INFECTION AND IMMUNITY, May, 1974, p. 903-908

Vol. 9, No. 5
Printed in U.S.A.

Release of Histamine from Hamster Mast Cells by Concanavalin A and Phytohemagglutinin

W. A. HOOK, S. F. DOUGHERTY, AND J. J. OPPENHEIM

Laboratory of Microbiology and Immunology, National Institute of Dental Research, Bethesda, Maryland 20014

Received for publication 8 January 1974

Concanavalin A (Con A) and phytohemagglutinin (PHA) released histamine from hamster mast cells incubated in a serum-free medium. Concentrations of Con A and PHA approximating those optimal for transforming lymphocytes also released maximal amounts of histamine without apparent cytotoxicity. Higher concentrations of mitogen inhibited both lymphocyte transformation and histamine release. Incubation at 37 C for 15 min released histamine, although longer times were more effective. Supernatants from cultured hamster splenocytes stimulated with Con A also released histamine from added mast cells. However, the effect could be inhibited by the addition of 0.1 M methyl alpha-D-mannoside or by passing the spleen cell culture supernatants through Sephadex G-75 to remove Con A. This mitogen-induced release of mast cell histamine is therefore not mediated by a lymphokine but probably results from a direct interaction of mitogens with receptors on mast cells.

Mast cells release histamine and other pharmacologically active substances into surrounding tissues upon alteration of the mast cell surface. Hamster mast cells release histamine upon exposure to a variety of biological and chemical agents including polypeptides isolated from granulocytes, compound 48/80, and mellitin (7). In immediate hypersensitivity reactions, histamine is secreted subsequent to the reaction of allergen with reaginic antibody on the surface of the cells (1, 5). It was recently reported by Keller (6) that mast cells obtained from rats sensitized with the nematode *Nippostrongylus brasiliensis*, but not those from normal rats, rapidly released histamine when triggered by concanavalin A (Con A).

We have tested the lectins Con A and phytohemagglutinin (PHA), which are mitogenic for lymphocytes (10, 13), and have found that they also release histamine from mast cells collected from nonimmunized hamsters. The mechanism of release by these lectins was studied because this provides another probe for investigating the process of histamine secretion.

MATERIALS AND METHODS

Preparation of cell suspensions. Female, 10- to 12-week-old hamsters (LVG:LAK) were purchased from Lakeview Hamster Colony, Newfield, N.J. For one experiment, virus- and parasite-free animals of the same age and strain were tested. Peritoneal mast cells were collected, as described previously, from the unstimulated abdominal washings of normal hamsters and washed three times with Veronal-buffered saline containing 0.1% gelatin and 2 U of heparin per ml (3). After the final centrifugation at 150 × *g*, cells were pooled from three or more animals and suspended to twice the original volume in medium RPMI 1640 containing 0.0001% $Ca(NO_3)_2 \cdot 4H_2O$ but without $MgSO_4$ and phenol red. Molten gelatin was added to a final concentration of 0.1%, and the modified medium, designated 1640-G, was used as diluent for all experiments unless specified otherwise. The cell suspensions consisted of approximately 10% mast cells and the remainder consisted of polymorphonuclear and other types of leukocytes. Purified mast cells were obtained where specified by differential centrifugation at 330 × *g* into 30, 40, and 50% Ficoll layers as described by Uvnäs and Thon (16). The resulting mast cell pellet was resuspended to one-tenth the original cell volume in 1640-G medium and was found by phase-contrast microscopy to contain >90% mast cells.

Reaction mixtures. Suspensions containing 5 × 10^4 hamster mast cells in 1 ml were added in triplicate to plastic tubes (no. 2058; 12 by 75 mm; Falcon Plastics, Oxnard, Calif.). Cells were exposed to 0.1-ml volumes containing appropriate amounts of mitogens. These were: 5 μg of Con A (Calbiochem, San Diego, Calif.); 10 μg of PHA (G. Hitchings, Burroughs Wellcome, Tuckahoe, N.Y.); 50 μg of pokeweed mitogen (PWM) purchased from GIBCO (Grand Island, N.Y.); 20 μg of purified protein derivative (PPD) concentrate of the DT strain (Lederle Labs, Pearl River, N.Y.); and 100 μg of *Salmonella typhosa* strain 0901 endotoxin W or 0.1 U of streptolysin O (Difco, Detroit, Mich.). Tubes containing mitogen and cells were incubated at 37 C for 1 h or as specified. For experiments requiring more than 1 h, tubes were

incubated with loose-fitting caps in an humidified atmosphere of 5% CO_2 in air. After incubation, cells were pelleted by centrifugation at 500 × *g* for 15 min, and the supernatant fluids were decanted and assayed for histamine content.

