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SHAPE CHANGES IN GOOSE ERYTHROCYTES

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Goose erythrocytes were subjected to agents and treatments that produce echinocytosis in human erythrocytes. In the presence of the ionophore A23187 and calcium at greater than micromolar concentrations, goose red cells retained their normal ellipsoidal symmetry, but developed extensive semiregular membrane wrinkles or corrugations. Metabolic NTP (nucleoside triphosphate) depletion, induced either by iodoacetamide or by incubating the cells without a substrate, initially produced a similar cell corrugation, but after prolonged incubation most cells became spherical with the nucleus displaced to the cell periphery. The echinocytic agents indomethacin and dimyristoylphosphatidylcholine had no effect on the gross morphology of goose erythrocytes.

Chicken erythrocytes treated with calcium and the ionophore A23187 undergo shape changes [1,9], indicating that a treatment that leads to echinocyte formation in human red cells [2–4] also has morphological effects in nucleated red cells. Echinocyte formation in human red cells can additionally be induced by several expedients (including ATP depletion [5] and treatment with dimyristoylphosphatidylcholine [6] or indomethacin [7]) some of which appear to act by independent mechanisms [8]. In this study we examine the effects of these agents on the morphology of goose red cells, and compare these effects with the effects of calcium loading.

Blood of the domestic goose (*Anser anser*) was obtained from Microbiological Media (San Ramon, CA) or from the State Veterinary Institute (Helsinki, Finland). The red cells were pelleted and washed three times by resuspension in buffer containing 145 mM NaCl, 15 mM KCl, 11.1 mM glucose and 10 mM Hepes (pH 7.4, high-Na^+ buffer), or with a buffer containing 145 mM KCl, 15 mM NaCl, glucose and Hepes (pH 7.4, high-K^+ buffer). Suspensions were stored until used, always within 5 days of sampling. Cells were examined microscopically before each experiment, and only suspensions consisting of 95–100% smooth ellipsoidal cells were subjected to later treatments.

Effects of intracellular calcium on the shape of goose red cells. Red cells were suspended in the above buffers at hematocrit 20 and made permeable to calcium by addition of A23187 (10^{-5} M). Calcium was added to some samples to yield nominal concentrations from micro- to millimolar (as designated in Fig. 2), and samples were incubated at 40 °C for 2 h. After 0, 15, 30, 60, and 120 min incubation, 5-μl samples were fixed in 4 volumes of ice-cold 1% glutaraldehyde, and the cells examined microscopically as described [8]. To detect any changes in cell volume that might influence cell shape, portions of each sample were measured into tared centrifuge tubes, and plasma and red cells separated by centrifugation. The cell

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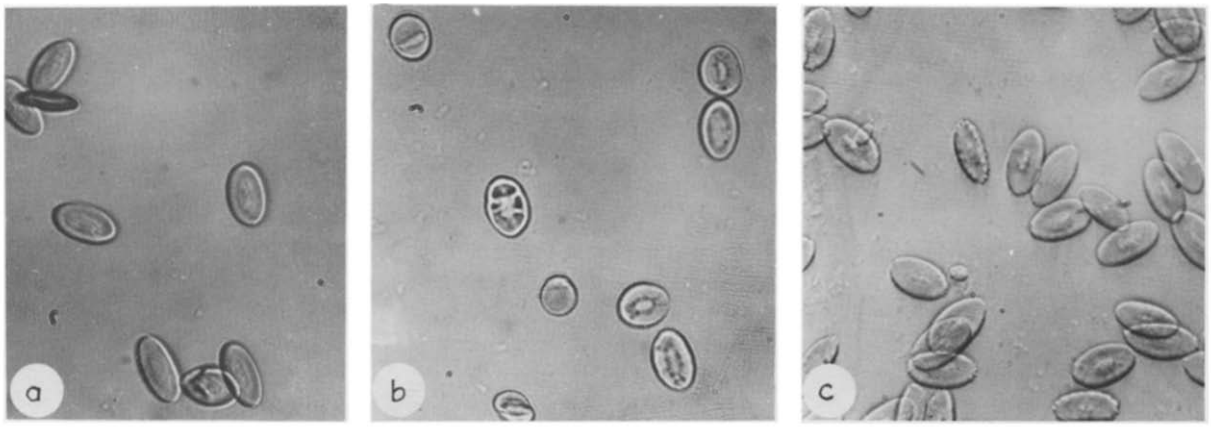

