PLASMA CELL IMMUNOGLOBULIN SECRETION

Arrest is Accompanied by Alterations of the Golgi Complex*

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The biosynthesis of Ig occurs on polysomes attached to the rough endoplasmic reticulum (RER)[1] of plasma cells (1, 2) and is followed by segregation of Ig in the cisternal space of the RER (3) and transport to the smooth-surfaced elements of the Golgi complex (4). The ensuing intracellular pathway is difficult to study, because plasma cells do not concentrate or store Ig before discharge. In secretory cells which store secretory products before release, discharge is known to occur by exocytosis and to be dependent upon extracellular Ca^{++} (5), energy (6), changes in cytoplasmic cyclic nucleotide levels (7, 8), and often on the integrity of cytochalasin- and colchicine-sensitive structures (9).

The present study explores the influence on Ig secretion by plasma cells in culture of a variety of hormones, cyclic nucleotides derivatives, extracellular Ca^{++} depletion, agents acting on microtubules or microfilaments, as well as respiratory inhibitors and carboxylic ionophores (10–13) which selectively perturb the intracellular Ca^{++} or Na^+/K^+ levels. Plasma cell secretion was found to be insensitive to a number of factors which regulate secretion by cells with a storage compartment. In this sense, plasma cells are nonregulated secretory cells. On the other hand, Ig secretion is markedly inhibited by lowering the intracellular Ca^{++} level, by partial Na^+/K^+ equilibration, or by blocking the energy source. In the first two situations, the decrease in Ig secretion is accompanied by a striking alteration of the ultrastructural appearance of the Golgi complex, different in each case.

It is postulated that interference with the traffic of Ig-containing vesicles to, within, and from the Golgi complex rapidly leads to an inhibition of Ig secretion. A different site and mechanism of inhibition of vesicular traffic is proposed for each inhibitory condition.

Materials and Methods

Chemicals. L-[4.5-³H(N)]-leucine NET-135H, ⁴⁵CaCl₂ NEZ-013, protosol, Omnifluor from New England Nuclear, Boston, Mass.; ouabain from Sigma Chemical Co., St. Louis, Mo.; lipopolysac-

[1] *Abbreviations used in this paper*: cAMP, adenosine 3'-5'-cyclic phosphate; CCCP, carboxyl cyanide m-chlorophenylhydrazone; cGMP, guanosine 3'-5'-cyclic phosphate; chase medium, Dulbecco-modified Eagle's medium supplemented with 20 µg/ml bovine serum albumin and nonessential amino acids; EGTA, ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N', tetraacetic acid; ³H-Ig, Ig labeled biosynthetically with L-[4,5-³H]-leucine; RER, rough endoplasmic reticulum.

charide W (*Escherichia coli* 0127:B8) from Difco Laboratories, Detroit, Mich.; adrenaline from Merck Chemical Div., Merck & Co., Inc., Rahway, N. J.; carbamylcholine from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; 8-Br guanosine 3'-5'-cyclic phosphate from ICN Pharmaceuticals, Inc., Irvine, Calif.; dibutyryl cGMP from Plenum Sci. Co., N. Y.; RO-201724 and ionophore X-537A through the courtesy of J. Berga of Hoffmann-La Roche Inc., Nutley, N. J.; monensin, nigericin, and A 23187 through the courtesy of R. J. Hosley and R. L. Hamill of The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.; cyclic soma-tostatin from Serono Laboratories, Inc., Boston, Mass.; cytochalasin B from ICI Research Lab, Cheshire, England; dibutyryl adenosine 3'-5'-cyclic phosphate, carboxylcyanide m-chlorophen-ylhydrazone (CCCP), colchicine, bovine glucogen, histamine, bovine insulin, prostaglandin E_1, serotonin, and theophylline from Calbiochem, San Diego, Calif.; fetal calf serum and media from Gibco Diagnostics, The Mogul Corp., Chagrin Falls, Ohio; bovine albumin (fraction V) from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind.; and Nonidet P-40 from Kolb A.G., Hedingen, Switzerland. All other chemicals were of reagent grade.

Origin of Cells. The murine IgA myeloma MOPC-315 was obtained from Dr. A. Williamson and maintained in Dulbecco-modified Eagle's medium supplemented with nonessential amino acids and 20% fetal calf serum under standard conditions (14). Cells were harvested toward the end of exponential growth. Murine nonmalignant plasma cells were obtained from the spleens of mice injected intraperitoneally either with 150 μg *E. coli* lipopolysaccharide 3 days before killing, or with 2×10^4 live *Trypanosoma brucei* 1 wk before killing. In both cases, the nucleated cell suspensions obtained from the spleen contain about 10% plasma cells (predominantly IgM secreting in the first case, IgG in the second).

