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Methods for Rapidly Altering the Permeability of Mammalian Cells

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Various agents alter mammalian cells so that they rapidly become nonspecifically permeable to substances that ordinarily do not penetrate intact cells. Thus, toluene renders liver cells permeable to nucleotides and macromolecules. Tween 80 and Tween 60 act in similar fashion, and the effect is reversible. Dextran sulfate reversibly alters the permeability of Ehrlich ascites tumor cells, which offers a tool for studying the control of macromolecular syntheses and other processes. Brief exposure to external ATP alters the permeability of certain transformed mouse cells but not of untransformed cells. The effect of ATP is rapidly reversible.

Key words: permeability, detergents, ATP

The purpose of this review is to call attention to methods which have become available for altering the permeability of mammalian cells within a few minutes. We are particularly concerned with passive permeability and we are interested in agents which render the cell nonspecifically permeable to substances that ordinarily do not penetrate intact cells, such as nucleotides, actinomycin D, etc. Thus we have excluded papers which describe the stimulation or inhibition of normally occurring uptake systems by serum, hormones, or other agents. These matters have been reviewed by others (see, for example, Ref. 1). Only passing reference is made to conditions which affect the uptake of macromolecules; this has become a large and developing field (see, for example, Refs. 2 and 3). We are mainly concerned with the use of agents such as toluene, Tween 80, Tween 60, detergents, dextran sulfate, and ATP in increasing the passive permeability of animal cells. Agents whose effect is reversible are of particular interest to us because they make possible some interesting biological studies of control mechanisms. Normally impermeant cofactors can be inserted at will into living cells with certain agents to be discussed below.

TOLUENE

The bacterial literature contains many publications on the use of toluene to increase cell permeability. As an example, Moses and Richardson (4) were able to demonstrate semiconservative replication of DNA in toluenized E. coli, using labeled deoxyribonucleoside triphosphates, ATP, Mg^{2+} and K^{+}. Synthesis was temperature sensitive at the restrictive temperature in certain dna_{ts} E. coli mutants.

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Replicative synthesis showed a 20-fold stimulation by ATP. If 1% Triton X-100 was present as well, Moses observed that the cells also became permeable to macromolecules (5). The DNA repair reaction in bacteria treated in this way was inhibited by exogenous antibody to DNA polymerase I.

Toluene has been used in Deutscher's laboratory to render isolated individual liver cells permeable to charged molecules and macromolecules (6, 7). Liver cells in culture were treated with 7–9% toluene for 2 min at 0°C. These cells became permeable to t-RNA and to [^{14}C] ATP. Exogenous t-RNA and [^{14}C] ATP entered the toluenized cells and reacted with internal tRNA nucleotidyl transferase and aminoacyl-tRNA synthetase. The reaction products then left the cell. Table I shows that at least 75% of the total synthetase activity for several amino acids remained within the cell after toluene treatment, while Table II indicates that the total units of aminoacyl-tRNA synthetase found after toluene treatment of cells was somewhat greater than that found by homogenization of an equivalent weight of intact liver.

These cells could be maintained for periods of up to 1 h and they appeared to be relatively intact upon electron microscopic examination. The toluene treatment did, however, extract a considerable fraction of membranal 5′-nucleotidase and cellular phospholipid. The cells, of course, were not viable.

DETERGENTS: TWEEN 80 AND TWEEN 60

Kay (8) reported that marked alterations in cell permeability were brought about by Tween 80 in Ehrlich-Lettré ascites cells. His work is of particular interest because he demonstrated that growth of these cells was normal after the treatment and they were viable as transplants. Thus, the effect was reversible. Increased permeability was demonstrated by uptake of the dye Lissamine green. Acid soluble nucleotides decreased by 50%, and amino acids were also removed by the Tween treatment. However, while levels of glutamine, aspartic acid, glycine, glutamic acid, leucine, and tyrosine were drastically reduced, the level of cellular serine was not altered. There was a reduction of [^{14}C] formate incorporation into protein and DNA, while RNA synthesis was stimulated.

