cies. At stations 5 and 6 the deficiencies were especially pronounced, reaching values nearly 10 dpm per 100 liters lower than parent ^{226}Ra activities. Radon deficiencies at stations 7 and 8 were measured after the main front had passed and were smaller, although considerably deeper, than at stations 5 and 6.

Radon depletions found on the west-central Florida shelf resembled or exceeded the most intense radon degassing reported by the GEOSECS expeditions (*13*). At 29 GEOSECS stations in the Atlantic and Pacific, the maximum depth of radon depletion was 50 to 90 m; the west-central Florida shelf had depletions at depths of 50 to 95 m. However, maximum GEOSECS radon deficiencies were about −6 dpm per 100 liters, whereas the west-central Florida shelf showed substantially greater radon depletions. But the percentage depletions of ^{222}Rn below equilibrium with its parent ^{226}Ra were reasonably similar for the two data sets. Using values from Table 2, we found that the maximum percentage depletions at stations 5 through 8 were 24 to 50 percent, which fall in the middle of the GEOSECS range for maximum percentage radom depletion: 10 to 70 percent (average value, 29 percent).

To be certain of the cause of the high radon degassing on the west-central Florida shelf, one would need more hydrographic and circulation data than are currently available. However, it is quite reasonable to suggest that the storm front encountered on the March cruise played an important role by enhancing turbulence, gas exchange, or the sinking of radon-deficient surface waters. Since the standing crops of ^{222}Rn on the west-central Florida shelf are much larger than reported elsewhere in the ocean, it is also possible that storms across the shelf produce a larger transport of radon to the atmosphere per unit area of sea surface than found elsewhere.

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References and Notes

1. Y. H. Li, G. Mathieu, P. Biscaye, H. J. Simpson, *Earth Planet. Sci. Lett.* **37**, 237 (1977).
2. D. E. Hammond, H. J. Simpson, G. Mathieu, *J. Geophys. Res.* **82**, 3913 (1977).
3. W. S. Moore, *Earth Planet. Sci. Lett.* **2**, 231 (1967).
4. R. J. Elsinger and W. S. Moore, *ibid.* **48**, 239 (1980).
5. J. S. Hanor and L. H. Chan, *ibid.* **37**, 242 (1977).
6. K. G. Knauss, T. L. Ku, W. S. Moore, *ibid.* **39**, 235 (1978).
7. P. E. Biscaye, D. R. Olsen, G. Mathieu, in *First American-Soviet Symposium on Chemical Pollution of the Marine Environment*, compiled by K. K. Turekian and A. I. Simonov (Publication EPA-600/9-78-038, Environmental Protection Agency, Washington, D.C., 1978), p. 125.
8. D. F. Reid, thesis, Texas A&M University (1979).
9. ———, W. S. Moore, W. M. Sackett, *Earth Planet. Sci. Lett.* **43**, 227 (1979).
10. K. A. Fanning, R. H. Byrne, J. A. Breland II, P. R. Betzer, W. S. Moore, R. J. Elsinger, T. E. Pyle, *ibid.* **52**, 345 (1981).
11. W. S. Broecker, in *Symposium on Diffusion in Oceans and Fresh Water*, T. Ichiye, Ed. (Lamont Geological Observatory, Palisades, N.Y., 1965), p. 116.
12. P. Biscaye, personal communication.
13. A. E. Bainbridge, W. S. Broecker, R. M. Horowitz, Y. H. Li, G. Mathieu, J. Sarmiento, *GEOSECS Atlantic and Pacific Surface Hydrography and Radon Atlas* (National Science Foundation, Washington, D.C., 1977).
14. R. F. Kaufman and D. J. Bliss, *Effects of Phosphate Mineralization and the Phosphate Industry on Radium-226 in Ground Water of Central Florida* (Publication EPA/520-6-77-010, Environmental Protection Agency, Washington, D.C., 1977).
15. W. K. Henry, *Mon. Weather Rev.* **107**, 1078 (1979).
16. Nearly 1 year later, in February 1981, Tampa Bay waters again had high excess radon activities (Table 2).
17. S. Upchurch, personal communication.
18. We thank G. Mathieu and R. Lupton of Lamont-Doherty Geological Observatory for assistance in setting up the radon counting facility at the University of South Florida. We thank P. Biscaye for constructive advice and criticism of the work and the Florida Institute for Oceanography for shiptime aboard the R.V. *Bellows*. Supported under Department of Energy contracts EE-77-S-05-5486, 79EV05486, 80EV05486, and 81EV05486.