Incubation Procedure. Cells were labeled at 10^7 cells/ml for 1 h at 37°C under 5% CO_2-95% air in minimal Eagle's essential medium (containing only 8 nM leucine) supplemented with 1 mg/ml bovine serum albumin, 100 μCi/ml L-[4,5-^{3}H]-leucine. At the end of the labeling period, cells were sedimented, washed twice in Dulbecco-modified Eagle's medium with 20 μg/ml bovine serum albumin and nonessential amino acids (chase medium), distributed among chase tubes at 2×10^6 cells/ml and returned to 37°C. During the labeling period and chase incubations, no increase in trypan blue-positive cells was noted. Thus, there is little contribution to ^{3}H-Ig release which results from cell death, under the conditions employed. At appropriate intervals, cells were sedimented and ^{3}H-Ig was estimated in the supernates and, where appropriate, in cytoplasmic extracts. For MOPC-315, specific precipitation was accomplished by adding samples of serum of mice bearing the 315 tumor, and therefore containing the myeloma protein, and a rabbit anti-serum raised against the myeloma protein (for control precipitates, aliquots of normal mouse serum and rabbit anti-mouse albumin were added); for the nonmalignant plasma cells, specific precipitation was accomplished by successive addition of rabbit anti-mouse Ig serum (or normal rabbit serum for controls) followed by sheep anti-rabbit Ig serum, or by formalinized staphylococci of the strain Cowan I (15).

Immunoprecipitates were held overnight at 4°C, then washed three times with phosphate-buffered saline-0.1% Nonidet P-40, dissolved in protosol, and counted in the presence of Omnifluor. The difference between the amount of radioactivity in the specific and control precipitates (^{3}H-Ig) was on the order of 4:1. Data represent the average of at least two experiments, each performed in duplicate.

Results

Factors Influencing Ig Secretion Rate. The rates of release of radioactive Ig after a labeling period of 1 h are shown in Fig. 1. The chase period required for 50% release of ^{3}H-Ig is 3 h for MOPC-315 myeloma cells and about 40 min for nonmalignant plasma cells. The reason for this difference is not known, but is not related to the class of Ig synthesized, since the rate of secretion by IgG or IgM myeloma cells is often slow (16, 17) while both the IgG- and the IgM-producing nonmalignant cells secrete rapidly.

The rate of ^{3}H-Ig secretion is not influenced by a broad variety of hormones or mediators, high concentration of cyclic nucleotide derivatives, nor by the presence of colchicine, cytochalasin B, or cycloheximide (Table I). Since dibu-
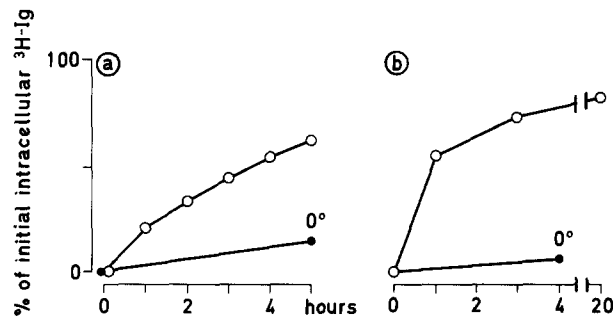
FIG. 1. Time-course of secretion. Cells were pulse-labeled for 1 h with L-[4,5-^{3}H]-leucine, washed, and returned to chase medium. At appropriate intervals samples were sedimented and the ^{3}H-Ig was estimated both in the cells and in the medium. The total amount of ^{3}H-Ig is conserved during the experiments. (a) MOPC-315; (b) nonmalignant cells.

TABLE I

Conditions Found to be without Influence on Discharge of ^{3}H-Ig

Dibutyryl cAMP, mM	Serotonin, mM
Dibutyryl cAMP + theophylline, mM	Histamine, mM
Dibutyryl cAMP + RO-201724*, 0.1 mM	Somatostatin, 10 μg/ml
Dibutyryl cAMP + prostaglandin E$_1$, 5	Cycloheximide, mM
$\times$ 10^{-5} M	Colchicine, mM
	Cytochalasin B, 5 μg/ml
Dibutyryl cGMP, mM	
8-bromo cGMP, mM	KCl‡, 25 mM
	KCl, 50 mM
Carbamylcholine, 0.1 mM	
Adrenaline, 0.1 mM	$-$Ca^{++} + EGTA, mM
Insulin, 10^{-8} M	$-$Ca^{++} + SrCl$_2$, 0.1 mM
Glucagon, 10^{-9} M	$-$Ca^{++} + MgCl$_2$, 10 mM

Labeling conditions are as for Fig. 1.

For the myeloma, discharge was measured over a 2-h chase interval. For nonmalignant cells, over a 1-h interval. Under the indicated conditions, rates of secretion were within 10% of controls. The last two conditions have been tested only for nonmalignant cells. $-$Ca stands for chase medium lacking calcium.

* A potent phosphodiesterase inhibitor (37).

