generation inhibitor" found in guinea pig serum by Okada *et al.* (*11*). This inhibitor, which is believed to form a complex with cell-bound C3b and inhibit the generation of the C5 binding site, elutes from Sephadex G-200 in a position similar to A·C3bINA. It does not block the immune adherence activity of C3b; we have some data to indicate that A·C3bINA also does not block immune adherence.

A·C3bINA is the third plasma protein described which forms complexes with C3b, the others being properdin and factor B (*12*). The binding of the last two leads to activation or stabilization of the alternative pathway convertase. Binding of A·C3bINA prevents the participation of C3b in both the alternative and classical pathways.

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1. The components of complement are abbreviated as C1, C2, C3, . . . C9. Fragments of components are represented by suffixes with small letters, such as C3a, C3b, C3c, and C3d. The activated form of a component is indicated by a bar over the symbol; thus $\overline{C42}$ indicates an active complex of C4 and C2 and is equivalent to the classical pathway convertase. The corresponding enzyme for the alternative pathway formed from C3b, factor B, and properdin (P) is written $\overline{C3bBP}$. Hemolytic intermediates are formed on sheep erythrocytes (E) coated with rabbit antibody (A), for example, $EAC\overline{42}$.
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Sialic Acid: Effect of Removal on Calcium Exchangeability of Cultured Heart Cells

Abstract. *Specific removal of sialic acid from cultured heart cells with purified neuraminidase increases cellular calcium exchangeability. Potassium exchange is unaffected or slightly decreased. Sialic acid removal also permits lanthanum, normally restricted to the cellular surface, to enter the cells and displace more than 80 percent of cellular calcium. The results indicate a specific role of cellular surface components in the control of calcium exchangeability in the heart.*

The surfaces of most animal cells are negatively charged. A major source of this negativity in cells from diverse tissues is the ionized carboxyl groups of sialic acid (*1*). The sialic acids occupy peripheral, terminal positions in the oligosaccharide portions of the glycoproteins and glycolipids that make up a large component of the coat material of the cell surface (*2*). Sialic acid accounts for a considerable component of cation binding on the cell surface with a particular affinity for calcium as demonstrated for red blood cell membrane (*3*).

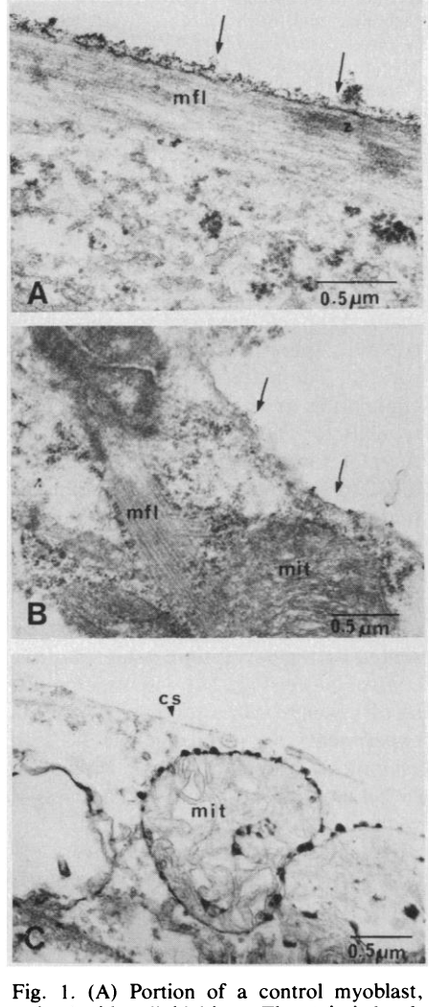

Fig. 1. (A) Portion of a control myoblast, stained with colloidal iron. The stain is localized to the cell surface (arrows); *mfl*, myofilaments; *Z*, Z-line material (× 30,000). (B) Portion of myoblast treated with neuraminidase, then stained with colloidal iron. There is an almost complete lack of staining of cell surface (arrows); *mfl*, myofilaments; *mit*, mitochondria (× 26,000). (C) Portion of a myoblast treated with neuraminidase and then stained with La. Note the intracellular accumulation of La around the mitochondria (*mit*) outer membrane; *cs*, cell surface (× 24,000).