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Cytoplasmic Calcium in the Mediation of Macula Densa Tubulo-Glomerular Feedback Responses

Abstract. *Within each nephron of the mammalian kidney, a feedback mechanism operating between the macula densa segment of the distal tubule and the afferent arteriole participates in the regulation of glomerular filtration rate. Retrograde microperfusion studies in rats were conducted to test the hypothesis that activation of macula densa cytoplasmic calcium is involved in the transmission of feedback signals to the vascular elements. Perfusion into distal tubules with a hypotonic solution (70 milliosmolar) elicited moderate decreases in glomerular pressure of 6 ± 0.8 millimeters of mercury. With the addition of a calcium ionophore (A23187) glomerular pressure decreased by 16 ± 1.1 millimeters of mercury. When a solution devoid of calcium but containing A23187 was used, the feedback response was inhibited. Thus, cytoplasmic calcium within the receptor cells may participate in the transmission of feedback signals to the contractile cells.*

In each nephron of the mammalian kidney, the macula densa segment of the distal tubule is in close proximity with the vascular elements of its own glomerulus. One intrinsic system regulating renal vascular resistance at the level of the single nephron involves an interaction between these structures. This mechanism has been termed tubulo-glomerular feedback. It is generally considered that the macula densa cells of the distal tubule detect changes in tubular fluid composition and then transmit signals to the glomerular vasculature causing changes in vascular tone (*1*).

In the tubular fluid flowing by the macula densa cells, the concentration of sodium chloride (NaCl) and the total solute concentration (osmolality) are usually about one-third of the plasma concentrations. As the rate of flow through the loop of Henle increases, the NaCl concentration and osmolality also increase (*2*). The macula densa cells appear to be responsive to flow-related alterations in the concentration of tubular fluid and, by effecting alterations in the glomerular filtration, participate in the regulation of fluid flow along the nephron. Thus, in response to extrinsic situations that cause increases in glomerular filtration rate (GFR), the fluid flow rate and tubular fluid electrolyte concentrations at the macula densa increase, thereby leading to feedback-mediated increases in afferent arteriolar resistance and a return of GFR toward normal.

There is some controversy regarding the specific constituent of the tubular fluid that is sensed by the receptor system. Our studies have indicated that the feedback receptor mechanism does not have a specific requirement for either sodium or chloride concentration and that the receptor system may be responsive to the osmolality of the tubular fluid (*3*). However, other investigators (*4*) have suggested that chloride concentration is important.

The sequence of cellular events that transmit the signal from the luminal receptor system across the macula densa cells and through the extraglomerular mesangial cells to the vascular contractile elements remains unknown. We reasoned that one means for the transduction of signals by the macula densa cells might involve an alteration in the concentration of an intracellular messenger system. Since previous studies have established the importance of cytoplasmic calcium as a coupling agent in many stimulus-response mechanisms (*5*), we considered the proposal that intracellular

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calcium participates in the transmission of macula densa feedback signals.

For these studies the calcium ionophore A23187 was introduced into the tubular lumen, specifically to increase the receptor cell luminal membrane permeability to calcium (*6*). Although the calcium activity within the macula densa cells has not been measured, presumably it is within the range of $10^{-5}M$ to $10^{-9}M$ that has been reported for other cells (*7*). The A23187 was introduced by retrograde perfusion into an early distal tubule with a solution also containing calcium (4 meq/liter) to ensure substantial entry of Ca^{2+} into the macula densa cells. To the extent that intracellular Ca^{2+} constitutes an intermediary step in the transmission of feedback signals, we predicted that elevations in intracellular Ca^{2+} would augment the tubulo-glomerular feedback responses observed during perfusion with a solution that normally produces only minimal responses.