‡ KCl replaces equimolar amounts of NaCl in the chase medium.

tyryl cAMP, colchicine, and cytochalasin B were observed to arrest cell division of MOPC 315 cells (data not shown), their lack of influence on Ig secretion can probably not be attributed to their failure to enter the cells. Table I also shows that ^{3}H-Ig secretion procedes at normal rates in the presence of excess KCl, in Ca^{++}-free medium and in media where calcium has been replaced by divalent cations used as calcium antagonists (5).

By contrast, Ig secretion is slowed by addition to the culture medium of any of the following carboxylic ionophores: A 23187, monensin, or nigericin, as shown in Fig. 2. The inhibitory effect of A 23187 is observed only in medium lacking Ca^{++} and containing ethyleneglycol-bis(β-aminoethyl ether)-N,N,N',N' tetraacetic acid (EGTA). Treatment of plasma cells with A 23187 in Ca^{++}-
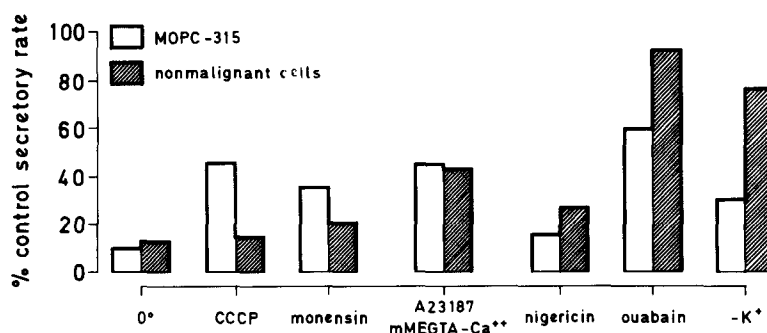
Fig. 2. Conditions which inhibit Ig secretion. The protocol is as for Table I. Doses employed are as follows: CCCP, 10 μM; monensin, μM; A 23187, 10 μM (nonmalignant cells), 0.2 μM (myeloma); nigericin, μM; ouabain, mM. 10 μM CCCP reduces L-[4,5-^{3}H]-leucine incorporation by 90% over a 1-h period. $-$K$^+$ designates chase medium lacking potassium.

containing medium was found to be toxic for normal plasma cells but without effect on the viability or rate of secretion of the myeloma. CCCP, an uncoupler of oxidative phosphorylation (18) also inhibits Ig secretion. Both normal and malignant plasma cells respond similarly, though the degree of inhibition is somewhat less with myeloma cells, as judged after a 2-h chase interval (Fig. 2). Fig. 2 includes data on two other conditions designed to cause partial intracellular Na$^+$/K$^+$ equilibration: incubation in the presence of ouabain (1 mM) or in K$^+$-free medium. Both slow ^{3}H-Ig secretion by myeloma cells, although their effect on normal plasma cells is modest or minimal in the conditions used.

From model studies, it is known that A 23187 facilitates exchange of divalent cations such as Ca^{++} for protons (11), while monensin and nigericin facilitate Na$^+$- and K$^+$-proton exchange (10, 12, 13). To evaluate the actual metabolic modifications of plasma cells treated with these ionophores, experiments were performed mostly with myeloma cells, since they represent the most homogeneous cellular population available. The results, shown in Table II, indicate that in the presence of A 23187 in a Ca^{++}-free medium, the cells rapidly lose ^{45}Ca^{++}, while their content of Na$^+$ and K$^+$ is unaffected. By contrast, after addition of monensin, there is an increase in intracellular Na$^+$ and a concomitant loss of K$^+$, while the ^{45}Ca efflux remains the same. Comparable alterations in Na$^+$ and K$^+$ levels accompany incubation of the myeloma cells with ouabain (1 mM) or in K$^+$-free medium (data not shown). Protein synthesis is unchanged in the presence of monensin, but almost completely arrested in Ca^{++}-depleted medium containing A 23187, although the respiratory rate is unaffected (Table II).

Morphological Studies. These studies were performed to determine the ultrastructure of normal and malignant plasma cells after block of Ig secretion. In some experiments, this was combined with autoradiography after a short pulse with L-[4,5-^{3}H]-leucine, in an attempt to determine the possible intracellular site of accumulation of the ^{3}H-Ig whose secretion has been prevented.

Cells treated with CCCP show no morphological changes. In contrast, both A 23187 (in calcium-depleted medium) and monensin induce major alterations of the Golgi complex.

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TABLE II

Metabolic Parameters of Ionophore-Treated Cells

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Treatment	$^{45}Ca^{++}$ Efflux $t_{1/2}$ (MOPC 315)	K^+ per cell (MOPC 315)	Na^+ per cell (MOPC 315)	L-[4,5-^{3}H]-leucine incorporation into Ig (normal plasma cells)	O_2 consumption (MOPC 315)
	min			*%*	
Control	46	100	100	100	100%
Monensin, μM	48	44	185	100	ND
A 23187, 0.2 μM $-Ca^{++}$ + EGTA, mM	5	100	100	10	107%

Rates of calcium efflux from the myeloma were established by incubating the cells for 6 h at 37°C in normal culture medium with calcium reduced to 0.1 mM and supplemented with $^{45}Ca^{++}$ at 1 μCi/ml. Cells were then rapidly washed four times at 24°C with chase medium and returned to 37°C for estimation of efflux under appropriate conditions for up to 2 h. The half-time ($t_{1/2}$) for efflux is indicated. Na^+ and K^+ were estimated after 1 h of pretreatment with ionophore by washing samples of 10^7 cells once in 0.3 M sucrose and hydrolyzing them in 10 N HNO_3 (38). The hydrolysates were diluted with 1 N HCl and analyzed with a flame photometer (Instrumentation Laboratory Inc., Lexington, Mass.). Control levels of K^+ and Na^+ were 10×10^{-11} and 6×10^{-11} meq per cell, respectively.