TABLE I. Location of Aminoacyl-tRNA Synthetases During Assay*

Amino acid	Supernatant	Pellet
	units/g cells	
Arginine	2.48	7.95
Lysine	2.20	5.51
Alanine	1.78	5.14
Threonine	2.62	7.50

*After toluene treatment the cells were suspended in 5 volumes of assay mixture without labeled amino acid and preincubated for 5 min at 37°C. The cells were then centrifuged at 100 × g for 2 min and the cell pellet was resuspended in 5 volumes of the same medium. Ten microliters of the cell and supernatant fractions were then assayed for aminoacyl-tRNA synthetase activities.

TABLE II. Comparison of Aminoacyl-tRNA Synthetase Activity in Cells Made Permeable by Toluene Treatment or Opened by Other Methods*

	Homogenization of intact liver		Sonication of individual liver cells		Nonidet P-40 treatment of individual liver cells		Toluene treatment of individual liver cells	
Amino acid	Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet
	units/g of liver							
Arginine	6.51	0.74	14.8	0.63	15.0	0.23	1.30	10.2
Lysine	9.08	0.13	11.3	0.19	13.3	1.60	1.21	10.32
Alanine	4.15	0.75	6.21	0.54	6.46	0.48	0.54	4.3
Threonine	7.63	0.86	11.9	0.49	12.6	0.98	1.19	10.1

*Isolated cells were suspended in 5 volumes of Buffer A containing 20% glycerol. Homogenization of intact liver (0.5 g) was done in a Dounce homogenizer using 10 strokes of a loose fitting pestle. Sonication of individual liver cells (0.5 ml) was performed using a Branson Sonifier cell disruptor at a setting of 1 for a total of 60 sec in 30-sec bursts with a 30-sec cooling period. Nonidet P-40 treatment involved incubating 0.2-ml aliquots of cells with 50 μl of a 10% Nonidet P-40 solution for 10 min in ice. Toluene treatment of individual liver cells was performed on a 0.2-ml aliquot as described earlier. All samples were centrifuged at 100 $\times$ g for 2 min, the pellets were resuspended in 0.2 ml of buffer, and both the supernatant and the pellet fractions were assayed for aminoacyl-tRNA synthetase activities.

Incorporation of $^{32}P_i$ into RNA was stimulated in both the nuclear and cytoplasmic fractions.

Kay reported an extraordinary stimulation of $^{32}P_i$ incorporation into phospholipids (two- to fourfold), in both nuclear and cytoplasmic fractions. Curiously enough, only phosphatidyl serine was labeled. He concluded from this that Tween 80 disrupts membrane structure and certain membrane components are reconstituted in the course of recovery. Tween 80 may affect other cell structures as well, since cardiolipins, which are mitochondrial constituents were completely lost as a result of the treatment with Tween 80.

In a very careful study, Malenkov et al. (9) incubated Ehrlich and hepatoma 22 cells with 1% Tween 60 in neutral phosphate buffer for 30–60 min. Tween made the cells susceptible to lysis by trypsin and they stained diffusely with neutral red instead of revealing the usual neutral red granules. When the treated cells were incubated with fluorescent albumin and washed, diffuse fluorescence of cytoplasm and nucleus was observed. The electrophoretic mobility of the cells decreased, as did their electrical resistance.

These changes were reversed within 2 hr at 37°C in the presence of a culture medium supplemented with 20% bovine serum. Thus, the treatment with Tween 60 made animal cells permeable to molecules as large as albumin, and the effect was reversible.