Experiments were performed on anesthetized rats prepared for microperfusion studies. The left kidney was placed in a plastic cup and tubular segments were visualized through a binocular microscope. A solid immobile wax cast was inserted into a middle proximal tubule segment and the intratubular pressure from an early proximal tubule of the same nephron was measured with a micropressure servo-null system (Fig. 1). Once the tubule had been blocked with wax, intratubular pressure increased for 1 to 2 minutes until an equilibrium was reached between the pressure in the glomerular capillaries and the intratubular stop flow pressure (SFP). Under these conditions, changes in SFP are a direct reflection of changes in glomerular capillary pressure. A second wax block was placed in a late distal tubule segment of the same nephron and an oil-containing pipette used to collect tubular fluid was positioned in a late proximal tubular segment downstream from the wax block. After measuring SFP in the absence of microperfusion, we inserted a pipette containing the perfusion solution into the earliest accessible surface distal tubular segment and perfusion was initiated at 15 nl/min. The fluid appearing in the late proximal tubular segment was aspirated with the collection pipette in order to allow uninterrupted flow through the perfused segment. Perfusion was maintained for 2 to 5 minutes and SFP was averaged over this period. Subsequently we withdrew the perfusion pipette, thus terminating the perfusion so that SFP could be evaluated for recovery.

Although the direction of perfusion in these experiments is opposite to the normal flow of fluid along the nephron, the retrograde microperfusion technique has the advantage of the perfusion site being very close to the macula densa area, thus minimizing changes in the composition of the perfusate reaching the receptor site. The perfusion solutions tested were: (i) an isotonic NaCl solution (290 mOsm/kg), (ii) a hypotonic solution with a low amount of NaCl and an osmolality of 70 mOsm/kg, (iii) the hypotonic solution with the addition of $5 \times 10^{-4}M$ A23187 calcium ionophore (*8*), and (iv) the hypotonic solution with $5 \times 10^{-4}M$ A23187 but containing no calcium. The first three solutions contained 4 meq of Ca^{2+} per liter. The statistical significance of the differences obtained between groups was analyzed with the unpaired *t*-test.

Figure 2A shows that with the isotonic NaCl solution (top panel) SFP decreased by 12 mmHg upon initiation of distal nephron perfusion. In contrast, during perfusion with the hypotonic solution (70 mOsm/kg) (middle panel) the feedback mediated decrease in SFP was only 5 mmHg. However, when the hypotonic solution included A23187 (bottom panel), the magnitude of the feedback mediated decrease in SFP was markedly enhanced; in this particular nephron, SFP

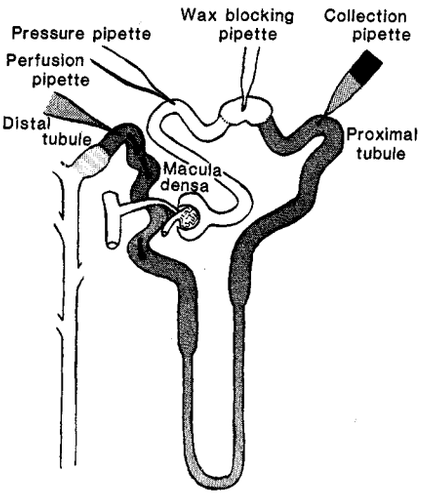

Fig. 1. Diagram of a nephron illustrating the in vivo microperfusion technique. Only proximal and distal tubules are present on the surface of the rat kidney and can be punctured with micropipettes. By insertion of wax in the middle proximal and late distal tubular segments, it is possible to obtain solid immobile blocks for the duration of the microperfusion procedure. Pressure in the upstream tubular segment is measured with a micropressure system (Instrumentation for Physiology and Medicine, San Diego) and the signal recorded on a polygraph (Grass Instruments). The early distal tubule is perfused with the use of a variable speed microperfusion pump (built by Walter Klotz, Munich, West Germany) capable of accurate delivery of fluid in the nanoliter range.