Measurements of protein synthesis are cumulative, starting at the moment of ionophore addition and continuing for 1 h at 37°C. Incorporation was linear with time for this period. O_2 consumption was measured after 1 h of pretreatment with ionophore, by using a Gibson Oxygraph K-1C, membrane YSI-5352 (Yellow Springs Instrument Co., Yellow Springs, Ohio). Control rates were 4 nmol O_2/min per 10^7 cells.

ND signifies not determined. $-$Ca stands for chase medium lacking calcium.

With A 23187 in the absence of Ca^{++} there is a massive accumulation of vesicles in the Golgi region, and Golgi cisternae are rare (Figs. 3b and c). Many of these vesicles have the size of normal Golgi vesicles, but others have a diameter up to four to five times greater. Transitional elements of the RER are numerous, and frequently abut on fields of the smaller vesicles. The possibility that the vesicles accumulated in the Golgi area are of endocytic origin was investigated by preincubating myeloma cells overnight in medium containing horseradish peroxidase and subsequently performing ionophore treatment in peroxidase-containing medium. Only a very minor proportion of the vesicles display peroxidase activity under these conditions.

In contrast, monensin-treated cells show very large, smooth-surfaced vacuoles in the Golgi region (Figs. 3e and g). These vacuoles often have an irregular perimeter suggesting that they may arise by fusion of smaller smooth-surfaced vacuoles. They usually contain a flocculent precipitate which may adhere to the inner aspect of their limiting membrane, and occasionally form a dense granule. Flattened Golgi cisternae are lacking, but transitional elements of the RER and a few Golgi vesicles are present. The mitochondria of these cells have a condensed appearance. Similar dilated vacuoles are also found after treatment of the myeloma cells with inhibitory doses of nigericin or ouabain (Fig. 3f).
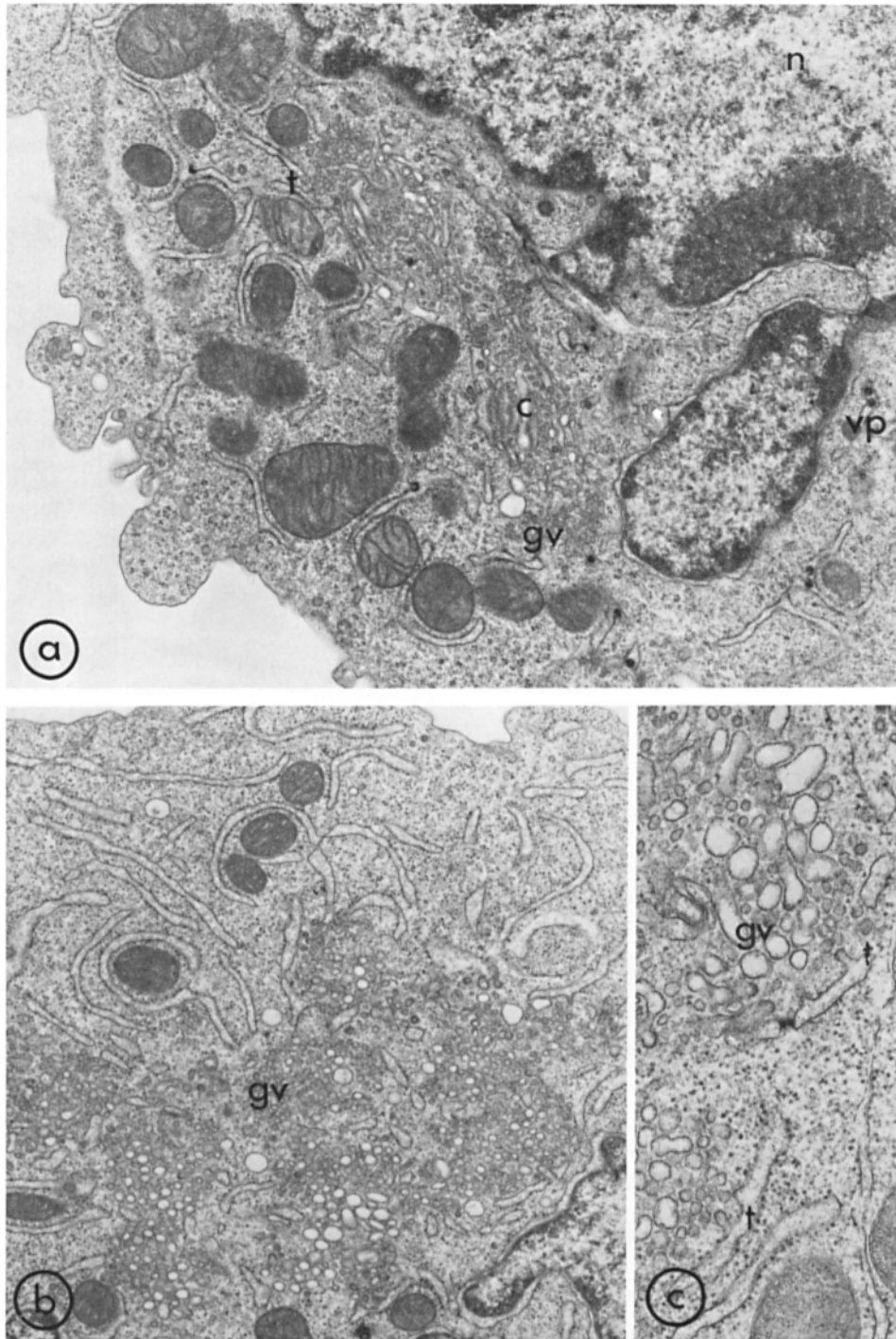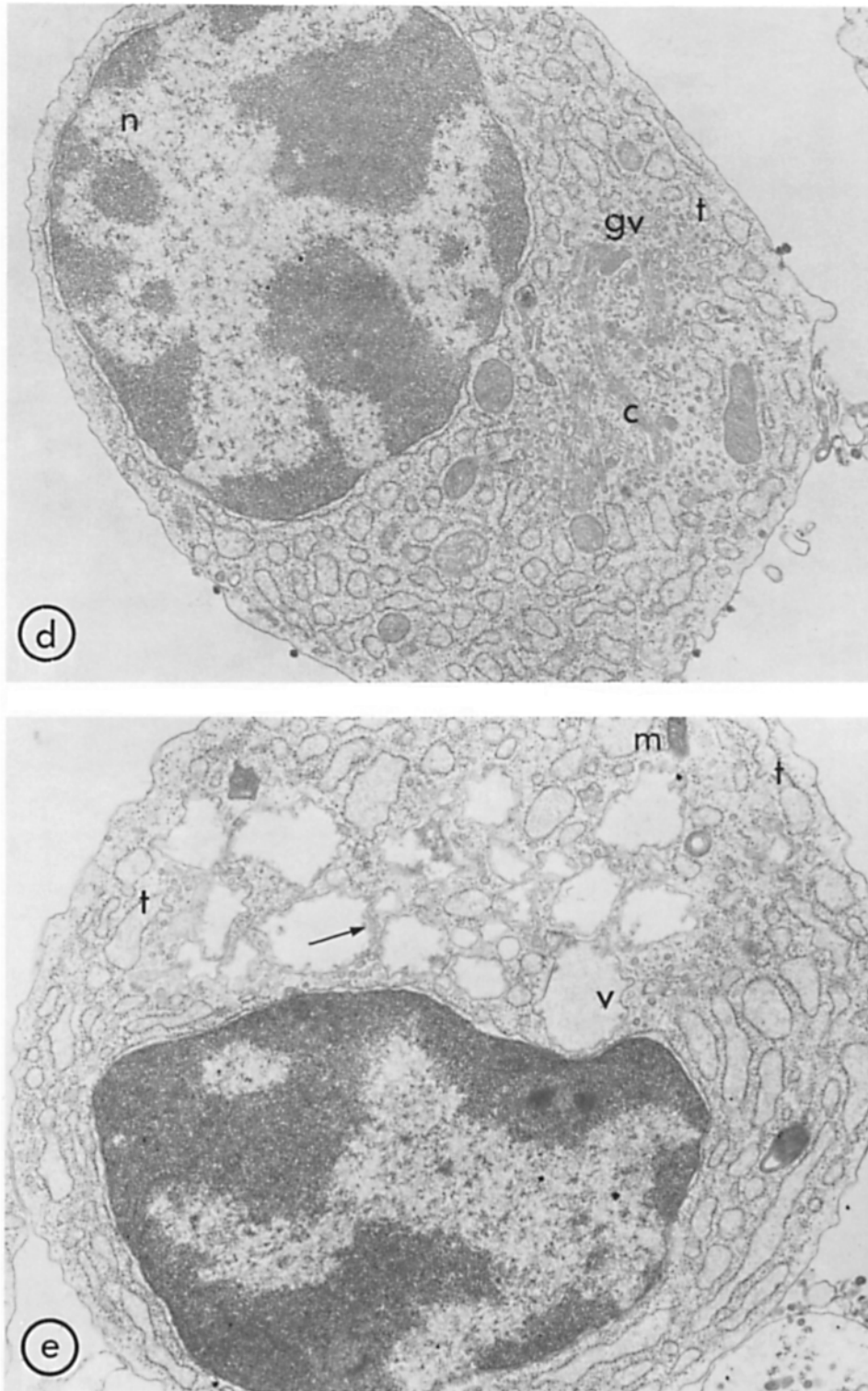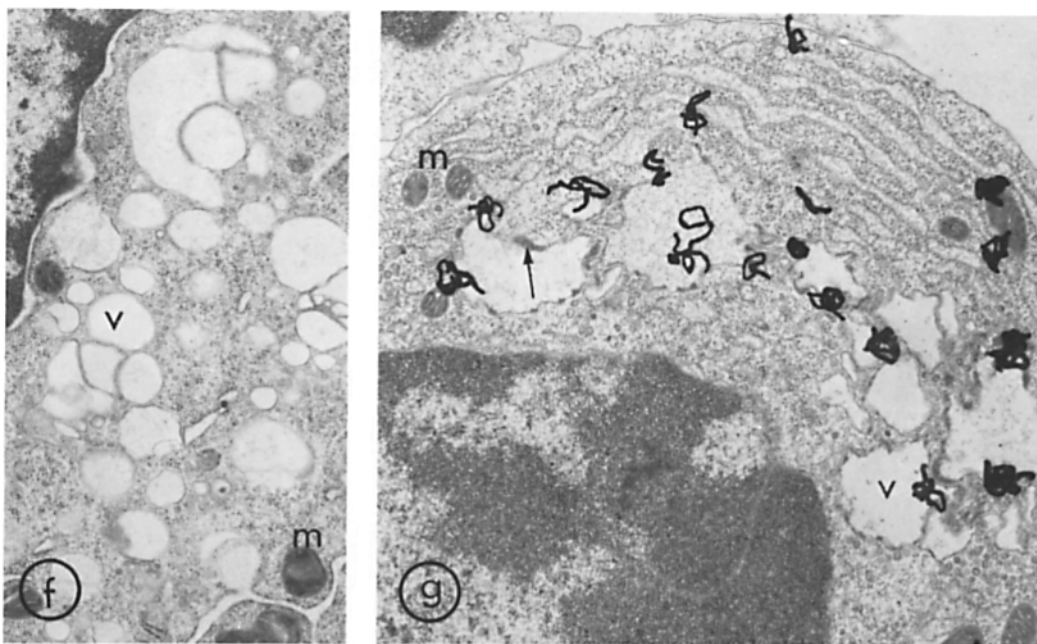
Fig. 3. A–C