Fig. 1. Shapes of goose red cells. (a) Normal cell. (b) i, Corrugated cell typical of calcium loading or ATP depletion; ii, spherical cell typical of long-term ATP depletion. (c) Cells treated for 2 h with DMPC (5 mg/ml).

pellet was weighed, dried to a constant weight at 105 °C and reweighed. Water content of the cells was then calculated from the wet and dry weights of the samples, taking into account trapped water volume (determined as inulin space [10]).

In the presence of A23187 and extracellular Ca^{2+} above 10^{-6} M, goose red cells undergo a rapid morphology change to present a corrugated appearance (Figs. 1b and 2). The fraction of cells showing this change after 30 min incubation increases with increasing nominal calcium concentration on the range from 10^{-6} to 10^{-4} M. When 10^{-4} M extracellular calcium is complexed with EGTA (10^{-3} M), added A23187 ($5 \cdot 10^{-5}$ M) has no effect on cell shape. This indicates that the morphology change is induced by calcium rather than by the ionophore itself.

In human and toad red cells, calcium loading results in a rapid decrease in cell volume [11] that can be prevented by high concentration of extracellular K^+ (see, for example, Ref. 12). In the present study, possible effects of cell volume changes were examined in two ways. The calcium-dependent morphology change was found to be essentially identical for goose cells suspended in high Na^+ and K^+ buffers (Fig. 2). When cell volumes were measured directly using a cell water assay [10], no significant differences were found in cells incubated for 2 h in the presence or absence of the ionophore and 10^{-5} M Ca^{2+} (Table I), although this treatment produced extensive corrugation already after 30 min; thus, the morphology change does not arise from cell shrinkage. The observed absence of calcium-induced volume changes in goose cells is in agreement with an earlier report on chicken erythrocytes [13].

Effects of ATP depletion on the shape of goose red cells. Red cells were suspended at hematocrit

TABLE I

WATER CONTENT (%) OF GOOSE RED CELLS INCUBATED IN HIGH-Na^+ BUFFER IN THE ABSENCE (CONTROL) AND PRESENCE OF 10^{-5} M Ca^{2+} and A23187

$X \pm$S.E. given, $n = 6$. There were no statistically significant differences in water contents of the two groups, when tested with paired t-test.

	Water content (%)				
	0 min	15 min	30 min	60 min	120 min
Control	60.6 ± 0.7	60.6 ± 0.7	60.1 ± 0.5	61.3 ± 0.4	62.3 ± 0.4
Ca^{2+} + A23187	60.6 ± 0.8	60.1 ± 0.8	60.4 ± 0.8	62.4 ± 0.8	62.3 ± 0.6