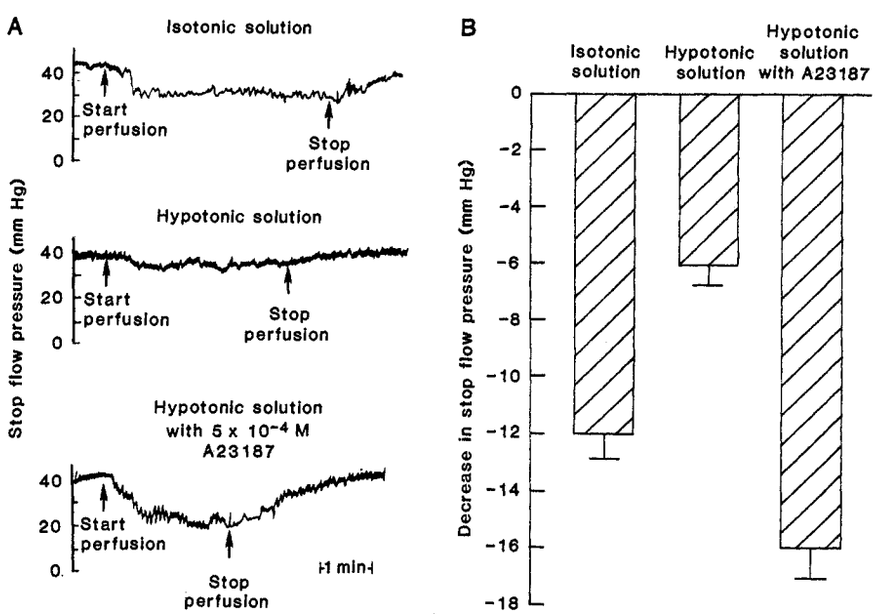

Fig. 2. (A) Examples of feedback-mediated decreases in stop flow pressure (SFP) obtained during perfusion with the isotonic solution, hypotonic solution, and hypotonic solution containing A23187. The SFP values measured before initiation of perfusion were similar. Perfusion was stopped by removal of the pipette, and in each tubule SFP returned to the previous control values. (B) The average decreases in SFP obtained with the solutions indicated. For each tubule, the decrease in SFP was calculated as the difference between the control value and the SFP value obtained during perfusion. The decrease in SFP obtained with A23187 (N = 17 tubules) was significantly greater than that obtained with the hypotonic solution (N = 13) or with the isotonic solution (N = 9). The decrease in SFP in tubules perfused with the isotonic solution was significantly greater than the decrease in the tubules perfused with the hypotonic solution.

decreased by 20 mmHg. Figure 2B presents the average decreases in SFP obtained during retrograde perfusion with these three solutions. During perfusion with the isotonic solution SFP decreased from 39 ± 1.2 to 27 ± 1.1 mmHg. The hypotonic solution elicited decreases in SFP from 42 ± 1.2 to only 36 ± 1.1 mmHg. With the hypotonic solution containing A23187, SFP decreased from 41 ± 0.7 to 25 ± 1.1 mmHg. The average decrease in SFP obtained when the calcium ionophore was present was significantly greater than the average SFP feedback responses obtained with either the hypotonic solution alone or with the isotonic NaCl solution. In all cases, the responses were reversible upon termination of perfusion, and SFP returned to 36 ± 1.1 mmHg in the isotonic solution series, 42 ± 1.1 mmHg in the hypotonic solution series, and 40 ± 1.1 mmHg in the A23187 series.

The return of SFP to preinfusion levels after perfusion with the hypotonic solution containing A23187 is important since it indicates that the reduction in SFP cannot be attributed to nonspecific cellular damage caused by A23187 and high intracellular Ca^{2+} concentration. Also, the same pattern of SFP feedback responses could be demonstrated by repeatedly perfusing the same nephron two to four times. This suggests that A23187 was not causing cell damage and that the functional integrity of the feedback mechanism was being maintained over periods of 15 to 30 minutes.

The ionophore A23187 not only enhances cell membrane permeability to calcium but can also lead to alterations in the transport of other ions (*9*). To determine if the accentuation of SFP responses was due to a specific calcium mediated effect, we evaluated feedback responses during perfusion with the hypotonic solution containing A23187 but in the absence of calcium. Control SFP averaged 39 ± 0.6 mmHg, and during perfusion SFP decreased to only 34 ± 1.5 mmHg (N = 14 tubules). In one half of the tubules tested, SFP did not change in response to perfusion or decreased by only 1 to 2 mmHg. These results are thus consistent with the view that the large decrease in SFP during perfusion with the solution containing A23187 and calcium is associated with the cellular entry of calcium and is not due to some nonspecific effect.

