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Uptake of Protein by Mammalian Cells: An Underdeveloped Area

The penetration of foreign proteins into mammalian cells can be measured and their functions explored.

H. J.-P. Ryser

More than 20 years have elapsed since Avery, MacLeod, and McCarty published the first observation of genetic transformation in pneumococci (*1*). This discovery, once confirmed and broadened (*2*), initiated the search for similar phenomena in cells of higher organisms. It prepared the way for major discoveries such as the initiation of infection (*3*) and malignant transformation (*4*) in mammalian cells by means of nucleic acids extracted from infectious or oncogenic viruses. Efforts to achieve genetic transformation in animal cells led to several claims of success (*5*), and although results on this subject have been difficult to reproduce, they have at least suggested that genetic transformation of animal cells is within reach.

There is no need to point out the implications of this line of research. One of its secondary benefits, however, deserves to be emphasized. By giving us the first definitive demonstration that macromolecules can exert specific biological effects in host cells, these investigations have changed our understanding of membrane function. They have compelled cell biologists to recognize that the study of membrane transport is no longer restricted to the diffusion and the active transport of small solutes but must reckon with the penetration of macromolecules.

Mechanisms that can account for this type of transport were proposed more than 10 years ago (*6*), and there is now near-consensus among cell biologists that macromolecules are drawn into cells by membrane movements associated with vesicle formation. The process is generally described as endocytosis or, depending on its dimensions, as micropinocytosis, pinocytosis, or phagocytosis. It is believed that endocytotic vesicles or vacuoles containing foreign macromolecules receive intracellular digestive enzymes by fusing with lysosomes (*7*). The largest part of the ingested macromolecules, it is assumed, undergoes intracellular digestion, whereas a smaller fraction escapes destruction and finds access to specific sites of action. The smallest vesicles containing ingested macromolecules are of the order of 0.05 micron in diameter and are seen only with the electron microscope. Larger vesicles, however, are visible with the light microscope, and several semiquantitative studies of pinocytosis have relied on the simple enumeration of such vacuoles (*8*). Because the quantitative evaluation of electron-microscopic data must contend with considerable difficulties and limitations, there is today a remarkable abundance of morphological data contrasting with a dearth of reliable quantitative studies of endocytosis. The emphasis on morphological aspects may explain why the biologists committed to the traditional study of transport fail to be captured by this problem. The sequence of vesicle formation, migration, and fusion and the superimposed process of digestion is altogether too complex to lend itself to a mathematical analysis of transport kinetics. On the other hand, virologists, who care about the ultimate biological function of a foreign macromolecule, tend to neglect the physiology of uptake and seldom consider this initial step as a subject worthy of special investigation. These may be some of the reasons why, 20 years after Avery's momentous discovery, little has become known about the physiology of macromolecular transports across animal cell membranes.

Selection of a Model and Technical Stumbling Blocks

Investigators measuring the uptake of macromolecules face a few technical problems which, on occasion, must have seemed so elementary that they were ignored. One of them is the distinction between adsorption and uptake. Macromolecules tend to be adsorbed heavily to the surface of living cells. The process is rapid, complete within seconds, and reversed by repeated washing. It shows little or no dependence

The author is assistant professor of pharmacology at Harvard Medical School, Boston, Massachusetts.

upon temperature. The activity measured after exposure of isolated cells for 1 minute to a labeled macromolecule and to a standard washing procedure can be defined as representing residual adsorption. This adsorption is far from negligible, for in the case of albumin it is roughly of the same order as the net uptake occurring during the subsequent hour (*9*).

A second important technical requirement is preservation of the integrity of the cells throughout an experiment. Injured cells can bind macromolecules in amounts far exceeding the modest degree of physiological adsorption or uptake. Proteins, for instance, seem to invade injured cells and to bind more strongly to cytoplasmic structures than to cell surfaces (*10*). Nucleic acids and histones bind to the nuclei of damaged, but not of normal, cells. Accordingly, experiments that do not carry adequate "health certificates" must be considered meaningless. This consideration imposes severe limitations on the conditions under which measurements of uptake can be performed (*11*). Tissue culture permits one to keep cells in optimum conditions and to assess their integrity in dye-exclusion tests or by measuring the growth of cells subcultured after exposure to experimental conditions.

