KC (1972) Mechanism of inactivaton in aerosols of bacteriophage T1. J Gen Virol 15:235–242

Trouwborst T, Kuyper S, De Jong JC, Plantinga AD (1974a) Inactivation of some bacterial and animal viruses by exposure to liquid-air interfaces. J Gen Virol 24:155–165

Trouwborst T, Kuyper S, Teppema JS (1974b) Structural damage of bacteriophage T1 by surface inactivation. J Gen Virol 25:75–81

Wagner EF (1978) Regulation der Genaktivitäten und der Membranenergie in der T1-Virus Entwicklung. Thesis, Technische Universität Graz

Wagner EF, Schweiger M (1980) Development of *E. coli* virus T1: ATP-mediated discrimination of gene expression. J Biol Chem 255:540–542

Wagner EF, Ponta H, Schweiger M (1977a) Development of *E. coli* virus T1: The pattern of gene expression. Mol Gen Genet 150:21–28

Wagner EF, Ponta H, Schweiger M (1977b) Development of *Escherichia coli* virus T1: Repression of host gene expression. Eur J Biochem 80:255–260

Wagner EF, Auer B, Schweiger M (1979) Development of *E. coli* virus T1: Escape from host restriction. J Virol 29:1229–1231

Wagner EF, Ponta H, Schweiger M (1980) Development of *E. coli* virus T1: The role of the protonmotive force. J Biol Chem 255:534–539

Walling L, Christensen JR (1981) The synthesis of coliphage T1 DNA: Studies on the roles of T1 genes 1,2 and 4. Virology 114:309–318

Wookey P, Rosenberg H (1978) Involvement of inner and outer membrane components in the transport of iron and in colicin B action in *Escherichia coli*. J Bact 133:661–666

Current Topics in Microbiology and Immunology

Editors: M. Cooper, W. Henle, P. H. Hofschneider, H. Koprowski, F. Melchers, R. Rott H. G. Schweiger, P. K. Vogt, R. Zinkernagel

Volume 96
Gene Cloning in Organisms Other Than E. coli

Editors: P. H. Hofschneider, W. Goebel

1982. 63 figures. VII, 259 pages
ISBN 3-540-11117-4

Volume 97

1982. 28 figures. IV, 204 pages
ISBN 3-540-11118-2

Contents: *M. R. Macnaughton:* The Structure and Replication of Rhinoviruses. - *J. A. Holowczak:* Poxvirus DNA. - *H. Persson, L. Philipson:* Regulation of Adenovirus Gene Expression. - *K. H. Nierhaus:* Structure, Assembly, and Function of Ribosomes.

Volume 98
Retrovirus Genes in Lymphocyte Function and Growth

Editors: E. Wecker, I. Horak

1982. 8 figures. VIII, 142 pages
ISBN 3-540-11225-1

Contents: *D. L. Steffen, H. Robinson:* Endogenous Retroviruses of Mice and Chickens. - *H. L. Robinson, G. F. Vande Woude:* The Genetic Basis of Retroviral-Induced Transformation. - *H. C. Morse III:* Expression of Xenotropic Murine Leukemia Viruses. - *E. Wecker, I. Horak:* Expression of Endogenous Viral Genes in Mouse Lymphocytes. - *E. Fleissner, H. W. Snyder, Jr.:* Oncoviral Proteins as Cellular Antigens. - *A. Schimpl:* Regulation of Lymphocyte Proliferation and Differentiation by Lymphokines. - *J. N. Ihle, J. C. Lee:* Possible Immunological Mechanisms in C-Type Viral Leukemogenesis in Mice. - *I. L. Weissman, M. S. McGrath:* Retrovirus Lymphomagenesis: Relationship of Normal Immune Receptors to Malignant Cell Proliferation. - *A. Coutinho:* From the Point of View of an Immunologist. - *R. A. Weiss:* Perspectives on Endogenous Retroviruses in Normal and Pathological Growth.

Volume 99

1982. 30 figures. III, 200 pages
ISBN 3-540-11419-X

Contents: *H. Garoff, C. Kondor-Koch, H. Riedel:* Structure and Assembly of Alphaviruses. - *I. Nakamura, G. Cudkowicz:* Fine Specificity of Auto- and Alloreactive Cytotoxic T-Lymphocytes: Heteroclitic Cross-reactions Between Mutant and Original H-2 Antigens. - *H.-P. Mühlbach:* Plant Cell Cultures and Protoplasts in Plant Virus Research. - *S. Siddell, H. Wege, V. terMeulen:* The Structure and Replication of Coronaviruses. - *H. Wege, S. Siddell, V. terMeuler:* The Biology and Pathogenesis of Coronaviruses.

Volume 100
T Cell Hybridomas

A Workshop at the Basel Institute for Immunology Organized and edited by H. V. Boehmer, W. Haas, G. Köhler, F. Melchers, J. Zeuthen With the collaboration of S. Buser-Boyd

1982. 52 figures. XI, 262 pages
ISBN 3-540-11535-8

Volume 101
Tumorviruses, Neoplastic Transformation and Differentiation

Editors: T. Graf, R. Jaenisch

1982. 27 figures. VIII, 198 pages
ISBN 3-540-11665-6

Contents: The Nature of the Host Range Restriction of SV40 and Polyoma Viruses in Embryonal Carcinoma Cells. - The Immune Response to C-Type Viruses and Its Potential Role in Leukemogenesis. - Mammary Tumor Formation and Hormonal Control of Mouse Mammary Tumor Virus Expression. - Avian Osteopetrosis. - Abelson Leukemia Virus. - Uptake, Fixation and Expression of Foreign DNA in Mammalian Cells: The Organization of Integrated Adenovirus DNA Sequences. - Subject Index.

Springer-Verlag
Berlin
Heidelberg
New York

Virology Monographs

Volume 18
S. Dales, B. G. T., Pogo

Biology of Poxviruses

1981. 27 figures. VIII, 109 pages
ISBN 3-211-81643-7

The book contains an up-to-date, critical analysis of the biology of poxviruses which is accessible both to specialists and to readers with general interest in microbiology. Salient historical discoveries into the nature of poxviruses and the diseases they cause is related to contemporary investigations. Emphasis is placed on unique molecular biological and biochemical features associated with developmental cycles of these DNA cytoplasmic viruses. The sequence and regulation of the genome replication and transcription, as they pertain to virion-associated and induced enzymatic activities, are considered in considerable detail. The program of development involving distinctive phases of assembly is evaluated in the context of temporally coordinated synthesis, processing and modification of $>$ 100 polypeptides constituting the virion. Phenomena related to the disease process are considered, among them genetic variability, cytopathology, transmission of infectivity and immunological responses. Attention is drawn to potential natural reservoirs of smallpox-like agents among the orthopoxviruses.

Springer Series in Microbiology

Editor: M. P. Starr

Basidium and Basidiocarp

Evolution, Cytology, Function, and Development
Editors: K. Wells, E. K. Wells
With contributions by numerous experts

1982. 117 figures. XI, 187 pages
ISBN 3-540-90631-2

Basidium and Basidiocarp is a comprehensive interdisciplinary review of recent studies of the basidiomycetes. This major class of fungi is examined from the taxonomic, cytologic, molecular biologic, physiologic, and biochemical points of view in order to provide a broad picture of modern findings and the experimental procedures underlying them. Among the areas covered are phylogenetic theory, basidial cytology, DNA replication, and the physiology of stipe elongation.

Springer-Verlag Berlin Heidelberg New York