FIG. 3. D-E

FIG. 3. Electron micrographs of thin sections of malignant (a, b, c, f) and nonmalignant (d, e, g) plasma cells. (a, d) Control cells, note relatively compressed Golgi cisternae (c), abundant small smooth-surfaced vesicles (gv) and transitional elements (t) of the RER; (b, c) cells incubated 90 min in medium lacking Ca^{++} and supplemented with mM EGTA and A 23187 at 0.2 μM. Note the abundance of smooth-surfaced vesicles (gv), both of normal dimensions and larger; (e, g) cells incubated 90 min in medium supplemented with μM monensin. Note large smooth-surfaced vacuoles (v) containing a flocculent precipitate, often adherent to the inner aspect of the limiting membrane (arrows) and condensed mitochondria (m); (f) cells incubated 90 min in the presence of mM ouabain. Other abbreviations: nucleus (n), viral particles (vp). The morphological alterations of the myeloma treated with monensin and nonmalignant cells treated with A 23187 and EGTA in the absence of Ca^{++} resemble (e) and (b), respectively. All cells were fixed 30 min at 0°C in 1% OsO_4, 0.1 M pH 7.3 Na cacodylate. The tissue was further processed by standard procedures and photographed with a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mount Vernon, N. Y.). In the case of (g), the cells are taken from an autoradiographic experiment, as explained in Table III. The illustration corresponds to a 90-min chase time-point. An Ilford IL-4 emulsion was employed (Ilford Ltd., Ilford, England).

For nonmalignant cells, nigericin produced a similar dilation, but ouabain was without effect.

For autoradiographic studies, nonmalignant spleen cells were pulse-labeled for 5 min, then washed in chase medium and either fixed at once or chased 90–120 min at 37°C under control or inhibitory conditions. The distribution of grains is given in Table III and an example is shown in Fig 3 g. At the end of the pulse there is predominant labeling of the RER with very few grains over the Golgi complex. As expected, after the control chase, there is a marked relative increase over the Golgi complex. In the ionophore-treated cells, this relative increase in labeling of the Golgi complex is also found, and, in the case of monensin, is accentuated, suggesting that ^{3}H-Ig is retained in the altered Golgi complex. By contrast, there is little transfer of grains to the Golgi complex in cells incubated with CCCP during the chase interval.