A third methodological feature of importance is the possibility that a labeled macromolecule will break into small molecules that are then reincorporated into new cellular macromolecules. Such a phenomenon may give a false indication of the uptake and distribution of the labeled material. In this regard, iodinated proteins are excellent tracers, for the iodine is carried exclusively by tyrosyl radicals and iodinated tyrosines are not subject to reincorporation in newly synthesized proteins (*12*). A common, stable, and commercially available radioiodinated protein, human serum albumin-I^{131}, was used as a tracer in most of the experiments discussed below.

The emphasis on proteins as a macromolecular model is quite appropriate because some aspects of the penetration of nucleic acids have been recently reviewed (*13*). Less attention has been given to the penetration of protein, perhaps because the topic was never heralded by resounding discoveries comparable to those of genetic transformation. Yet proteins are so very much more prominent in the immediate environment of living cells that one has to consider that they might serve important intracellular functions. The presentation of our data on the penetration and fate of albumin-I^{131} will be followed, therefore, by a discussion of the possible role of other foreign proteins in animal cells.

Table 1. Stimulation of albumin uptake by histones and homopolymers of L-arginine and L-lysine. Cells were incubated for 2 hours at 37°C in the presence of albumin-I^{131} (Eagle's medium without horse serum). Uptake of albumin was measured before or after addition of one of four basic proteins to the medium. The figures represent increases in uptake (multiples of controls) due to the added compound.

Basic protein	Stimulation by a compound rich in	
	L-Lysine	L-Arginine
Histones	1.2	4.6
Homopolymers	3.0	14.5

Characteristics of Albumin Uptake by Tumor Cells in Culture

To measure the cellular penetration of albumin-I^{131}, monolayers of an established tumor-cell line, Sarcoma S 180 II, were exposed to albumin-I^{131} added to their own culture medium for periods of 1 minute to 2 hours. The cells were then detached, washed, and processed for measurement of the radioactive material insoluble in acid (*9*).

Exposure at 2°C for 1 minute or for 60 minutes gave identical results; so did a 1-minute exposure at 37°C. This immediate binding showed all the characteristics of simple adsorption. By contrast, exposure at temperatures higher than 2°C led to an increase in labeling that was dependent on time and temperature and that was no longer reversed by washing and was, therefore, considered to represent albumin uptake by intact cells. This net uptake amounted, on the average, to 10^4 to 10^5 molecules per cell and per hour (*9, 14*). The temperature dependence was characterized by a temperature coefficient (Q_{10}) of 1.6 to 1.8. Conventional metabolic inhibitors such as iodiacetate, NaF, 2,4-dinitrophenol, and cyanides, when given in concentrations that inhibit phagocytosis of white blood cells, failed to reduce the uptake of albumin. It appears, therefore, that the uptake of albumin requires less energy than phagocytosis does and that it can be supported entirely by the cellular pool of energy-rich compounds present in normal cells. Next to low temperature, the most efficient inhibitor of uptake was the presence of another negatively charged polypeptide. Poly-L-glutamic acid, for instance, in a concentration of 30 μg/ml, decreased albumin uptake by 51 percent. Basic polypeptides, on the other hand, markedly enhanced the process.

The addition of 3 μg of crude calf-thymus histone or salmon-sperm protamin per milliliter of incubation medium (Eagle's, with 1 percent horse serum) more than doubles the amount of albumin taken up in 1 hour by a sarcoma monolayer (*15*). When tested separately, the two main subfractions of calf-thymus histones, those rich in arginine and lysine, respectively have quite different effects, the former being considerably more active than either the crude histone or that rich in lysine (Table 1). This difference cannot be attributed to the different ratios of basic to acidic amino acids, nor to contamination with acidic constituents present in certain preparations of histone rich in lysine. The simplest assumption is that the arginyl and lysyl radicals have different membrane activities. When tested in the form of homopolymers, L-arginine is indeed considerably more active than L-lysine and also more active than the histone rich in arginine (Table 1). Polyarginine is not more active, however, than poly-L-ornithine, poly-L-histidine, or poly-D-lysine; all have approximately equal effects. It is striking to find the polymer of D-lysine clearly more active than that of L-lysine. This difference was observed in all experimental conditions but was particularly marked at high concentrations (1 to 10 μg/ml).