Spleen cell cultures. For certain experiments, supernatants were collected from cultures of mitogen-stimulated hamster spleen cells. Replicate cultures of 5×10^6 spleen cells from three or more hamsters were pooled and incubated in 1 ml of RPMI 1640 without serum for 4 days at 37 C in 5% CO_2 and air. The cultures were centrifuged at 500 × *g* for 10 min, and the supernatants were decanted and added to 5×10^4 mast cells. A second incubation at 37 C for 1 h was then performed to release histamine. Lymphocyte transformation in response to mitogens was quantitated in replicate cultures. These cultures were incubated for 4 days, and 1 μCi of [^{3}H]thymidine (specific activity, 6.0 Ci/mmol; Schwarz/Mann, Rockville, Md.) per ml was added for the final 16 h of incubation. The counts per minute of [^{3}H]thymidine in the acid-precipitable fraction were determined (11).

Test for cytotoxicity. Peritoneal mast cells after incubation were suspended in a mixture of 0.2 ml of 1640-G and 0.2 ml of 0.4% trypan blue in 0.85% saline. Cell suspensions were examined immediately by phase-contrast and direct microscopy with siliconized slides and cover slips to determine the percentage of dead or degranulated mast cells. In addition, 5×10^7 peritoneal mast cells were labeled at 37 C for 30 min with 300 μCi of ^{51}Cr. They were then washed three times with 1640-G, sometimes purified on Ficoll gradients, and reacted with appropriate mitogen, and the percentage of isotope released from the cells was determined (2).

Inhibition of histamine release. Where specified, Con A or PHA was incubated with mast cells at 0 or 20 C for 1 h rather than at 37 C, or incubated in the presence of 0.1 M methyl alpha-D-mannoside (MAM), (Sigma Chemical Co., St. Louis, Mo.) or with the immunoglobulin G fraction of rabbit antiserum to Con A (kindly provided by Bruce Mackler and Robert Kelly, Kennedy Inst. of Rheumatology, London, England). This antiserum was prepared by adsorbing Con A to autologous rabbit erythrocytes and injecting the mixture intravenously four times at 3- to 4-week intervals. Supernatants from Con A-stimulated spleen cell cultures were freed of Con A by passing 2-ml volumes of the supernatants through columns (1 by 25 cm) of Sephadex G-75 (Pharmacia, Piscataway, N.J.). The eluent used was 0.85% NaCl. The removal of Con A from the eluate was determined from decreased optical density measurements at 280 nm, disappearance of Con A-induced agglutination of red blood cells, and reduction in the mitogenic effects of supernatants on hamster lymphocytes.

Histamine assay. Histamine was measured fluorometrically by the method of Lichtenstein (8). The total available histamine contained in 5×10^4 mast cells was released by boiling them for 5 min and was approximately 0.1 μg in each experiment. Assays were performed in triplicate, and the results were recorded as the mean percentage of available histamine released ±1 standard error of the mean.

RESULTS

Hamster mast cells were incubated in the presence of a variety of mitogens and antigens at concentrations of 1 to 200 μg/ml. Optimal histamine release was obtained with 1 to 5 μg of Con A and 2.5 to 10 μg of PHA/ml (Fig. 1). A similar dose response was obtained with cells obtained from virus- and parasite-free hamsters of the same strain and age. Increasing the concentration of Con A or PHA to 50 μg/ml or more inhibited histamine release. At higher concentrations of Con A or PHA (>10 μg/ml), the uptake of tritiated thymidine by cultured splenic lymphocytes was also inhibited significantly. The histamine release and lymphocyte transformation response to multiple doses of a variety of mitogens and antigens were tested. PWM itself contained 0.093 μg of histamine-like material per 50 μg, but PWM did not appear to release additional histamine from mast cells above this high background level in spite of its high mitogenic activity. Streptolysin O (0.4 U) released only 3.4% of the available histamine and was relatively inactive in transforming hamster leukocytes at thise dose. PPD and lipopolysaccharide were also ineffective in transforming lymphocytes from unimmunized hamsters and did not release histamine above baseline values. Con A and PHA again were markedly active in both assays and released 32.4 ± 5.6 and 23.9 ± 4.2% histamine, respectively. Thus, with the exception of PWM, only stimulants that were mitogenic for hamster lymphocytes also released histamine from hamster mast cells.