TABLE III

Autoradiography of Nonmalignant Plasma Cells

Incubation conditions	Ratio of grains over Golgi complex to grains over the RER and Golgi complex
	%
Exp. 1	
1 5 min pulse	10
2 5 min pulse + 90 min control chase	33
3 5 min pulse + 90 min chase μM monensin	50
4 5 min pulse + 90 min chase 10 μM A 23187 $-Ca^{++}$, mM EGTA	36
Exp. 2	
1 5 min pulse	13
2 5 min pulse + 120 min control chase	32
3 5 min pulse + 120 min chase 10 μM A 23187 $-Ca^{++}$, mM EGTA	36
Exp. 3	
1 5 min pulse	10
2 5 min pulse + 120 min control chase	36
3 5 min pulse + 120 min chase 10 μM CCCP	17

Cells were pulse-labeled 5 min at 10^8 cell/ml at 37°C, 2 Ci/ml L-[4,5-^{3}H]-leucine, washed in chase medium, and reincubated (nos. 2-4) at 5 × 10^6 cell/ml, 37°C. Fixation was in 1% OsO_4, 0.1M pH 7.3 Na cacodylate for 30 min at 0°C. Photographs were taken of plasma cells with a visible Golgi complex and autoradiographic grains. At least 200 grains were counted for each condition. A circle was drawn circumscribing each grain and the grain was assigned to the subcellular structure underlying the center of the circle. On the average, 22% of the grains were nuclear, and of the remaining 78%, 83% were assigned to either the RER or the Golgi complex. A typical photo from exp. 1 condition 3 is given as Fig. 3 g. $-Ca$ stands for chase medium lacking calcium.

Discussion

There are only a few studies on the physiology of Ig secretion. Ig discharge has been reported to be acutely sensitive to respiratory inhibitors (19) and temperature reduction (20), but unaffected by inhibition of protein synthesis (16) or high doses of colchicine or cytochalasin B (21). The present observations are in agreement with these data, and show in addition that secretion rate is not influenced by cyclic nucleotide derivatives or agents thought to alter the cellular level of cAMP and cGMP, by the absence of extracellular Ca^{++}, or by increase in extracellular K^+. Since these agents participate in the triggering of discharge in a variety of secretory cells under regulatory control, the present data support the notion that there is little or no short-term physiological regulation of the discharge rate of Ig by plasma cells. The present data contradict the reports that Ig secretion can be slowed by histamine, catecholamines, and prostaglandins (22), or by colchicine (23). It should be pointed out, however, that these studies used an entirely different assay, the plaque-forming test, which cannot be taken as a direct measure of the Ig secretion rate.

The present data are consistent with the hypothesis that Ig secretion is mediated by a constant traffic of smooth vesicles to the cell surface, where Ig is discharged by exocytosis. Thus, the inhibitory effects of ionophore treatments are accompanied by striking alterations of the Golgi complex, which are best ascribed to disturbance of an ongoing vesicular traffic of Ig between the RER, the Golgi complex, and the plasma membrane. Since each condition leading to a block of Ig secretion is associated with a different cell morphology, these treatments may shed light on the underlying events of membrane fission and fusion involved in vesicular traffic.

With A 23187 in the absence of Ca^{++}, the much enlarged pool of smooth vesicles suggests that the site of the block in the secretory pathway is at the level of the Golgi complex; an hypothesis which is consistent with the pulse-chase autoradiographic data. Since extracellular Ca must be absent to observe this inhibition of Ig secretion, the effect of this ionophore can be best attributed to the observed Ca^{++} depletion (Table II), rather than to magnesium flux or to the mere presence of the lipophilic ionophore in cellular membranes. Intracellular Ca^{++} is known to be involved in a broad variety of cellular events, some of which can be ruled out as primary mechanisms in the observed block in Ig secretion. For example; (a) mitochondrial function is likely not to be grossly altered in the conditions used, since cell respiration remains normal (24); (b) protein synthesis is blocked by A 23187, as previously reported (25), but it is known that inhibition of protein synthesis does not interfere with Ig secretion (16); (c) Ca^{++} plays an important role in the extent of polymerization of tubulin (26), however, the insensitivity of Ig secretion to high doses of colchicine makes an interference with microtubule function an unlikely explanation of the observed effect. Finally, there is considerable evidence that Ca^{++} plays a role in membrane stability and fusion (27). However, the block in Ig secretion cannot be attributed simply to a lack of fusion of Golgi vesicles with the plasma membrane since the smooth vesicles accumulate in the Golgi area and not at the periphery of the cells.

Judging from the persistence of transitional elements of the RER and the autoradiographic data, membrane fission continues at the RER-Golgi junction, generating an abundance of smooth-surfaced ^{3}H-Ig-containing vesicles. These observations lead to the following hypothesis to explain the block in secretion, which appears to result from Ca^{++} depletion: vesicles formed from the RER transitional elements and containing Ig neither fuse to contribute to Golgi cisternae (as is the case in many secretory cells [28]), nor migrate to the cell membrane, but progressively accumulate in the Golgi area.