The stimulation of albumin uptake by 3 μg of poly-D-lysine or poly-L-ornithine per milliliter is approximately tenfold. Even more striking are the low thresholds of action. At a concentration of 0.01 μg/ml four out of five basic polyamino acids significantly affect albumin uptake measured over a period of 2 hours. With polypeptides of high molecular weight, such as a poly-D-lysine of molecular weight 210,000 or a poly-L-ornithine of molecular weight 200,000, the thresholds of activity lie below 5.10^{-11} mole/liter which, in our system, represents some 2×10^3 molecules per cell. One cannot escape the conclusion that basic compounds of this nature are highly active upon cell membranes.

The mechanisms responsible for stimulation of protein uptake were studied

in two ways. First, it was established that the basic macromolecules are taken up themselves and that their own rate of uptake is proportional to their effect on the transport of other macromolecules (*15*). So far, however, the experimental evidence does not support the notion that albumin and basic polyamino acids are taken up in association. No complexes have been detected in vitro, and no simple stoichiometry is apparent in the uptake of the two compounds. Results of fluorescence microscopy suggest that basic macromolecules labeled with fluorescein are localized in cytoplasmic vacuoles (*15*), and electron microscopy reveals that treatment of the cells with basic compounds increases the amount of ferritin and albumin-I^{125} found in pinocytotic vesicles (*16*).

Correlation between Effect and Molecular Size of Basic Polymers

The other approach to the study of the mechanism of stimulation is based on the use of homologous polyamino acids of different sizes. Neither ornithine nor a diamine, like spermine (molecular weight, 203), influence albumin uptake. Apparently, a minimal molecular size is required for the basic compounds to evoke a membrane effect. Polyornithines, ranging in molecular weight 4,000 to 200,000 when tested in identical amounts (3 μg/ml), revealed a positive correlation between effect and size (*17*). This dependence was particularly striking when the results were expressed on a molar basis: they fell on a straight line that intersected the coordinates at a molecular weight of about 1,000. This extrapolation gives an estimate of the minimum size required for action. Such a novel requirement may hold a clue to the mode of action of basic macromolecules. Since the size determines the number of positive charges capable of interacting simultaneously with the cell surface, it is assumed that a polymer must make multiple attachments to the cell surface in order to act and initiate albumin uptake (*17*). Data obtained with basic polymers of different structure, like diethylaminoethyl dextran, are consistent with this postulate (*17*). As stated earlier, basic compounds are themselves taken up by cells at rates correlating with their power of stimulation. It can be inferred, therefore, that the size of the basic compounds also determines their own rate of penetration into cells. Thus, larger macromolecules are taken up more readily than smaller ones, a finding at first difficult to reconcile with classical notions on transport across membranes. The same relation, however, has been observed with other macromolecules. When ferritin solutions differing only in degree of aggregation were presented to cells in identical concentrations and under identical conditions, the ferritin with larger aggregates was taken up at a much higher rate (*18*). This finding recalls older observations on the uptake of DNA, which indicated that more DNA penetrated into cells when presented in the form of supramolecular complexes than when used in a molecular solution (*19*). This rule may explain other biological observations such as the fact that antigens presented in particulate form or as complexes with RNA are considerably more active in initiating immunity than their soluble analogues are (*20*).

Table 2. Relative rate of uptake of different proteins. The uptake of several proteins was measured under comparable conditions in the grams per milligram of cell protein per hour. Ferritin was determined by an assay for iron. The basic compounds were labeled with fluorescein. The molecular weights of the polymers of L-lysine, D-lysine, and L-ornithine were 70,000, 113,000, and 200,000, respectively.