Incubation of mast cells with 5 μg of Con A for as little as 15 min resulted in significant histamine release (Fig. 2). Increased release was achieved by incubation for greater lengths of time and was accompanied by a concomitant

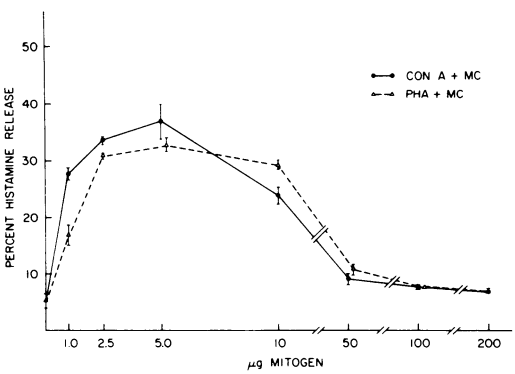

FIG. 1. *Histamine release by hamster mast cells incubated for 1 h at 37 C with increasing amounts of mitogen.*

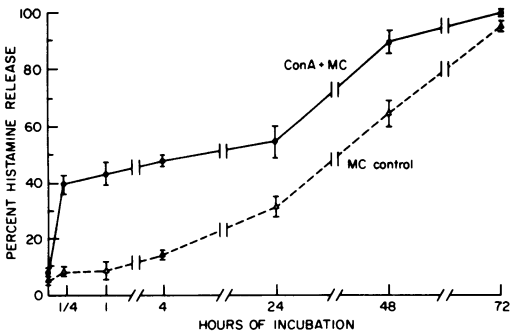

FIG. 2. *Effect of increasing incubation time at 37 C on histamine release from hamster mast cells (MC) with and without 5 μg of concanavalin A (Con A).*

increase in the spontaneous release of histamine by controls incubated without mitogen. Phase-contrast microscopy and vital staining of these control and Con A-exposed mast cells indicated that at 1 h there was little or no uptake of trypan blue nor degranulation. After 24 h or more of incubation, the increased histamine release by both control and Con A-exposed mast cells was associated with marked degranulation, a higher incidence of trypan blue-stained cells, and a decrease in mast cell count. Microscope examination of mast cells incubated in the presence of histamine-releasing doses of Con A and PHA at 1 and 24 h revealed that the only striking difference from controls was the marked agglutination of cells, making quantitation difficult. These observations indicated that lectin-induced histamine release was not based on cytolysis.

To determine whether mast cells were indeed the only source of histamine in the suspensions used or whether its release depended on the participation of other leukocytes, mast cells were purified with Ficoll gradients. Partially purified peritoneal cells consisting of 90% or more mast cells released histamine in response to Con A in a manner similar to unpurified cell suspensions which contained only 10% mast cells (Table 1). In addition, ^{51}Cr was not released by either purified or unpurified mast cells which were concomitantly stimulated to release histamine by exposure to Con A.

To determine whether the mechanism of histamine release was indirect and mediated by mitogen-induced lymphokines on mast cells, hamster splenocytes were cultured for 4 days with increasing doses of Con A, and the supernatants were tested for the presence of such a factor. In fact, the supernatant from a culture incubated with 5 μg of Con A released 25% of the available histamine from mast cells, whereas the supernatant of unstimulated splenocytes released only 7.4% (Table 2). However, the increase in histamine-releasing activity of the supernatants of activated lymphocytes was due to the presence of residual Con A. Removal of this Con A by passage of supernatants through Sephadex G-75 reduced the release of histamine completely. Removal of all but the highest concentrations of Con A was demonstrated by the inability of the Sephadex G-75-treated supernatants to agglutinate guinea pig erythrocytes or to transform hamster lymphocytes (Table 2). Addition of 5 μg of fresh Con A to the supernatants after gel filtration released 18 to 29% of the available histamine, indicating that no inhibitor of histamine release was present in the supernatants after gel filtration.