OTHER DETERGENTS

A number of investigators have used other surface active agents to increase the permeability of animal cells to normally impermeant molecules and ions. For example, Hodes et al. (10) found that a series of sodium alkyl sulfates and sulfonates, as well as a

series of nonionic phenoxy polyoxyethylene ethanols promoted the penetration of a dye, nigrosin, through the plasma membrane of Ehrlich ascites cells. However, these were relatively dangerous reagents. Increased dye entrance occurred at concentrations only slightly lower than those necessary to produce membrane lysis.

Seufert (11) studied the effect of anionic, cationic, nonionic, and amphoteric surface active substances on synthetic lipid bilayers. All 4 types of detergents lower the membrane resistance and develop potentials to varying degrees, at least transiently. A mechanism for channel formation as a result of detergent-lipid interaction was proposed. This is an example of many publications in which the properties of lipid bilayers have been altered by a vast number of lipid and nonlipid materials. We cannot review this literature here.

GENETIC APPROACHES COUPLED WITH THE USE OF DETERGENTS

Genetic methods have been used to alter the permeability properties of mammalian cells. This does not fall within the province of this review, which is limited to procedures that rapidly alter the permeability to small molecules. However, in some cases the genetic change has been analyzed with the help of detergents and these matters will now be discussed.

A good example is provided by work from a group in Toronto (12–14) on the isolation of mutants resistant to colchicine, a drug that inhibits mitosis. In some cases the basis for resistance was a defect in the system responsible for uptake of the drug by the cells. Such permeability mutants showed reduced ability to take up ^{3}H-labeled colchicine into intact cells with no decrease in intracellular binding activity (12). In their studies, ethylmethanesulfonate was employed as the mutagen and colchicine resistant (CH^R) lines of stable phenotype were isolated from Chinese hamster ovary (CHO) cells. Successive single-step selections were performed for increasing resistance. The CH^R lines were resistant to other drugs including actinomycin D, vinblastine, and colcemid. The degree of cross-resistance was correlated with the degree of colchicine resistance; thus, the mutation appeared to be associated with a generalized decrease in nonspecific permeability.

Ling and Thompson (13) went on to show that Tween 80 potentiated the toxicity of colchicine, both in wild type Chinese hamster ovary cells and in the mutant. This was apparently due to a four- to sixfold stimulation in the rate of colchicine uptake. A similar potentiation had previously been shown by Riehm and Biedler (14) in the case of CHO cell lines resistant to actinomycin D. It should be noted that several workers have described actinomycin resistant lines (for example, Refs. 15, 16).

In a later study, Carlsen, Till, and Ling (17) performed a careful study of the kinetics of colchicine uptake into parental cells and drug-resistant CHO mutants. They concluded that colchicine permeates the cells by an unmediated process for various reasons, including: a) No substrate saturation was observed. b) Sulfhydryl reagents did not inhibit. This is hardly diagnostic, but a good many carrier-mediated systems are known to be sensitive to sulfhydryl reagents. c) No competition was noted for colcemid, a compound of quite similar structure. (This argument also is suggestive, but not conclusive.) d) Colchicine uptake is greatly stimulated by nonionic detergents and local anesthetics.

These authors believe that the detergents and local anesthetics act by causing an increase in membrane fluidity. It is of interest that the maximal rate of colchicine uptake obtainable with Tween 80 in the colchicine-resistant mutant was only half that of the parental line. Apparently part of the alteration associated with the genetic change to colchicine resistance cannot be reversed by the detergent.

USE OF OSMOTIC SHOCK

An interesting paper by Kaltenbach (18) describes the use of a form of osmotic shock to alter the permeability of Ehrlich ascites tumor cells. His procedure was as follows: The ascites tumor was collected in cold buffered saline, and washed by centrifuging twice at 200 × g. The ascites tumor cells were modified by suspending them in distilled water for 4 min and then adding an equal volume of double strength saline to restore isotonicity. The fraction of cells "modified" by this treatment was determined by counting those that took up the dye when suspended in 0.2% nigrosin solution, which stained them black. The degree of change was controlled by varying the time of exposure to distilled water.