Albumin	1
Ferritin	65
Lysine-rich histone	150
Poly-L-lysine	400
Crude histone	800
Arginine-rich histone	1000
Poly-D-lysine	1500
Poly-L-ornithine	2000

Selectivity of the Uptake Process

Next to the size, the charge of a macromolecule is of obvious importance, since all basic proteins have higher rates of uptake than albumin (Table 2). However, neither charge nor size can account for the difference between poly-L-lysine and poly-D-lysine, or between histones rich in arginine and those rich in lysine (Table 2). More subtle structural factors seem to control the selectivity of uptake in these cases. It is important to recall that the apparent lack of selectivity had long been considered an obstacle to the assignment of a specific physiological function to pinocytosis. Discussions of endocytosis usually emphasize the notion of bulk transport of solutes present in the medium. The evidence given in Table 2 for the selective penetration of different proteins suggests, however, that pinocytosis is initiated by a specific event, such as the interaction of a macromolecule with a membrane receptor. Cell membranes do harbor sites that react specifically with exogenous compounds (drugs, immunoglobulins, viruses). It is reasonable to assume that a variety of proteins participate in similar interactions prior to their uptake.

The Value of Albumin as a Model for Other Macromolecules

Data obtained with macromolecules other than proteins suggest that the described results have general validity. Thus, the uptake of C^{14}-inuline, or of bacterial DNA labeled with P^{32}, is also stimulated by basic polyamino acids (*21*). The pattern of stimulation is similar in all cases and poly-L-lysine is again more active than histones rich in lysine or than diethylaminoethyl dextrans, but less active than poly-D-lysine and poly-L-ornithine. Polyglutamic acid, on the other hand, inhibits the uptake of DNA. Also, the response to low temperature, or to metabolic inhibitors, is identical in the case of inuline, albumin, basic proteins, and DNA. The only exception noted so far is in the effect of iodoacetate on the uptake of DNA with high molecular weight (*21*) and of aggregated ferritin (*18*), both of which are significantly

Table 3. Radioactivity (I^{131}) released by cells into the medium. Cells were first labeled with albumin-I^{131} during 60 minutes at 37°C (Eagle's medium with 0.5 percent horse serum, 5.5 μc of albumin-I^{131}, and 10 μg of histones per milliliter). They were then washed and incubated at 37°C in medium free of label (Eagle's, with 5 percent horse serum). Samples of this medium were removed at intervals for measurement of radioactivity. "Macromolecules" are defined as belonging to a fraction excluded by Sephadex G-75. "Small molecules" were of a size no larger than 400 as indicated by the elution pattern on Sephadex G-15 (*22*).

Time of sampling (min)	Total activity (count/min per milliliter)	Percentage of total activity found in the form of:	
		Macromolecules	Small molecules
0	3854	100	0
60	21462	31	67
120	27120	25	74

inhibited. Inasmuch as these two compounds share the property of being the largest of all the materials tested, it can be assumed that the transport of very large macromolecules or molecular aggregates calls for a more active energy metabolism and that, in this respect, the uptake phenomenon resembles phagocytosis. This finding suggests that phagocytosis and pinocytosis may differ not only in the dimensions of their membrane movements or the nature of the carried material, but also in the amounts of energy required by the transport function.

Metabolic Fate of Foreign Proteins in Host Cells

These data, especially the finding that uptake of macromolecules can be markedly stimulated, should be useful to investigators concerned with the intracellular functions of foreign macromolecules. Uptake, however, is only a first necessary step toward performing an intracellular function. Because ingested macromolecules are localized in cytoplasmic vacuoles endowed with digestive activities, it is important to explore their fate, that is, their chance of reaching a site of intracellular action.

Experiments were carried out to determine the half-life of albumin-I^{131} inside sarcoma cells. The iodine label was again particularly suitable because of its total lack of reincorporation into cellular proteins. Cells labeled over a period of 1 hour were washed and reincubated in medium free of the isotope. The radioactivity of the cell protein, as well as that of the medium, was measured over the following 3 hours (*22*).

The amount of I^{131} in the cell protein decreased rapidly within 60 minutes, and the activity recovered from the medium contained an increasing amount of small molecular components (Table 3). When the initial amount of albumin-I^{131} was increased by the addition of histones to the medium during the labeling period, a greater absolute loss of cellular radioactivity was observed. It appears, therefore, that intracellular digestion can respond to an increasing challenge and efficiently degrade foreign proteins to amino acids or oligopeptides (*22*). Comparable results have been obtained in other cell systems (*23*).