With monensin, the ultrastructural lesions observed indicate that a different block in the intracellular vesicular traffic occurs. Transitional elements are present, and the autoradiographic data indicate that the initial event of membrane fission from the RER occurs. The morphologic and autoradiographic observations suggest that the block in Ig secretion results from an excessive fusion of the derived Golgi vesicles, either with each other or with pre-existing cisternae, thus generating the dilated vacuoles which constitute a storage compartment for the Ig. The large size of these vacuoles suggests that little membrane removal by fission ensues. Moreover, these vacuoles appear unable

to fuse with the plasma membrane. Why this exaggerated fusion of Golgi vesicles occurs in monensin-treated cells is a matter of speculation. The importance of an alteration of the intracellular Na^+/K^+ ratio is suggested by the similar effect of monensin and nigericin. Moreover, this effect is also observed, at least with myeloma cells, with two other entirely different conditions which also alter the Na^+/K^+ ratio, namely incubation of cells in the presence of ouabain or in K^+-free medium. It is of interest to note that a similar vacuolization of the Golgi complex has been observed in ouabain-treated neurons (29). The pertinent consequences of a perturbation of the intracellular Na^+/K^+ ratio are not known, but this ratio is known to be of crucial importance in some regulated secretory cells: elevation of extracellular K^+ and consequent membrane depolarization can be used to trigger discharge (30–32), i.e., to promote fusion of secretory granules with the plasma membrane.

Another carboxylic ionophore, X-537A, has been found to slow Ig secretion and to produce large vacuoles in plasma cells (A. M. Tartakoff, unpublished observation). X-537A has also been shown to induce lesions of the Golgi complex in a variety of other cells (33–35). However, this ionophore interacts with both monovalent and divalent cations (10, 11), and thus its effect is likely to be more complex.

In contrast to the inhibition of Ig secretion discussed above which appears to result from ionic perturbation, the secretion block observed in the presence of CCCP, an uncoupler of oxidative phosphorylation, is not accompanied by striking ultrastructural alterations. Under these conditions of energy depletion, multiple events of membrane fission and fusion may be blocked. For example, cellular metabolism is known to be essential for intracellular transport from the RER to the Golgi complex and for exocytosis in some regulated secretory cells (36). The autoradiographic data shown in Table III strongly supports the hypothesis that the principal effect of CCCP may correspond to a pre-Golgi block.

The observations made in the present report with plasma cells seem to be of general significance, since parallel observations have been made with cultures of chick tendon fibroblasts secreting collagen, and, to a certain extent, with activated mouse macrophages secreting various enzymes (A. M. Tartakoff and P. Vassalli, unpublished observation). Neither of these cell populations is known to be subject to rapid short-term regulation of secretory rate.

In this sense, it is possible to contrast (a) regulated secretory cells possessing a storage compartment whose release can be either triggered or inhibited by agents which promote or inhibit the fusion of storage granules with the plasma membrane and (b) nonregulated secretory cells, whose secretion is mediated by a constant traffic of small vesicles from the Golgi complex to the cell membrane, and can be inhibited by interfering with this traffic. The cytoplasmic-ionic perturbations observed in the present study to inhibit vesicular traffic in plasma cells might also be used to investigate the role of such traffic in the early stages of intracellular transport of secretory proteins in the case of regulated secretory cells.

Summary

Conditions influencing Ig secretion by plasma cells have been studied with suspensions of murine plasma cells and myeloma cells by determining the

release of ^{3}H-Ig after a pulse of biosynthetic labeling with L-[4,5-^{3}H]-leucine. Ig secretion is insensitive to a variety of hormones, mediators, cyclic nucleotide derivatives, extracellular calcium depletion, and agents acting on microtubules or microfilaments; i.e., to a number of factors which are involved in the regulation of secretion by cells with a storage compartment. On the other hand, Ig secretion is markedly inhibited by conditions which (a) lower intracellular calcium levels (ionophore A 23187 in Ca^{++}-free medium), (b) induce partial sodium/potassium equilibration (the ionophores monensin and nigericin and, in the case of myeloma cells, ouabain and incubation in K^+-free medium) or (c) uncouple oxidative phosphorylation. The first two situations are accompanied by striking alterations of the ultrastructural appearance of the Golgi complex, different in each case. These ultrastructural observations, together with autoradiographic experiments after a short pulse with L-[4,5-^{3}H]-leucine, have led to the following hypothesis: (a) under Ca^{++} depletion ^{3}H-Ig passes to Golgi vesicles but these vesicles are incapable of fusion or migration and therefore accumulate in exaggerated numbers in the Golgi area; (b) under partial Na^+/K^+ equilibration, ^{3}H-Ig passes to Golgi vesicles which have an exaggerated tendency to fuse with other Golgi elements, thereby generating large vacuoles which store increasing amounts of Ig; (c) under energy block, multiple membrane fission and fusion events are inhibited and there is therefore, little intracellular transport of ^{3}H-Ig or alteration of cell ultrastructure.

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