The modified cells were able to glycolyze glucose; however, the formation of lactic acid became dependent upon the addition of glycolytic cofactors such as ATP and NAD. Presumably these nucleotides had leaked out of the shocked cells so that it was necessary to add them as supplements. Furthermore, the treated cells were able to convert fructose diphosphate to lactic acid. The control cells were unable to accomplish this conversion because normally a permeability barrier exists for fructose diphosphate and other phosphate esters.

DEXTRAN SULFATE

Several publications have appeared from Racker's laboratory on the use of dextran sulfate in order to increase the permeability of Ehrlich ascites tumor cells (19–21). In the paper of Schnolnick et al. (19) the effect of dextran sulfate and other sulfated polysaccharides on glycolysis was investigated. All of them inhibited aerobic glycolysis, probably by an effect on the cell surface. It appeared highly unlikely that materials of such high molecular weight and charge would permeate into the cells. The inhibition of glycolysis was reversed by AMP and P_i added during the assay of glycolysis. Presumably, dextran sulfate caused leakage of nucleotides. Consistent with this, the ATP level was quite low in dextran sulfate-treated cells, but greatly increased when AMP was present during the treatment with dextran sulfate.

In a later study, McCoy et al. (20) noted that dextran sulfate caused enhanced permeability to $Rb^+$and increased staining by erythrosin B. Respiration was reduced and thus was reversed by P_i alone. In agreement with the earlier studies, restoration of glycolysis required both P_i and AMP. The damage induced by dextran sulfate was reversible, for the lesion was repaired by injection of dextran sulfate-treated Ehrlich cells into mice. The cells could also be repaired by incubating them with ascites fluid that had been heated for 7 min in a boiling water bath and then centrifuged to remove coagulated proteins. The repaired cells were able to carry out glycolysis without a supplement of AMP and were not stained by erythrosin B. The uptake of Rb^+ was also restored. Furthermore, while the dextran sulfate-treated cells showed stimulation of Ca^{2+} uptake by external ATP, this effect was abolished after repair with ascites fluid. The repair activity was composed of both dialyzable and nondialyzable factors (see below).

Exposure of Ehrlich ascites tumor cells to dextran sulfate also impaired the active transport of α-aminoisobutyric acid, and it rendered the cells permeable to sorbitol, (unpublished experiments carried out by M. Kasahara, P. C. Hinkle, and L. A. Heppel). Subsequent incubation with ascites fluid restored active transport and repaired the permeability barrier in dextran sulfate-treated cells. Ascites fluid was ineffective after dialysis or addition of ethylene glycol bis(β-aminoethylether)-N,N′-tetraacetic acid, suggesting the involvement

of Ca^{2+} ions in repair. Treatment with $CaCl_2$ and glucose under specific conditions restored the transport activity of dextran sulfate-treated cells as effectively as was the case for ascites fluid. McCoy et al. (20) were able to confirm the observation that ethylene glycol bis(β-aminoethylether)-N,N′- tetraacetic acid abolished the repair activity of ascites fluid and agreed that Ca^{2+} is one of the dialyzable components required for the repair process. However, they observed that Ca^{2+} alone was not effective, Ca^{2+} plus dialyzed ascites fluid gave partial repair, and undialyzed ascites fluid was optimal for the repair process.

A major purpose in developing methods for changing permeability to small molecules was to study the regulation of metabolic processes under conditions where general cell organization was not greatly disrupted. Accordingly, McCoy and Racker (21) examined protein synthesis in dextran sulfate-treated ascites tumor cells. They determined that a suitable K^+/Na^+ ratio was needed for incorporation of [^{14}C] valine into protein in the treated cells. This was expected in view of the known requirement for K^+ observed in cell-free systems. The process was inhibited by rotenone, which blocks mitochondrial respiration and ATP synthesis, which was another expected finding. However to overcome the rotenone inhibition both inorganic phosphate and either glucose or glucose-6-phosphate were required. This was unexpected, since there is sufficient residual phosphate present in dextran sulfate-treated cells to support respiration. The virtually complete dependency on added P_i was attributed to the accumulation of phosphorylated intermediates in the presence of glucose, which depletes residual intracellular P_i and nucleoside triphosphates.