The sizable and rapid protein digestion that follows penetration represents no doubt an efficient protection of mammalian cells against active macromolecules of the environment. It also constitutes an important limiting factor in attempts to use foreign macromolecules as vectors of biological activity. The failure to achieve reproducible genetic transformations in mammalian cells may be due in part to the efficiency of this intracellular digestion presumably carried out by lysosomes. By contrast, the relative ease of producing transformation in bacteria may be related to the absence of a comparable lysosomal system in microorganisms. A better understanding of the physiology of intracellular digestion can be expected to improve the prospects of future attempts at transforming animal cells. The procedures commonly used to enhance the effect of viral nucleic acids in host cells share the property of inducing a rather nonspecific cell damage, characterized by an abnormal permeability to dyes and by vacuolization (*11*). The role of this and other factors and their relation to both uptake and fate of macromolecules must be actively investigated.

One would be mistaken to believe that foreign proteins are always and necessarily limited in their effects by capture and destruction in cytoplasmic vacuoles. In fact, the function of certain foreign proteins may be facilitated rather than limited by a rapid and complete intracellular breakdown. On the other hand, certain specialized cells accumulate ingested foreign proteins and are able to use them for special purposes.

Functions Enhanced by an Intracellular Breakdown

Since a number of hormones, cofactors, and drugs can form complexes of various strength with plasma proteins, it has been suggested that they might be carried into cells in protein-bound form. This suggestion of a carrier function of blood proteins, described by Bennhold some 30 years ago (*24*), was criticized on the ground that complexes of this sort are reversible and that it is impossible, even when their dissociation constants are very low, to exclude minimum dissociations followed by an active transport of the unbound agent (*25*). This criticism, however valid, fostered the view that transport mediated by protein did not exist because it was not demonstrable. This point was recently reexamined and it was shown that, under appropriate conditions, such a transport can indeed be demonstrated. Ferritin, an iron-containing protein of high molecular weight was used for this purpose. When isolated in the classical way by precipitation with cadmium sulfate, this compound contains about 3 to 7 percent of cadmium that cannot be removed entirely by any purification procedure (*26*). This contaminant is responsible for the marked cellular toxicity of most commercial ferritin preparations. By contrast, ferritin isolated without recourse to $CdSO_4$ is innocuous (*26*). Cadmium toxicity can be accurately quantitated in dye-exclusion tests, and it was used as a measure of the penetration of protein-bound cadmium into cells. Two cell populations were incubated in a $CdCl_2$ solution or in a cadmium-ferritin solution having the same total amount of cadmium. A strong chelating agent was added to both solutions in a concentration large enough to bind all free cadmium, thus rendering the $CdCl_2$ solution innocuous. When the cell populations were washed and reincubated in medium free of cadmium, cell damage developed only in the population exposed first to ferritin. That the damage was due to the penetration of protein-bound cadmium was further established in experiments performed at low temperature. According to expectation, incubating cells in the presence of cadmium-ferritin at 2°C prevented cell death by inhibiting protein uptake. Although free cadmium can enter the cells and does not illustrate the case of an agent transported exclusively in protein-bound form, it shows nevertheless, that a small foreign molecule can be made to penetrate into cells in the form of a protein complex. The drastic effect in this case is contingent upon the degradation of ingested ferritin and the release of free cadmium in the cell. Hence, intracellular digestion of a foreign macromolecule can be tied to a function. This view raises a number of questions. Are the breakdown products of ingested proteins endowed with biological activities of their own? Are the active oligopeptides, normally present in the body, breakdown products of circulating proteins, which are taken up and digested in a controlled fashion at specific locations?

It is known that the thyroid hormone, an iodinated tyrosine derivative, is released from a protein precursor, thyroglobulin. It has been postulated that thyroglobulin is taken up by epi-

thelial cells of the thyroid gland (*27*) and that its breakdown in the cells results in the release of the active hormone. Although this hypothesis has to await further confirmation, it is currently highly regarded and has the merit of focusing attention on a possible function of protein uptake. The liberation of polypeptide hormones into the bloodstream might follow the same pattern.