The effect on histamine release of conditions known to interfere with mitogenic activity on Con A and PHA was tested. Incubation at 0 or 25 C or inactivation of mitogen by boiling almost completely inhibited histamine release by both Con A and PHA (Table 3). Exposure of mast cells to mitogens at 0 or 25 C, followed by removal of unbound mitogen by two washings and incubation at 37 C for 30 min, resulted in less than 4% histamine release in the case of

TABLE 1. *Histamine and ^{51}Cr release from purified and mixed mast cells (MC) incubated with Con A*[a]

Added to MC	% ^{51}Cr released from:		% Histamine released from:	
	Pure MC	Mixed MC	Pure MC	Mixed MC
1640-G (control)	13 ± 2.3	15 ± 0.6	4.9 ± 0.6	5.9 ± 0.3
100 μg of Con A	1.6 ± 0.9	0	6.0 ± 1.1	0.9 ± 0.2
2.5 μg of Con A	1.3 ± 0.3	0	20 ± 4.3	16 ± 0.6
Frozen and thawed MC (six times)	64 ± 2.1	71 ± 4.0	93 ± 0.8	64 ± 9.0

[a] Hamster MC (5×10^4) were added to triplicate tubes containing Con A and incubated at 37 C for 1 h. All results except for 1640-G (control) were corrected by subtracting background ^{51}Cr or histamine release from mast cells incubated in the absence of mitogen (control). Percent histamine release is given as the mean of triplicate samples ± 1 standard error.

TABLE 2. *Removal of histamine-releasing activity of supernatants from 4-day Con A-splenocyte cultures by passage through G-75 dextran gel*[a]

Con A (μg) incubated with splenocytes	Treatment of supernatant	Histamine release (%) from added mast cells (MC)		Lymphocyte transformation (mean counts/min)	Erythrocyte agglutination[b]
		MC only	Con A (5 μg + MC)		
0	None	7.4 ± 0.3	ND[c]	612	–
5	None	25 ± 7.6	ND	33,135	+
0	G-75	0	29 ± 2.7	364	–
0.5	G-75	0	25 ± 1.1	641	–
2	G-75	0	22 ± 5.5	941	–
5	G-75	0	18 ± 2.7	8,267	–
25	G-75	0	18 ± 4.4	577	–
50	G-75	0	26 ± 1.5	2,014	–
100	G-75	1.4 ± 0.3	22 ± 1.5	23,033	–

[a] Spleen cells (5×10^6) were cultured for 4 days at 37 C in RPMI 1640 and centrifuged to remove cells, and supernatants were passed through G-75. MC (5×10^4) were added, and mixtures were incubated for 1 h at 37 C to release histamine. Values below the space were corrected for background histamine release by cells incubated in the absence of mitogen.

[b] Plus (+) indicates the presence of more than 1.6 μg of Con A, as detected by agglutination of erythrocytes. Minus (–) signifies no agglutination.

[c] Not done.

Con A, indicating that binding by Con A did not occur at these lowered temperatures. Con A and PHA differed in their ability to adsorb to mast cells at 0 or 25 C. A 12 and 15% release was seen when mast cells were exposed to PHA (Table 3), suggesting that PHA binds mast cells with greater activity than Con A. Rapid freezing and thawing, six times, of both Con A and PHA did not destroy their ability to release histamine. Addition of antiserum to Con A or of MAM, a competitive inhibitor of Con A, markedly inhibited Con A activity, but PHA inhibited only to a limited extent (Table 3).

DISCUSSION

A variety of agents can induce histamine release from mast cells by different mechanisms. For example, a cationic protein from neutrophil lysosomes initiates distinct steps of intracellular activation and nonlytic release of histamine (14). In the case of reaginic (immunoglobulin E [IgE]) antibody, the release of histamine from rat mast cells can also be separated into two or more steps: sensitization of basophils with antibody, followed by challenge with antigen, resulting in the secretion of histamine (1). There are other antibody-mediated reactions not involving reagins, in which histamine secretion occurs if mast cells are exposed to the cleavage products of the third (C3a) or fifth (C5a) components of complement that are generated by immune aggregates (13). Hamster serum incubated with bacterial endotoxin will also generate a substance of approximately 60,000 molecular weight which will release histamine from hamster mast cells (4). In contrast, the polypeptide mellitin from bee venom (17) and substances such as detergents release histamine by direct lysis.