Cytoplasmic calcium controls or regulates many cellular processes. In certain systems, intracellular calcium functions as an intracellular messenger system, serving as a link between a specific extracellular or plasma membrane stimulus and a cellular effector mechanism (*10*). Our studies suggest that the macula densa intracellular calcium receptor system may be another example of this type of mechanism. Activation of the effector mechanism, as assessed by changes in SFP, presumably resulted from an A23187 mediated influx of Ca^{2+} into the macula densa cells. The elevation in macula densa cytoplasmic Ca^{2+} could have been responsible for the transmission of signals to the mesangial or smooth muscle cells leading to the large decreases in SFP. With the termination of perfusion, SFP returned to preinfusion levels. During this part of the procedure we observed the collapse of the distal tubular lumen which resulted from the continued absorption of fluid after cessation of perfusion. The absence of additional reabsorbate probably led to a reduction in calcium influx, and intracellular Ca^{2+} returned toward normal levels by means of the intracellular Ca^{2+} regulatory mechanisms (*11*).

Although these experiments support the suggestion that changes in intracellular Ca^{2+} may be important in mediating macula densa feedback responses, it is unlikely that normal feedback responses to changes in tubular fluid concentration actually require influx of Ca^{2+} into the macula densa cells. Previous microperfusion studies have shown that isotonic solutions devoid of Ca^{2+} are capable of producing normal feedback responses (*12*). Thus, it seems likely that increases in luminal fluid concentration directly elicit alterations in the level of cytoplasmic Ca^{2+} without necessitating the entry of calcium from the luminal fluid.

Figure 3 shows that four general steps are postulated to occur during the transmission of feedback signals. Under this scheme, alterations in the cytoplasmic calcium of the recepter cell (step 2) serve to link changes in distal tubular solute concentration (step 1) to the vascular contractile response (step 4). This process also includes a Ca^{2+} mediated stimulus-response coupling between the receptor cells and the vascular contractile elements (step 3). However, the nature of this signal transmission step which involves passage through the extraglomerular mesangial cell layer remains unknown.

There are several potential mechanisms for the coupling of changes in tubular fluid concentration to changes in cytoplasmic calcium concentration. It is possible that changes in luminal fluid concentration could alter cellular fluid volume which in turn might affect changes in cytoplasmic calcium concentration. However, it is equally possible