Function Linked to the Absence of Intracellular Breakdown

Different cells and tissues differ in their capacity to handle ingested macromolecules. There is evidence that certain specialized cells are quite inactive in this regard. Electron-microscopic studies have shown, for instance, that egg cells of several species can accumulate extracellular macromolecules in the course of their differentiation. The active inward-directed vesiculation seen in these cells leads to the formation of large vacuoles filled with material of extracellular origin (*28*). This process is believed to contribute to the formation of egg yolk. Time-sequence studies suggest that distinct pinocytotic vesicles ingest different material at successive phases of differentiation (*28*). Although the content of these vacuoles has not been positively identified, its appearance and immunological characteristics suggest that it is extracellular protein. The oocyte may, therefore, illustrate the case of a cell in which a negligible turnover leads to a positive balance of extracellular proteins needed to fulfill specific nutritional functions.

The yolk of chicken eggs also fulfills an immunological function. It contains immunoglobulins of maternal origin, which reach the circulation of the embryo by passing through the yolk-sac membrane (*29*). A similar transfer of immunity takes place in the gut of newborn mammals. Antibodies contained in the mother's milk are absorbed by the epithelial cells and passed along to the circulation (*30*). This passive immunization is limited, however, to the first hours or days of life. It is not known whether the abrupt cessation of protein transport results from an inhibition of cellular uptake or from an increased breakdown of ingested proteins.

It should be mentioned, incidentally, that immunoglobulins are also taken up by other tissues. They appear to have an important function in phagocytosis, coating foreign particles and making them attractive to leucocytes and macrophages (*31*).

Possible Metabolic Functions of Extracellular Proteins

It can be expected that further advance in animal cell biology will uncover other functions related to protein uptake. There are already strong indications that specific extracellular proteins may exert profound effects on the cell metabolism. It may be appropriate, therefore, to discuss some examples in the light of the results obtained with our model compounds. Two well-known cases deal with the inhibition of nucleic acid synthesis by virus-infected or noninfected cells. Several others are related to the stimulation of cell growth and differentiation. The discussion will also briefly consider the role of antigen uptake in immune response.

Interferon. When challenged by viral infection, most animal cells respond by synthesizing and releasing a protein which can protect them—and also protect other cells—against the challenging microorganism and other viruses (*32*). Proteins possessing this property have been isolated in relatively pure form; they have a molecular weight ranging from 20,000 to 63,000. These interferons have not so far been available in sufficient quantities to be labeled and used for determining their cellular binding and localization. Studies on the uptake of interferon have therefore relied essentially on the disappearance of biological activity from incubation media. Although these studies have led to contradictory results and interpretations, recent data based on a more precise knowledge of the relationship between dose and effect have now demonstrated that the successive passages of an interferon solution on eight different cell monolayers lowers its titer by about 6 units, that is, some 6 ng (*33*). This result is consistent with data on the activity recovered from cells exposed to higher concentrations of interferon (*34*). Moreover, the relation between length of exposure and effect also suggests that interferon is taken up by target cells (*35*). It is generally believed that interferon does not itself inhibit viral growth but rather induces the synthesis of a protein that interferes with the translation of the viral message (*36*). Although one could, by some stretch of imagination, explain this mechanism on the basis of an extracellular interaction of interferon with the cell membrane, it is altogether simpler to postulate that interferon penetrates into cells and acts as a derepressor in the synthesis of a specific inhibitory protein. The potential function of proteins as gene regulators has been emphasized by the recent finding that the repressor of the "Lac-operon" in *Escherichia coli* has been identified as a protein (*37*). The example of interferon has important implications, showing that small amounts of an extracellular protein may produce pronounced intracellular effects which outlive its presence in the cell.

Fiber antigen of adenovirus. Whereas the cellular origin of interferon is firmly established, a protein of viral origin can have a rather comparable effect. It was shown 8 years ago that a protein isolated from adenovirus 5, now called the fiber antigen (molecular weight of about 200,000), can protect cells against unrelated viruses when it is given either before or after challenge (*38*). That this antigen changes the cellular response to infection has now been confirmed by Levine and Ginsberg (*39*). These authors showed, in addition, that the fiber antigen inhibits the cellular synthesis of RNA, DNA, and proteins in both infected or uninfected cells. The kinetics of cellular fixation of fiber antigen labeled with C^{14} indicate that only small amounts of the protein become irreversibly bound by host cells within a 2-day exposure. Both the increase of binding with time and the relation of dose and effect suggest, in my view, that the fiber antigen is taken up and exerts its effect inside the cell. The action of this protein can be distinguished from that of interferon: it is not prevented by actinomycin D, and it affects the synthesis of both viral and cellular macromolecules (*39*). In addition, the time course of action of the two proteins are profoundly different, for irreversible effects of the viral antigen are seen only after a prolonged exposure of some 20 hours, whereas a 10-minute contact with interferon can lead to irreversible binding and prolonged effect (*35*). Both proteins stand out because of their ability to produce full effects in extremely small amounts. Interestingly enough, these amounts are comparable to the albumin uptake measured in our experiments (*14*).

