Enhancement of Phagocytic Activity by Elevated Ca^{2+} Levels and Its Relation to Antibody Formation (35246)

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Many membrane-associated events are significantly influenced by calcium ions. Thus, it is known that Ca^{2+} regulates the deformability and viscosity of the cell membrane, alters its permeability, acts as primary coupling factor in the transfer of extracellular membrane signals to intracellular responses, modifies membrane-associated enzymes, influences complement-dependent reactions as well as the maintenance of cell junctions and communicability [cf. (1) for refs.]. Prior studies have revealed that the elevation of Ca^{2+} levels, produced by repeated iv injections of $CaCl_2$, or a lowering of serum Ca^{2+} levels by sc injections of calcitonin, modifies antibody formation to sheep red blood cells (sRBC) in mice (1). The extent of these modifications, and their direction, was found to be greatly dependent on the time of initiation of changes in Ca^{2+} levels in relation to the time of injection of the antigen: elevation of Ca^{2+} levels started 8–12 hr prior to antigen administration, or begun 12–18 hr after antigen administration, delayed the appearance of antibody-forming spleen cells (AFC); in contrast, when the elevation of Ca^{2+} levels was begun at the time of antigen administration or 6 hr thereafter, the number of AFC, assayed 48 and 72 hr after immunization, increased remarkably (as much as 7-fold above the number found in animals receiving sRBC only). Calcitonin produced exactly the opposite effects, *ie.*, early or late administration enhanced responses, whereas its administration at or shortly after the time of antigen injection inhibited responses measured in terms of AFC. Alteration of Ca^{2+} levels also produces comparable modifications of antibody formation *in vitro* (M. Ishizuka and W. Braun, unpublished observations) and therefore the effects appear to represent direct effects on the cells involved in antibody formation.

Since members of phagocytic cell populations appear to play a role in the initiation of antibody formation to at least certain types of antigens (2–6), including sRBC (7), it became of interest to determine whether elevated Ca^{2+} levels could enhance phagocytosis and thus account, at least in part, for the stimulation of early events in antibody formation to sRBC.

Materials and Methods. Carbon clearance was studied in male Rockland Swiss mice, weighing approx 20 g, by the method of Biozzi *et al.* (8) Sixteen mg of colloid carbon (Guenther Wagner, Hannover, C 11/1431a), dissolved in 0.5 ml of 1% Difco gelatin, was injected into the tail vein and the phagocytic index, K, was determined on the basis of blood samples removed at different intervals thereafter. The corrected phagocytic index, α, which represents a measure of phagocytic activity per unit weight of tissue, was calculated as

$$\frac{\text{mouse wt}}{\text{combined wt of liver and spleen}} \times K^{-\frac{1}{3}}.$$

Calcium chloride ($CaCl_2$ dihydrate, Merck) was injected iv, 400 μg/animal, once every 6 hr for a maximum of five times. Five groups of 10 animals each were tested for carbon clearance and then sacrificed at 6-hr intervals, *i.e.*, following each injection of $CaCl_2$, and, in addition, 2 groups of 10 animals each were tested and sacrificed 10 and 20 hr after the last $CaCl_2$ injection. Eight groups of five animals each served as con-

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trols, receiving saline only, and were tested 6 hr prior to the start of the experiment and at each of the experimental test periods.

Results and Discussion. The data compiled in Table I show that clearance rates increased greatly, leading to a doubling of the K value, within 12 hr after the beginning of $CaCl_2$ injections and declined to normal values within 20 hr after termination of $CaCl_2$ injections.

Thus, phagocytosis is enhanced as the result of elevated levels of Ca^{2+} but the time required for the initiation of this effect suggests that stimulated phagocytosis may not be the major factor in the stimulation of antibody formation by elevated Ca^{2+} levels. The latter stimulation was optimal when the first $CaCl_2$ injection took place at time of iv antigen administration or 6 hr thereafter. According to the present data, however, more than 6 hr must elapse before increased Ca^{2+} concentrations result in increased phagocytosis, and, therefore, it is unlikely that very early events in antibody formation, involving phagocytic cells, are the major target of Ca^{2+}-enhanced antibody formation. Other data (9) indicate that the major contribution of these early, stimulator-susceptible events is over within less than 2 hr after iv antigen administration. Also, when the injection of $CaCl_2$ is begun 6–12 hr prior to antigen administration, no enhancement of antibody formation is found, in fact, an inhibition in the early increase of AFC can be observed under these conditions (1). Yet, according to the present data, this early Ca^{2+} elevation should lead to enhanced phagocytosis at time of antigen administration. What, in fact, appears to occur is that more active scavenger cells compete with the availability of antigen for those cells that are meaningfully involved in the early states of the cellular events leading to antibody formation.[2] The present results, therefore, support other indications (2, 5, 10, 11) that there is no necessary relationship between the rates and extent of overall phagocytic activity and antibody formation, even in the case of antigens that appear to require a macrophage-dependent step.

TABLE I. The Effect of Repeated $CaCl_2$ Injections (400 μg/mouse, iv) on the Clearance of Carbon from the Circulation of Mice.

K = phagocytic index, α = corrected phagocytic index.

Group (hr)	Group (injection)	K ($\times 10^{-3} \pm$ SD)	$\alpha \pm$ SD
Controls		13.00 ± 1.64	4.20 ± 0.42
0	1st	14.88 ± 0.70	4.38 ± 0.36
6	2nd	15.60 ± 0.60	4.37 ± 0.34
12	3rd	27.90 ± 0.76	5.22 ± 0.36
18	4th	27.20 ± 0.58	5.20 ± 0.73
24	5th	27.32 ± 0.70	5.27 ± 0.47
34 (10 hr after end of Rx)		27.18 ± 0.81	5.18 ± 0.34
44 (20 hr after end of Rx)		14.60 ± 0.48	4.16 ± 0.38

Summary. Elevated Ca^{2+} levels in mice produce an enhancement of carbon clearance. On the basis of the time required for the initiation of such effects, it appears that this increased phagocytic activity is not the major factor in previously observed modifications of antibody formation by altered Ca_2^+ levels.

[2] All of these comparisons are based on the assumption that there are no major differences in the timing of Ca^{2+} effects in CFW mice, with which the antibody studies were carried out, and in Rockland Swiss mice which were used in the present tests.

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