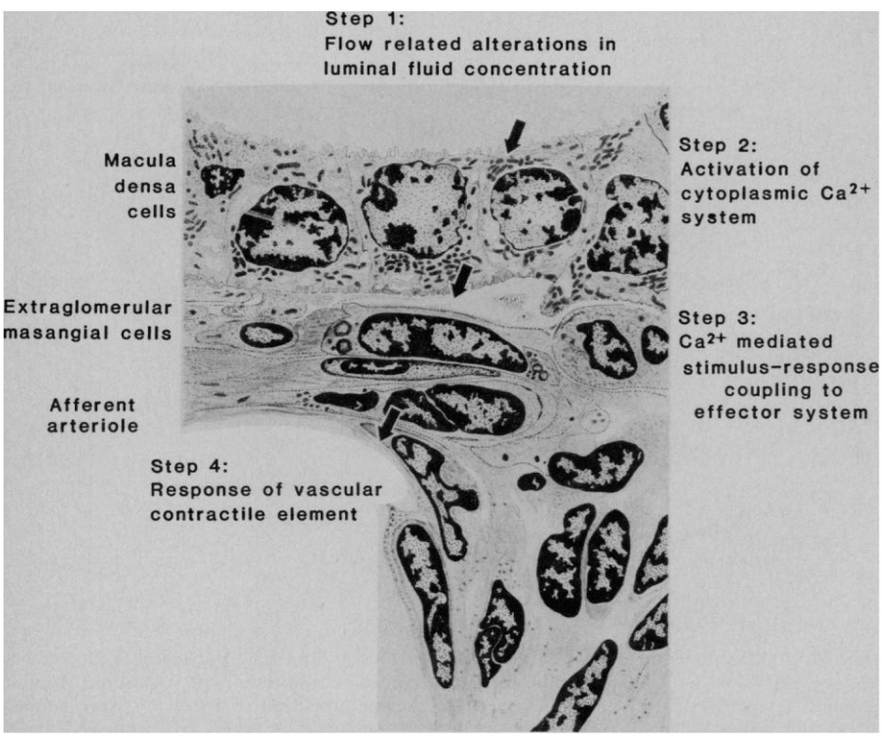

Fig. 3. Composite drawing of the macula densa juxtaglomerular apparatus reconstructed from electron micrographs (*13, 14*). The space at the top of the illustration represents the lumen of the distal tubule. Below the lumen there are three layers of cells. The first is a single layer of macula densa cells. Below that are several layers of extraglomerular mesangial cells. Adjacent is the afferent arteriole with its lumen. The four general steps involved in the transmission of feedback signals from the tubular epithelial cells to the contractile elements are described in the text.

that changes in membrane electrical potential difference or changes in the transport of specific ions into the receptor cells might serve a similar function in influencing the level of intracellular calcium. The present studies do not provide sufficient information to distinguish between these various alternative mechanisms. Nevertheless, our results indicate that the activity of the intracellular calcium system of the recepter cell serves a critical role in coupling the events occurring within the distal tubular lumen with the resultant alterations in glomerular vascular resistances.

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References and Notes

1. L. G. Navar, *Am. J. Physiol.* **234** (*Renal Fluid Electrolyte Physiol. 3*), F357 (1978); F. S. Wright and J. P. Briggs, *Physiol. Rev.* **59**, 958 (1979); L. G. Navar, D. W. Ploth, P. D. Bell, *Annu. Rev. Physiol.* **42**, 557 (1980).
2. P. D. Bell, L. G. Navar, D. W. Ploth, C. B. McLean, *Kidney Int.* **18**, 460 (1980).
3. P. D. Bell and L. G. Navar, in preparation; P. D. Bell, C. B. McLean, L. G. Navar, *Am. J. Physiol.* **240** (*Renal Fluid Electrolyte Physiol. 9*) F111 (1981).
4. J. Schnermann, D. W. Ploth, M. Hermle, *Pfluegers Arch.* **362**, 229 (1976); J. P. Briggs, J. Schnermann, F. S. Wright. *Am. J. Physiol.* **239** (*Renal Fluid Electrolyte Physiol. 8*), F427 (1980).
5. R. P. Rubin, *Pharmacol. Rev.* **22**, 389 (1970).
6. B. C. Pressman, *Annu. Rev. Biochem.* **45**, 501 (1976).
7. J. O'Doherty, S. J. Youmans, W. McD. Armstrong, R. J. Stark, *Science* **209**, 510 (1980).
8. The ionophore (Calbiochem-Behring) was dissolved in dimethylsulfoxide. The vehicle was also added to the first two solutions. Each solution contained the following: Mg^{2+}, 2 meq/l; SO_4^{2-}, 2 meq/l; HPO_4^-, 2 meq/l; K, 5 meq/l; and urea, 250 mg/l. The concentrations of the other ions were, in the isotonic solution: Na^+, 145 meq/l; Cl^-, 135 meq/l; and HCO_3^-, 10 meq/l. In the hypotonic solution the concentrations were: Na^+, 25 meq/l; Cl^-, 6 meq/l; and HCO_3^-, 25 meq/l. The bicarbonate concentration was increased in the hypotonic solution in order to increase the osmolality without increasing the chloride concentration.
9. P. W. Reed, *J. Biol. Chem.* **251**, 3489 (1976).
10. W. W. Douglas, *Br. J. Pharmacol.* **34**, 451 (1968); H. Rasmussen and D. B. P. Goodman, *Physiol. Rev.* **57**, 421 (1977).
11. E. Carafoli, K. Malstroin, E. Sigel, M. Crompton, *Clin. Endocrinol. (N.Y.)* **5**, 495 (1976).
12. P. D. Bell, C. Thomas, R. H. Williams, L. G. Navar, *Am. J. Physiol.* **234** (*Renal Fluid Electrolyte Physiol. 3*), F154 (1978); J. Schnermann and M. Hermle, *Pfluegers Arch.* **358**, 311 (1975).
13. R. Taugner, A. Schiller, B. Kaissling, W. Kriz, *Cell Tissue Res.* **186**, 279 (1978).
14. L. Barajas, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 78 (1981).
15. We thank C. McLean and C. Dastmalchi for assistance. This work was supported by grants HL 18426 and HL 07457 from the National Heart, Lung, and Blood Institute.