Quercetin, an inhibitor of the Na^+, K^+-ATPase, reduced the incorporation of [^{14}C] valine into protein in intact ascites tumor cells in a high Na^+ medium, but had little or no effect in dextran sulfate-treated cells in a high K^+-low Na^+ medium. These findings suggest a relationship between protein synthesis and operation of the Na^+,K^+-ATPase. The Na^+,K^+-ATPase is held to control the intracellular concentration of P_i and adenine nucleotides.

Experiments of this sort demonstrate the usefulness of treatments that alter permeability in the analysis of biosynthetic processes.

TREATMENT OF ANIMAL CELLS WITH EXTERNAL ATP

A very substantial literature has accumulated in which various effects of exogenous ATP on animal cells are described. In most cases these effects are ascribed to changes in permeability induced by ATP. The effects are usually highly specific and are not obtained by nonspecific chelating agents nor by other nucleoside triphosphates.

Effect of External ATP on Suspensions of TA_3 Ascites Tumor Cells

Stewart, Gasic, and Hempling (22, 23) reported that when exogenous ATP was added to suspensions of TA_3 ascites tumor cells in Ca^{2+} and Mg^{2+} free media, a significant increase in cell volume could be measured, which was reversible on addition of divalent cations. The effect was temperature sensitive and highly specific for ATP. An effect was seen with 0.2 mM ATP and it saturated at about 1 mM. In a later paper, Hempling, Stewart, and Gasic (24) studied this phenomenon further and observed that 1 mM ATP caused a dramatic loss of K^+ and Na^+ down their respective concentration gradients.

These authors devised a very specific theory to explain their data. They believed that ATP produced a major change in the passive permeability of the membrane for these ions and suggested that the effect was due to a response of a contractile protein in the mem-

brane to ATP, creating a hydrophilic passage for ions. Other strains of Ehrlich cells did not show this response to ATP. They suggested that differences in the outer coat, in the mucopolysaccharide layer, might provide a barrier for ATP in some strains and not in others.

Effect of External ATP on HeLa Cells

Aiton and Lamb (25) reported that exogenous ATP caused a 15-fold increase in efflux and influx of K^+. The ATP-stimulated K^+ efflux was transient, reaching a maximum within 1–2 min and declining with a half time of about 4 min. It is interesting that only 5×10^{-6} M ATP was required to produce half maximal stimulation of both influx and efflux, whereas concentrations near 1 mM were used in studies with mouse cell lines. Removal of external K^+ reduced the ATP-stimulated efflux by 60–70%, suggesting a large K^+:K^+ exchange component. If a second application of ATP was made, the response was less marked; after 6 h it was only 10% of the initial response. However, after 24 h the cells recovered their sensitivity to ATP so that the K^+ efflux was 80% as great as the initial response.

Trams (26) observed that 6×10^{-5} M ATP in the medium caused an immediate fivefold increase in efflux of $^{42}K^+$ from NN astrocytes. At suitable doses this effect of ATP was also seen in the N-18 neuroblastoma, HeLa cells, KB cells, glioblastoma GL-26, and L-929 fibroblasts.

Effect of External ATP on Ca^{2+} Accumulation by Chick Embryo Fibroblasts

Perdue (27) reported that cultured chick embryo fibroblasts accumulate Ca^{2+} in the presence of Mg^{2+} and ATP. The effect of ATP saturated at 3 mM and the optimal concentration of Mg^{2+} was 6 mM. The stimulation of uptake was highly specific: other nucleoside triphosphates were inactive. The uptake of Ca^{2+} was inhibited by Mn^{2+}, mersalate, oligomycin, and hydroxylamine. Perdue suggested that the function of this Ca^{2+} uptake may be to control motility in ameboid-like cells.