The ability of the trivalent cation lanthanum (La^{3+}) to bind at the surface of cultured heart cells, displace significant quantities of calcium (Ca^{2+}), and inhibit Ca^{2+} exchange (*4*) suggested that negatively charged molecules, specifically sialic acid, might be of importance in the regulation of Ca^{2+} permeability in the cells.

Colloidal iron applied at *p*H 1.7 is a specific stain for sialic acid (*5*); it was used to show the distribution of sialic acid at the cell surface (Fig. 1A) by electron microscopic techniques (*4*). The specificity of the technique was demonstrated by the almost complete elimination of the stain density (Fig. 1B) when the cells were exposed to a purified neuraminidase preparation (*6*). At concentrations applied to the cells the enzyme was assessed for protease activity with ^{125}I-labeled albumin as substrate. No proteolytic activity was detectable; phospholipase activity of the enzyme preparation was measured by incubating the neuraminidase for 90 minutes with [^{14}C]phosphatidylcholine as substrate. The extent of hydrolysis was compared to that obtained with phospholipase C, and the neuraminidase was found to contain the equivalent of 0.0025 unit of phospholipase activity per milliliter. Cultured heart cells (from newborn rats) were then incubated with 0.0025 unit of phospholipase per milliliter under conditions identical to those used with neuraminidase incubation. No effect on beating or on ^{45}Ca exchangeability (see below) of the cells was produced.

The ^{45}Ca exchange of the cultured cells was continuously monitored with the scintillation-disk flow cell technique (*4*), which depends on the growth of a cellular monolayer on one surface of each of two disks composed of polystyrene combined with scintillation material (*7*). The cells were cultured by a standard trypsin digestion technique (*8*). The proportion of myoblasts was increased by in-