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Neutrophil Activation Monitored by Flow Cytometry: Stimulation by Phorbol Diester Is an All-or-None Event

Abstract. *The population dynamics of single-cell stimulation was analyzed by monitoring autofluorescence by flow cytometry. Stimulation of the respiratory burst in human neutrophils by 12-O-tetradecanoyl phorbol-13-acetate (TPA) caused a decline in highly fluorescent cells (characteristic of resting neutrophils) and a corresponding increase in the number of weakly fluorescent cells (characteristic of activated neutrophils). Increasing concentrations of TPA caused increasing numbers of cells to shift from the highly fluorescent population to the weakly fluorescent population without the appearance of intermediate populations. Thus the neutrophil respiratory burst, a component of neutrophil cytotoxic response, is triggered in an all-or none fashion.*

Phagocytosis of foreign bodies by macrophages and neutrophils is associated with a respiratory burst of superoxide (O_2^-) production because of the activation of a membrane-bound pyridine nucleotide oxidase. Superoxide, or subsequently formed "active oxygen" species such as hydrogen peroxide, hydroxyl radicals, and possibly singlet oxygen, participate in the oxidative cytotoxic mechanisms of these cells. These oxidative mechanisms are used by phagocytic cells to kill foreign organisms [for review, see (*1–3*)]. The formation and release of O_2^- from activated immunologic cells also appears to play a role in the pathology of autoimmune and arthritic diseases (*4*). For these reasons, the mechanism and control of this response have been the subjects of intense investigation.

Triggering of the respiratory burst does not require phagocytosis since surfaces that are coated with immunoglobulin G (IgG) and that do not undergo phagocytosis trigger the response (*5*). The response is also triggered by certain soluble agents such as chemotactic peptides, anionic detergents, calcium ionophore A23187, concanavalin A, and various phorbol diesters. In general, increasing concentrations of stimulating substances cause an increased mean metabolic response of a cell population (*1–3*, *6*). It has not been known whether this is due to a dose-dependent increase in the metabolic response of all cells, or whether individual cells are quantized in completely activated or resting modes and vary in the concentration of agonist required for activation.

To test whether the metabolic response in neutrophils is quantized, we monitored cell autofluorescence by flow cytometry. In principle, any fluorescence change due to metabolic stimulation could be monitored. We chose excitation wavelengths in the region of 350 nm and monitored emission > 425 nm in order to observe the decreases in intracellular reduced pyridine nucleotides [NAD(P)H] that have been reported to occur after stimulation of the neutrophil respiratory burst (*7–9*). The fluorescence of reduced pyridine nucleotides is much greater than that of the oxidized species and fluorescence changes have previously been monitored in intact cells (*10*).

As shown in Fig. 1a, the fluorescence distribution of neutrophils is clearly bimodal (*11*). Most of the cells belong to the highly fluorescent population. The fraction of weakly fluorescent cells varied between neutrophil preparations; in some cases, it was less than 0.05, in others (see Fig. 1a) it was as great as 0.3. Both cell populations were viable as determined by analysis of propidium iodide exclusion (*12*).

Next we added progressively increasing amounts of the respiratory burst stimulator 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) to the cell suspension prior to incubation. As shown in Fig. 1, b to f, TPA caused a dose-dependent decrease in the population frequency of the highly fluorescent cells and a corresponding increase in the population frequency of the weakly fluorescent cells. This result is consistent with the expected decrease in the ratio of reduced to oxidized pyridine nucleotides in metabolically stimulated neutrophils (*7–9*). No decrease in fluorescence was observed when the cells were incubated with TPA at 0°C. The lower fluorescence intensity of the stimulated neutrophils was similar in magnitude to that of the initial variable subpopulation of neutrophils with low fluorescence intensity. The presence of weakly fluorescent cells in the initial unstimulated neutrophil preparation may be due to stimulation of a fraction of the neutrophils in vivo or during the cell purification procedure.

Analysis of these results yielded additional information about the mechanism of stimulation of individual cells. A downward shift in fluorescence intensity of the entire neutrophil population as the concentration of TPA is increased would indicate that higher TPA concentrations

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