Miscellaneous Effects of External ATP

Many other effects of external ATP have been reported and a few of these will now be mentioned. Rorive and Kleinzeller (28) found that exogenous ATP affects the water and ionic contents of the cells of kidney tubules by an interaction with the cell membrane and Ca^{2+} ions. Exogenous ATP alters cell adhesion, aggregation, and movement in fibroblasts (29, 30). It changes ionic fluxes in mast cells (31) and it inhibits (H^+) secretion in the frog gastric mucosa (32).

Chang and Cuatrecasas observed that addition of only 5–50 μM ATP to isolated fat cells inhibits insulin-stimulated glucose oxidation under conditions that measure glucose transport (33). ATP inhibits the insulin-stimulated rate of 3-O-methylglocose transport. The authors speculate that their effect involves selective membrane phosphorylation.

Interesting studies have been carried out with dog red blood cells, which undergo a rapid increase in Na^+-K^+ permeability and an alteration in physical properties when exposed to at least 0.2 mM ATP (34). The effect is reversible on washing the cells. Other nucleotides and chelators are inactive, and the effect is prevented by Ca^{2+} and Mg^{2+} ions. Another study (35) reports that ATP causes a greater than eightfold increase in Na^+influx.

It is worth mentioning that exogenous ATP has been postulated as a neurotransmitter (36).

Effect of Exogenous ATP on Untransformed and Transformed Mouse Cells

Rozengurt and Heppel (37) observed that external ATP caused a rapid and manyfold increase in the permeability of transformed cells to p-nitrophenylphosphate. The effect was specific for ATP and was not found for other nucleoside triphosphates, nor was it obtained by the use of EDTA, inorganic pyrophosphate, or other chelating agents. This great increase in permeability was observed for 3T6, SV3T3, and PY3T3 cells, but not for 3T3 cells nor for mouse embryo secondary cultures. It was rapidly reversed when the cell cultures in petri dishes were transferred to serum-free growth medium. The effect of ATP was noted in Tris-saline or HEPES-saline mixtures buffered at pH 7.7–8.2. Magnesium ions did not interfere as long as the ratio of Mg^{2+} to ATP did not greatly exceed 1:1.

In a later study (38) it was found that external ATP produced an extensive and nonspecific permeability change in transformed cells leading to a depletion of intracellular pools labeled with [^{3}H] uridine, [^{3}H] adenosine, ^{86}Rb, or [^{3}H]-2-deoxyglucose. These pools consisted of over 90% phosphate esters which ordinarily do not penetrate the membrane barrier of mouse cells. When 3T6, SV3T3, and PY3T3 cells were exposed to as little as 0.2–0.3 mM ATP in Tris-saline, HEPES-saline, or phosphate-saline for several minutes, there resulted a 20–30-fold increase in rate of efflux of the acid-soluble uridine nucleotide pool. Little or no stimulation was seen with resting or growing, untransformed 3T3 cells or with secondary cultures of mouse embryo fibroblasts. Again, the presence of Mg^{2+} was allowed as long as the ratio of Mg^{2+} to ATP did not greatly exceed 1:1. The increase in permeability produced by ATP was highly specific; it was not obtained with GTP, CTP, UTP, ADP, inorganic pyrophosphate, EDTA, and other chelating agents, cAMP, and various analogues of ATP. The effect showed a pH optimum at about 8.2 and was temperature dependent. Stimulation of efflux persisted after removal of ATP. This fact made it possible to determine that the enhanced rate of efflux was as rapid at 20°C as at 37.5°C, in contrast to the effect of temperature on ATP activation itself. This abnormal change in permeability was readily reversed, within 3 min, by incubating the cultures with serum-free growth medium or with neutral Tris-saline containing Ca^{2+} and Mg^{2+}. In complete growth medium the acid-soluble pools were restored within 1 hr, and the treatment with ATP could be repeated. When cells that had lost their acid-soluble pools were incubated once more in Dulbecco's serum-containing medium, they grew and divided at a normal rate.