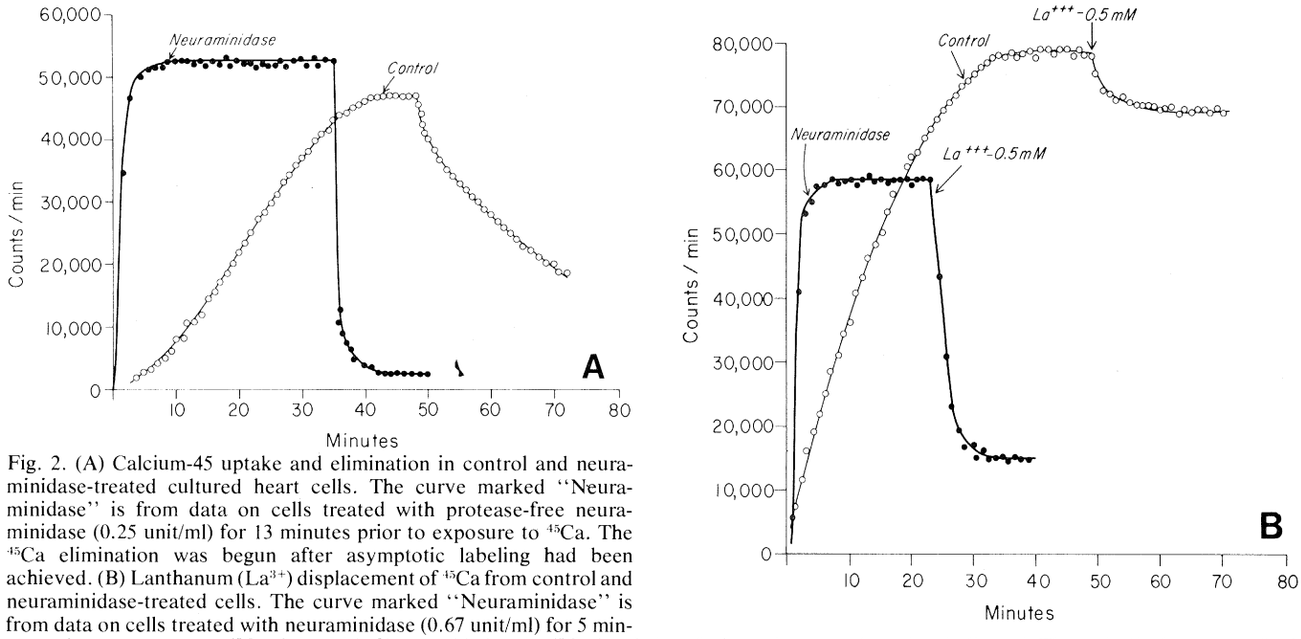

Fig. 2. (A) Calcium-45 uptake and elimination in control and neuraminidase-treated cultured heart cells. The curve marked "Neuraminidase" is from data on cells treated with protease-free neuraminidase (0.25 unit/ml) for 13 minutes prior to exposure to ^{45}Ca. The ^{45}Ca elimination was begun after asymptotic labeling had been achieved. (B) Lanthanum (La^{3+}) displacement of ^{45}Ca from control and neuraminidase-treated cells. The curve marked "Neuraminidase" is from data on cells treated with neuraminidase (0.67 unit/ml) for 5 minutes prior to exposure to ^{45}Ca; 0.5 m*M* La^{3+} was added to the ^{45}Ca-labeled perfusion solution at the points indicated. The La^{3+} displacement of calcium was increased by neuraminidase treatment.

cubation of the freshly dissociated cells in a petri dish for 2 to 3 hours. During this time the mesenchymal cells settled and attached, but the myoblasts remained floating (*9*), so that they could then be poured over the disks. At 3 days, the cells were 80 to 90 percent myoblastic, as defined by electron microscopic examination, and were confluent and beating synchronously. The scintillation disks form the walls of a flow cell with the surfaces to which the cells are attached directed inward in contact with the perfusate flowing through the flow cell. The perfusate was of the following composition (m*M*): NaCl, 133; KCl, 3.6; $CaCl_2$, 1.0; $MgCl_2$, 0.3; glucose, 16.0; and Hepes buffer, 3.0 (*p*H, 7.1). For isotopic studies ^{45}Ca (*10*) was added at 1.5 μc/ml.

The cell was inserted into the well of a spectrometer (*11*). The ^{45}Ca activity of the cell layer was determined by scintillation counting with an efficiency of 73 percent; the ^{45}Ca from the perfusate was quenched and the efficiency of the counting less than 5 percent. Therefore, the attachment of the cells to the isotopic detector (scintillator plastic) permitted continuous counting of ^{45}Ca uptake and elimination from the cell layer. The contribution of noncellular ^{45}Ca activity, that is, from the flow cell and disk surface, was measured with blank disks in the flow cell after each experiment.

Figure 2A shows the pattern of ^{45}Ca uptake and elimination from two cultures that had been treated identically, except that the "neuraminidase" curve represents data from cells exposed to the enzyme at a concentration of 0.25 unit/ml for 13 minutes prior to exposure to ^{45}Ca-labeled perfusate. The counts were normalized for cell mass and specific activity and, therefore, are directly comparable. The pattern of ^{45}Ca uptake and elimination as shown for the control is typical for cultured heart cells (*4*). Asymptotic ^{45}Ca activity was reached after 40 to 50 minutes of labeling. Elimination of ^{45}Ca was biphasic with a rapid component (the half-time being approximately 1.2 minutes) and a slower component (with a half-time of about 20 minutes) (*4*). The exchangeable Ca^{2+} represented by the asymptotic value from the control curve (Fig. 2A) was 16.3 mmole/kg (dry weight). In a series of 24 control experiments the exchangeable Ca^{2+} at asymptotic labeling was 16.2 ± 1.4 (S.E.) mmole/kg (dry cells). The ratio of wet to dry weight was 5.85.