Suspensions containing washed mast cells and other leukocytes from the unstimulated hamster peritoneum released a significant amount of histamine when incubated with the lectins Con A or PHA. Histamine release occurred with doses of Con A and PHA that were similar to the amount that stimulated hamster lymphocytes to incorporate [^{3}H]thymidine, as seen in this work and that described previously by Singh and Tevethia (15). Since lymphocyte activation involves the interaction of stimulants with cell surface receptors, this suggests that an analogous reaction may be occuring between receptors on the mast cells and the mitogens involved in histamine release. The ability of mitogens to act as histamine-releasing agents, as observed in our work, is due to nonlytic mechanism since excess Con A or PHA failed to release histamine. Furthermore, microscope and ^{51}Cr-release studies of mast cells incubated in the presence of Con A also indicated that this lectin was not cytolytic at the histamine-releasing doses tested.

The possibility that histamine release by mast cells was due to a mitogen-induced lymphokine was tested. Indeed, supernatants ob-

TABLE 3. *Inhibition of mitogen-induced histamine release*

Inhibition	Histamine released[a] (%) from MC incubated with:	
	Con A (5 μg)	PHA (5 μg)
1. Non-inhibited control	24 ± 1.2	26 ± 0.6
2. a. Incubation at 0 C	0	0.3 ± 0.1
b. Incubation at 0 C, wash, 37 C[b]	2.0 ± 0.9	12 ± 2.6
3. a. Incubation at 25 C	1.4 ± 0.9	2.3 ± 0.1
b. Incubation at 25 C, wash, 37 C[b]	3.5 ± 0.6	15 ± 6.6
4. Mitogen heated to 56 C, 30 min	0	24 ± 1.3
5. Mitogen heated to 100 C, 30 min	0	1.7 ± 0.5
6. Mitogen frozen and thawed six times	27 ± 1.6	33 ± 1.4
7. Antiserum to Con A (0.1 ml)	0	13 ± 1.5
8. 0.1 M methyl alpha-D-mannoside	0.9 ± 0.5	17 ± 1.4

[a] Values were corrected for a value of 3.7 ± 0.3% background histamine release by mast cells incubated in absence of mitogen.

[b] Mixtures were incubated at 0 or 25 C for 30 min, sedimented, and washed two times with 1640-G; the pellets resuspended to 1.1 ml and incubated at 37 C for 30 min.

tained after incubating Con A and hamster splenocytes for 4 days were capable of releasing histamine. However, this activity was removed by passing supernatants through dextran gel which absorbs Con A. In addition, the Con A-induced, histamine-releasing activity was blocked by incubating the mast cells in the presence of an inhibitor, namely, MAM, capable of specifically interacting with the binding sites of Con A (13). MAM reduced the histamine release by PHA only partially, indicating that it competes more effectively with binding by Con A. These experiments suggested that the lectin-induced release of histamine by mast cells was dependent on direct interaction with specific receptors on mast cells.

There are several observations that argue against interaction of lectins with the antibody-combining site of IgE. We have found that Con A and PHA also release 20 to 60% of the histamine from peripheral blood basophils of most normal humans (W. A. Hook, S. F. Dougherty, and J. J. Oppenheim, Fed. Proc. **32**:1000, 1973). However, the mechanism of histamine release by the lectins Con A and PHA presumably differs from the effect of allergens on blood leukocytes (9) because these donors do not manifest any clinical evidence of allergy to the lectins. Furthermore, it is also unlikely that Con A caused histamine release by reacting with preexisting, specific IgE antibody toward the mitogen because of the demonstrated inhibition by MAM, which competes only with binding to carbohydrate groups.

Our findings favor the view that the lectins interact directly with receptors on the surface of hamster mast cells. All the pooled cell populations from our "normal" immunized hamsters were reactive. Furthermore, work in progress shows that pooled cells from 9- to 10-week-old, virus- and parasite-free hamsters also released histamine in response to Con A. However, we can not exclude the possibility that "normal" hamsters have considerable amounts of IgE on their surface.