Function of antigen uptake in immune response. Results with adeno-

virus fiber antigen have the merit of suggesting that antigens can penetrate into cells and influence cell functions, albeit in ways unrelated to their antigenic nature. It has been postulated for a long time that the immune response itself is initiated by a cellular uptake of antigens. A clear understanding of the mechanism of initiation is still lacking, however, and the role of antigen uptake is obscured by the existence of primary and secondary responses and by the participation of several cell types in these processes. Thus, although macrophages take up particles and macromolecules, including antigens of all sorts, it is not yet known whether this uptake has any bearing on the primary immune response (*40*); nor has it been shown that such a response can be initiated in vitro by exposing peripheral lymphocytes to soluble antigens. A kinetic study of this interaction, making use of the data obtained with model compounds, would help clarify these questions. When investigating this point, it will be useful to remember the case of interferon, in particular the fact that the transient presence of small amounts of agent can evoke profound and lasting effects. Viewed in this light, the failure of earlier studies (*41*) to demonstrate labeled antigen in lymphoid cells undergoing immunological differentiation appears less surprising.

Proteins promoting cell division and differentiation. Lymphocyte transformations similar to those observed in the early stages of immune response can be produced by a number of proteins extracted from plants. One of them, phytohemagglutinin (PHA), with a molecular weight of 128,000, has been labeled with fluorescein to demonstrate its penetration into different types of white blood cells (*42*). The localization of fluorescence in cytoplasmic vacuoles indicates that PHA does not merely interact with the cell surface but penetrates into cells. Moreover, single lymphocytes labeled with fluorescein-PHA have been seen to transform into blast cells; this finding supports the view that uptake and transformation are causally related (*42*). Experimental inhibition and stimulation of protein uptake would strengthen the argument. Exposure to PHA leads within hours to an increase in RNA synthesis, followed by protein synthesis and mitosis. Similar mitotic effects of PHA have also been observed in cells other than blood elements (*43*).

A more selective growth factor, acting on nerve tissue, has been described by Levi-Montalcini and Cohen (*44*). This nerve growth factor, isolated in pure form from snake venom and from submaxillary salivary glands of mice, is a protein with an amino acid composition similar in pattern to that of serum albumin and a molecular weight of 20,000 or 44,000. It exerts a marked stimulation of mitosis on sympathetic nerve cells of both embryos and newborn chicks and mice, leading to a four- to sixfold increase in the volume of nerve tissue. The effect is dependent on time and concentration. The first indication of the effect is an increased RNA synthesis, detectable within 2 hours after exposure, and followed by an enhancement of protein and DNA synthesis. Since these early events are abolished by actinomycin D, it is postulated, as in the case of the interferon, that the nerve growth factor derepresses genes that regulate macromolecular biosynthesis. There are no data on the kinetics of its binding to isolated cells. However, the fact that this compound is normally present in nerve cells and is indeed essential for their survival, leaves little doubt that it enters the cells and acts at intracellular loci.

Another protein with different physicochemical properties has been isolated from the source that yielded the nerve growth factor. It was found to promote the growth of tissues other than nerve (*45*).