Makan and Heppel, in unpublished work, have demonstrated the usefulness of this technique in studies of the control of glycolysis and of the hexose monophosphate shunt in transformed cells. The acid-soluble pools were nearly completely depleted so that glycolysis became almost totally dependent on the addition to the medium of glucose, P_i, ADP, and NAD^+ in order to obtain a rate equal to that of untreated cells. Phosphorylated intermediates, such as glucose-6-phosphate and fructose-1,6-diphosphate served as substrates for lactic acid formation in ATP-treated cultures of transformed cells but were inactive when ATP was omitted. Table III describes experiments with citrate, a well-known inhibitor of glycolysis in extracts. It has no effect on control 3T6 cells, but after treatment with ATP, citrate inhibits glycolysis. In the case of the nontransformed, 3T3 cells, citrate has no effect on glycolysis in control as well as in ATP-treated cultures. Table IV demonstrates that in ATP-treated, transformed, 3T6 cultures both NADP and glucose-6-phosphate are able to enter the cells and form NADPH. The reaction is catalyzed by intracellular glucose-6-phosphate dehydrogenase; release of enzyme into the supernatant solution was excluded.

Effect of ATP on Ca^{2+} Uptake by Ehrlich Ascites Cells

Landry and Lehninger (39) observed that extracellular ATP supported Ca^{2+} uptake by Ehrlich ascites cells. They considered the possibility that ATP is effective only because it leaks through damaged cells. However, in view of the many reported effects of ATP on intact cell preparations they also presented the alternative possibility, namely "that extracellular ATP may have two effects: one to increase membrane permeability of the cell to both Ca^{2+} and ATP, and the other to supply energy during its oligomycin-sensitive hydrolysis in the mitochondria for the transport of cytosolic Ca^{2+} into the mitochondrial matrix."

TABLE III. Effect of Citrate on Rate of Glycolysis of ATP-treated Normal and Transformed Cells*

Additions	Normal cells (3T3)		Transformed cells (3T6)	
	−ATP	ATP treated	−ATP	ATP treated
	(lactic acid formed, nmoles/ml/10 min)			
(a) glucose, P_i, ADP	104	110	370	252
(b) glucose, P_i, ADP + 5 mM citrate	116	92	308	181
(c) glucose, P_i, ADP + 10 mM citrate	98	106	349	78

*Swiss mouse 3T3 (normal) or 3T6 (transformed) cells were subcultured into 33-mm Nunc dishes in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. In the late log or early confluent phase, the cell cultures were washed and treated with 1 ml of Medium A plus or minus 0.5 mM ATP. After 10 min at 37.5°C, the medium was replaced by Medium A minus ATP but containing the appropriate additions as shown. Further incubation was carried out for 10 min at 37.5°C. The supernatant fluid was then removed and assayed for lactate. Medium A contains: Tris-HCl, 0.1 M; NaCl, 0.05 M; $CaCl_2$, 50 μM; Dextran 500, 5 mg per ml; pH (at 23°C) 8.2.

TABLE IV. ATP-Induced Permeability to Glucose-6-Phosphate and $NADP^+$ and Subsequent Effect on the Hexose Monophosphate Shunt*

	Treatment	OD_{340} of supernatant
(A)	Controls: (did not receive ATP treatment in Stage I)	
	Stage II: Buffer A + 50 mM glucose-6-phosphate + 0.4 mM $NADP^+$	0.161
(B)	Cultures permeabilized with ATP in Stage I	
	Stage II: (a) Buffer A + 50 mM glucose-6-phosphate + 0.4 mM $NADP^+$	2.581
	(b) Buffer A only	0.055
	(c) Buffer A + 50 mM glucose-6-phosphate	0.083
	(d) Buffer A + 0.4 mM $NADP^+$	0.136
(C)	Supernatant of ATP-treated cultures: Stage I only	0.031
(D)	Reagent Blank (Buffer A + 50 mM glucose-6-phosphate + 0.4 mM $NADP^+$)	0.048