There is evidence from other laboratories favoring the view that lectin-induced histamine release is based on its interaction with the carbohydrate component on the Fc region of IgE. Keller has reported that mast cells from rats sensitized with *N. brasiliensis* released 18 to 24% of the available histamine within 2 to 10 min of incubation with 10 μg of Con A but did not release with PHA (7). However, cells from his non-parasitized animals failed to release histamine. He suggested that Con A, like specific allergen, reacted by cross-linking the Fc regions to IgE. Finally, there are the observations by Siraganian et al. (P. A. Siraganian, M. Brodsky, and R. P. Siraganian, J. Immunol., in press) that sensitivity of human basophils to histamine release by Con A correlated directly with sensitivity of cells to antiserum toward IgE, indicating that receptors for Con A were associated with IgE. We are currently testing this hypothesis by assaying the histamine release of hamsters that have been stimulated to produce "IgE" in response to immunization.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Julian Washington.

LITERATURE CITED

1. Bach, M. K., K. J. Block, and K. F. Austen. 1971. IgE and IgG antibody-mediated release of histamine from rat peritoneal cells. J. Exp. Med. **133**:752-771.
2. Canty, T. G., and J. R. Wunderlich. 1970. Quantitative *in vitro* assay of cytotoxic cellular immunity. J. Nat. Cancer Inst. **45**:761-772.
3. Hook, W. A., R. Snyderman, and S. E. Mergenhagen. 1970. Histamine releasing factor generated by the interaction of endotoxin with hamster serum. Infect. Immunity **2**:462-467.
4. Hook, W. A., R. Snyderman, and S. E. Mergenhagen. 1972. Further characterization of a factor from endotoxin-treated serum which releases histamine and heparin from mast cells. Infect. Immunity **5**:909-914.
5. Ishizaka, T., K. Ishizaka, S. G. O. Johansson, and H.

Bennich. 1969. Histamine release from human leukocytes by anti-γE antibodies. J. Immunol. **102:**884-892.
6. Keller, R. 1968. Interrelations between different types of cells. II. Histamine-release from the mast cells of various species by cationic polypeptides of polymorphonuclear leukocyte lysosomes and other cationic compounds. Int. Arch. Allergy Appl. Immunol. **34:**139-144.
7. Keller, R. 1973. Concanavalin A, a model "antigen" for the *in vitro* detection of cell-bound reaginic antibody in the rat. Clin. Exp. Immunol. **13:**139-147.
8. Lichtenstein, L. M. 1971. The immediate allergic response: *in vitro* separation of antigen activation, decay and histamine release. J. Immunol. **107:**1122-1130.
9. Lichtenstein, L. M., and A. G. Osler. 1964. Studies on the mechanisms of hypersensitivity phenomena. II. Histamine release from human leukocytes by ragweed pollen antigen. J. Exp. Med. **120:**507-530.
10. Nowell, P. C. 1960. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. Cancer Res. **20:**462-466.
11. Oppenheim, J. J., R. A. Wolstencroft, and P. G. H. Gell. 1967. Delayed hypersensitivity in the guinea pig to a protein-hapten conjugate and its relationship to *in vitro* transformation of lymphnode, spleen, thymus and peripheral blood lymphocytes. Immunology **12:**89-94.
12. Osler, A., H. G. Randall, B. M. Hill, and Z. Ovary. 1959. Studies on the mechanism of hypersensitivity phenomena. III. The participation of complement in the formation of anaphylatoxins. J. Exp. Med. **110:**311-339.
13. Powell, A. E., and M. A. Leon. 1970. Reversible interaction of human lymphocytes with the mitogen Concanavalin A. Exp. Cell Res. **62:**315-323.
14. Ranadive, N. S., and C. G. Cochrane. 1971. Mechanism of histamine release from mast cells by cationic protein (Band 2) from neutrophil lysosomes. J. Immunol. **106:**506-516.
15. Singh, S. B., and S. S. Tevethia. 1972. In vitro stimulation of hamster lymphocytes with concanavalin A. Infect. Immunity **5:**339-345.
16. Uvnäs, B., and I. L. Thon. 1959. Isolation of "biologically intact" mast cells. Exp. Cell Res. **18:**512-520.
17. Williams, J. C., and R. M. Bell. 1972. Membrane matrix disruption by mellitin. Biochim. Biosphys. Acta **288:**255-262.