A number of other examples of cell proliferation and differentiation have been attributed to ill-defined macromolecules present in blood serum and culture medium. Full-grown monolayers of 3T3, an established line of mouse fibroblasts, temporarily lose their inhibition of mitosis and go through a burst of synthetic activity when exposed to fresh culture medium (*46*). Similar stimulations, as well as inhibitions, of cell proliferation of cultured cells have been observed in other cell systems (*47*). There is evidence that macromolecules are involved in these effects and that, in certain cases, they are transferred directly between contiguous cells (*48*). Not enough is known about them, however, and they are only mentioned to indicate that our list of metabolic effects mediated by extracellular proteins is most certainly incomplete and likely to be lengthened in the near future. It can be expected that the demonstration of causal relations between uptake and metabolic effect will be arduous when such small amounts of protein are involved. However, the examples discussed may stimulate interest in the fact that exogenous proteins can act as regulators of cell function and as signals in cell to cell communication.

In contrast to the sparse uptake of these specific agents, foreign histones penetrate into cells with ease, and investigation of the metabolic consequences of their uptake is worthwhile. Effects of large concentrations of histone (100 μg/ml and more) on isolated cells and nuclei have been reported (*49*). Our data on the membrane action and uptake of histone at concentrations of 0.1 to 3.0 μg/ml suggest that the use of lower concentrations is warranted and may yield more subtle findings.

Independently of their potential use in the study of genetic and metabolic regulations, histones and basic polyamino acids are already valuable tools by virtue of their membrane effects. They are being used to explore several aspects of membrane structure and function, such as the conformation of proteins in the plasma membrane (*17*), and the structural transitions of the nuclear membranes (*50*). In addition, with their ability to enhance the penetration of other macromolecules, they will—as in the past (*51*)—be helpful in the study of the intracellular effects of an increasing number of large-sized compounds of biological importance.

Summary

Although it is accepted on the basis of biological and morphological evidence that mammalian cells will take up macromolecules, little is known about the kinetics, the specificity, and the functions of this uptake. With labeled proteins used as models, it is found that the transport proceeds at very low rates, requires little energy, and is markedly enhanced by polybasic compounds. Molecular charge and size are important factors: cells clearly favor cationic macromolecules of large molecular weights. Neither factor, however, can fully account for the selectivity detected in the uptake of different proteins. Ingested albumin undergoes rapid and extensive degradation. This fact suggests that macromolecules have only a limited chance to express their biological activity in target cells, a finding that is relevant also to the role of foreign nucleic acids and the possibility of achieving genetic

transformation in animal cells. There are concrete indications, however, that in spite of their short half-life, proteins can act as carriers, as precursors of active agents, and as regulators of metabolic functions in host cells. They may also be important in the control of growth and differentiation. These functions of exogenous proteins are still largely unexplored.

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52. The work discussed was supported by PHS research grant GM-08482 and performed during the tenure of a Lederle Medical Faculty Award (1964–1967). I thank Dr. O. Krayer for his lasting interest in this subject and Drs. R. Z. Lockart, Jr., H. S. Ginsberg, and P. Reade for discussions.

Are "Infinity Machines" Paradoxical?

Can processes involving an infinite sequence of operations or "acts" be completed in a finite time?

Adolf Grünbaum

Let us begin with a statement of the problem.

1) Consider a hypothetical machine, to be called the "π-machine," which prints *all* the digits 3.1415926535 . . . constituting the infinite decimal representation of π in such manner that the first digit is printed in ½ minute, the second in ¼ minute, the third in ⅛ minute, and so on. We disregard here the question of whether an infinite time might not be required for the more complicated process by which this progression of digits might first have been computed seriatim, via, say, Archimedes's method of exhaustion for determining the area of a unit circle. It suffices for our purposes that a progression of digits is to be printed as described, and, if necessary, these digits may be any digits whatever, provided that there is a denumerable infinity of them. Furthermore these $\aleph_0$ numerals might all have been inserted simultaneously into the printing press in a spatial arrangement discussed below. I refer here to any such process as "the π printing." And our problem is to determine the conditions, if any, under which the π printing could be completed in 1 minute.

2) Let a hypothetical mechanical device capable of reciting the sequence of natural numbers $n = 1, 2, 3, \ldots$ depart from the leftmost point 1 and move continuously to the right through a unit interval in 1 minute to the point 0. Now focus on the progression of points $1/n$ (where $n = 1, 2, 3, \ldots$) within that interval, a progression

Dr. Grünbaum is Andrew Mellon Professor of Philosophy at the University of Pittsburgh, Pittsburgh, Pennsylvania.