By contrast, when the cells were exposed to neuraminidase, the asymptotic values were reached within 8 to 10 minutes or four to five times more rapidly than control. The exchangeable Ca^{2+} represented by the asymptotic value was 18.2 mmole/kg (dry weight) and was 18.5 ± 1.4 mmole in 18 cultures treated with neuraminidase. This content was not significantly different ($P > .25$) from that of the control (16.2 ± 1.4). The increase in Ca^{2+} exchangeability after exposure to the enzyme is emphasized by the fact that 96 percent of ^{45}Ca was eliminated within 8 minutes.

The effect of neuraminidase on Ca^{2+} exchangeability is different from its effect on potassium (K^+) exchange. In four control cultures labeled with ^{42}K and washed out, the K^+ exchange was monoexponential with an average exchange constant of 0.059 min^{-1} (range, 0.053 to 0.068). In two cultures treated with neuraminidase (0.25 unit/ml) prior to ^{42}K labeling and elimination (as described for the Ca^{2+} exchange experiments) the monoexponential exchange constants were 0.036 and 0.066 min^{-1}, an indication of the contrasting effect of sialic acid removal on Ca^{2+} and K^+ exchange. The Ca^{2+} exchange is markedly increased whereas K^+ exchange is unchanged or, possibly, somewhat decreased. The effect of neuraminidase treatment on K^+ exchange in the heart cells is in agreement with the reported effect on leukemic cells (*12*) and Ehrlich ascites cells (*13*) where K^+ exchange was slightly decreased. Therefore, sialic acid removal, under the conditions of our study, did not disrupt cellular permeability in a nonspecific manner, nor did it produce cell death. Cultures were exposed to neuraminidase in the standard manner, the enzyme was removed by extensive washing, and the cells were incubated again in standard media. After 48 hours the cells appeared retracted, and Ca^{2+} exchangeability remained increased; nevertheless the cells were beating asynchronously.

The average sialic acid content (*14*) of cells from two typical cultures was 80.8×10^{-9} mole per milligram of cell protein. Exposure of these cells to neura-

minidase (0.25 unit/ml) for 15 minutes caused the release of 49.3×10^{-9} mole/mg into the supernatant or 61 percent of the total cellular sialic acid content as compared to the insignificant amounts of free sialic acid released from cells exposed to the perfusate solution alone.

Thus, removal of sialic acid from heart cells increased their Ca^{2+} exchangeability. Previous work (*4, 15*) showed that in intact cells La^{3+} does not penetrate intracellularly and displaced only surface-bound Ca^{2+}. The effect of La^{3+} on ^{45}Ca exchange in control and neuraminidase-treated cells is shown in Fig. 2B. Again, the ^{45}Ca exchange from two cultures is shown, normalized for cell mass and ^{45}Ca specific activity. The control achieved asymptotic ^{45}Ca activity at 40 to 45 minutes representing 21.1 mmole of Ca^{2+} per kilogram (dry cells). At 50 minutes 0.5 m*M* $LaCl_3$ was added to the perfusate. This produced a displacement of 11.5 percent (2.4 mmole) of the labeled Ca^{2+} within 10 minutes. In ten control experiments, 0.5 m*M* La^{3+} displaced 1.84 ± 0.13 mmole of Ca^{2+} per kilogram or 11.4 percent of labeled Ca^{2+}. A culture treated with neuraminidase (0.67 unit/ml) for 5 minutes before ^{45}Ca labeling (Fig. 2B) showed asymptotic labeling at 7 minutes, representing 15.8 mmole of Ca per kilogram (dry weight). The addition of 0.5 m*M* $LaCl_3$ at 23 minutes produced a displacement of 74.1 percent (11.7 mmole) of the labeled Ca^{2+} over the course of 10 minutes. In a series of 11 cultures first treated with neuraminidase, 0.5 m*M* La^{3+} caused the displacement of 83 percent of the labeled Ca or 15.6 ± 1.27 mmole/kg (dry weight). This displacement compared to control (1.84 mmole or 11.4 percent) is significant ($P <<< .001$).