*Swiss mouse 3T6 cells were subcultured into 33-mm Nunc dishes in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. In the late log or early confluent phase, the cell cultures were washed and treated with 1 ml of medium A plus 0.5 mM ATP. After 10 min at 37.5°C (Stage I) the medium was replaced by 1 ml medium A minus ATP but containing the appropriate additions as shown. Further incubation was carried out for 10 min at 37.5°C (Stage II). The supernatant fluid was then removed and the amount of NADPH that was formed was measured at 340 nm.

Resch (40) carried out similar experiments, and she prefers the first possibility, namely that ATP enters damaged cells, as mentioned by Landry and Lehninger. She also observed stimulation of Ca^{2+} uptake by ATP; CTP, UTP, and ADP were ineffective. However, she noted that the ATP-stimulated uptake, as well as basal uptake, increased with storage of the cells over one to several days. The stimulation of freshly harvested cells by ATP was often very small. The effect of aging for 1–3 days was reproduced by heating them for a short while at 30°C. In view of these observations one must be careful in interpreting the experiments just cited. Another point to keep in mind is that different laboratories have used different strains of Ehrlich ascites cells; Stewart, Gasic, and Hempling (21) reported that ATP caused a dramatic loss of K^+ and gain of Na^+ only in one out of several strains of Ehrlich cells.

Effect of External ATP on Isolated Chromaffin Granules

Pollard et al. (41) observed that isolated secretory vesicles from the adrenal medulla, known as chromaffin granules, release their contents when exposed to Ca^{2+},Mg^{2+}-ATP and high levels of chloride ion. They postulate that the mechanism involves a chloride gradient, and anion entry resulted from Mg^{2+}-ATP-mediated changes in transmembrane potential. At 37°C, this anion permeation step resulted in release of vesicle contents. The effect was specific for ATP, although AMPPNP was also active. Reagents such as SITS and pyridoxal phosphate, which block anion movement across red cell membranes, also blocked release of epinephrine and protein from these granules. They concluded that release from the granules occurred as a result of exposure to Ca^{2+}, followed by Mg^{2+}-ATP-evoked anion entry through a specific anion channel.

How Might ATP Become Available at the External Surface of Cells?

Since only small concentrations of ATP occur in the extracellular space the question arises whether any of the effects of exogenous ATP described in this review have physiological significance. Several possibilities for providing extracellular ATP have been advanced. Agren and Ronquist (42) have described the formation of extracellular ATP by tumor cells. Evidence also exists that ATP may be translocated from the cytosol to the exterior of the cell (26, 28). The existence of nucleotide pyrophosphatases (43), ectoATPase (44), and ATP requiring protein kinases (45) on the surface of animal cells almost makes it obligatory that some mechanism for providing external ATP should exist.

GENERAL CONCLUSIONS

This short review makes no pretense of completeness. Our purpose is simply to call attention to some interesting papers which are concerned with relatively rapid alterations in permeability of animal cells. There are suggestions in some instances that these rapid changes in permeability may operate physiologically as important control mechanisms. In other cases the main emphasis has been to demonstrate a technique for temporarily altering cell permeability so that small molecule pools can be depleted and impermeant molecules in any desired concentration can be inserted into the cell. In several cases the procedure for increasing permeability does not alter cell viability or subsequent rate of growth. Thus, the opportunity arises for taking a fresh look at control mechanisms for glycolysis, macromolecule syntheses, and other processes. One may compare the results of studies with reconstituted systems with investigations in which internal cell organization is disrupted as little as possible.

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