The increased ability of La^{3+} to displace Ca^{2+} indicated that removal of sialic acid from the cellular surface permitted La^{3+} to freely enter the cell and displace intracellular Ca^{2+}. A heart cell culture was exposed for 15 minutes to neuraminidase (0.25 unit/ml) and then to a solution containing 0.5 m*M* $LaCl_3$ for 10 minutes (Fig. 1C). The cell surface shows only sparse La deposits, but the interior of the cell, especially the mitochondria, shows heavy La deposits.

Our results indicate an important role for the complex of surface coat and external lamina in the regulation of Ca exchange in cultured heart cells. More specifically, the presence of the C_9 amino sugar sialic acid is required to control Ca^{2+} flux and to inhibit the entry of normally excluded cations such as La^{3+}. Sialic acid removal, on the other hand, does not increase the exchangeability of the major intracellular cation, K^+. This suggests a specific role for cell surface components in the control of ionic exchange in the heart.

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Cortisol Stimulation of Parathyroid Hormone Secretion by Rat Parathyroid Glands in Organ Culture

Abstract. *Addition of cortisol (10^{-6} to 10^{-8}M) and related glucocorticoid congeners to cultures of rat parathyroid glands stimulated dose-related increases in parathyroid hormone secretion; the addition of deoxycorticosterone or cortexolone was without effect. Cortexolone, however, inhibited the stimulatory activity of cortisol when both were added to the culture medium. This direct stimulatory effect of cortisol on parathyroid gland secretion may account in part for the increased concentration of parathyroid hormone in the serum of cortisol-treated animals.*

Cortisol and other active glucocorticoid congeners can produce hypocalcemia in parathyroidectomized animals but not in intact animals. This suggests that an interaction occurs between adrenocorticosteroid and parathyroid hormone (PTH) in calcium homeostasis (*1*). In recent studies, the concentration of immunoreactive PTH in the serum increased significantly when cortisol was administered to intact animals, including man, while the amount of calcium remained unchanged (*2*). The increase in PTH secretion has been attributed to an

Table 1. Effect of different adrenocorticosteroid congeners on PTH secretion by rat parathyroid gland (PTG) cultured in thyroparathyroidectomized rat serum (5 percent) in Eagle's basal medium containing 1.25 m*M* calcium and 1.0 m*M* magnesium. The ratio *T/C* is for the rate of PTH secretion in a 24- or 48-hour treatment period (*T*) over the rate of secretion in the 24-hour control period prior to the steroid treatment (*C*) for each culture dish. Values are means ± S.E. for the number of culture dishes (*N*) in each steroid-treated group. Control cultures received no steroids throughout the 72 hours; each experiment included three or four such control cultures.

Steroid and concentration (*M*)	Time period (hours)			
	24		48	
	N	*T/C*	*N*	*T/C*
Control cultures	15	1.46 ± 0.17	13	1.29 ± 0.18
Cortisol (10^{-8})	4	2.69 ± 0.95	4	2.20 ± 0.81
Cortisol (10^{-7})	11	4.83 ± 0.69*	9	4.68 ± 0.90*
Cortisol (10^{-6})	7	5.80 ± 0.84*	7	8.05 ± 1.35*
Dexamethasone (10^{-8})	4	5.20 ± 0.67*	4	5.16 ± 1.13*
Corticosterone (10^{-6})	4	4.68 ± 0.75*	4	4.80 ± 0.92*
Deoxycorticosterone (10^{-6})	4	1.62 ± 0.53	4	1.06 ± 0.21
Cortexolone (10^{-6})	7	2.06 ± 0.24	7	1.99 ± 0.31
Cortisol (10^{-7}) plus cortexolone (10^{-6})	4	1.80 ± 0.48†	4	1.14 ± 0.57†

*Significantly different from control group for the same period, $P < .01$. † Significantly different from cortisol (10^{-7}*M*) group, $P < .05$.