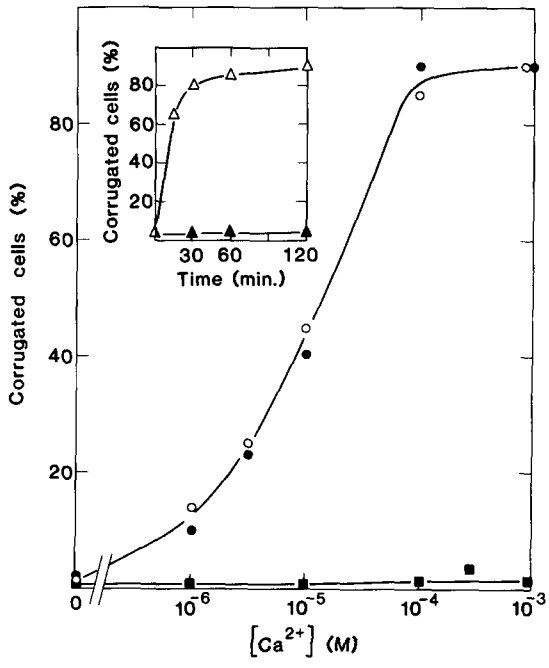

Fig. 2. Morphology change in goose red cells as a function of extracellular calcium concentration, after 30 min incubation in the absence (■) or presence (●,○) of A23187 (10^{-5} M), in high-Na^+ (○, 145 mM Na^+ plus 15 mM K^+) and high-K^+ (●, 145 mM K^+ plus 15 mM Na^+) buffers. Inset shows the time-course of calcium-induced corrugation of cells incubated with calcium (0.1 mM)+A23187 (10^{-5} M) (△) or with calcium alone or with A23187 and EGTA (1 mM (▲)). Values are means of six experiments.

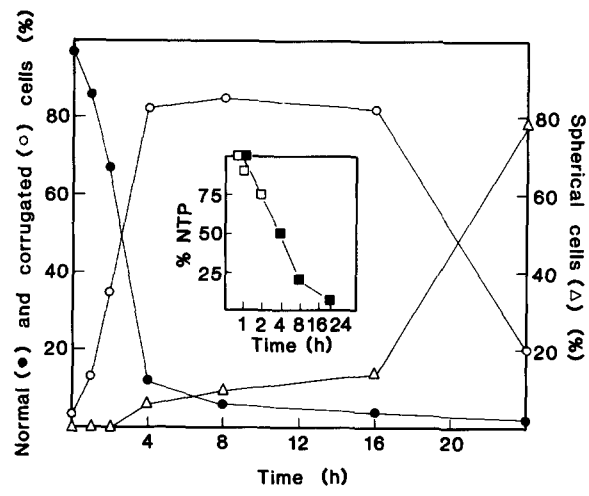

Fig. 3. Effect of iodoacetamide-induced ATP depletion on goose red cell morphology. Normal cells (●); corrugated, ellipsoidal cells (○); spherical cells (△). Inset shows the time-course of ATP depletion in cells loaded with calcium (1 mM) plus A23187 (10^{-5} M) (□), and in cells treated with iodoacetamide (6 mM) plus inosine (10 mM) (■). Values are means of five experiments.

20 in high-Na^+ buffer supplemented with 10 mM inosine and 6 mM iodoacetamide, which produces rapid ATP depletion [14]. For microscopic examination, samples were taken at 0, 1, 2, 4, 8, 16 and 24 h, and treated as described above. Nucleoside triphosphate (NTP) levels of Ca^{2+}-loaded and ATP-depleted cells were determined using sigma test kits (Sigma technical bulletin 366-UV). Although iodoacetamide attenuates the morphology change of chicken red cells, preventing the formation of spherical cells and loss of microvesicles [9], as goose cells lose ATP they initially develop the same corrugated appearance as induced by calcium loading (Figs. 1b and 3). After extended incubation the proportion of corrugated drops and most cells become spherical and small with nuclei displaced to the periphery of the cell (Fig. 1b). In their final form, these cells resemble the calcium-loaded chicken cells described by Allan et al. [1].

Corrugation and spherical cell formation also takes place when the cells are incubated at 40 °C in high-Na^+ buffer without the substrate (glucose). After 24 h incubation 74% of the cells were corrugated, and 8% spherical, after 48 h the percentages were 60 and 32, and after 72 h 5% and 95% (values means of four experiments), respectively. Spherocytosis seems to be due to the loss of membrane from the cells, as cell fragments were visible in the micrographs.

The corrugation and spherical cell formation of ATP-depeleted cells is not dependent on extracellular calcium, as these morphological changes occurred even in the presence of 10^{-3} M EGTA in the incubation medium.

Effects of other echinocytic agents. In human red cells both ATP depletion and calcium loading cause the formation of echinocytes and later spheroechinocytes. Echinocytes are also formed when human cells are treated with DMPC or indomethacin for short periods [6,7]. Ott and coworkers [6] showed that shape changes induced by DMPC occur without appreciable depletion of cellular ATP; they suggested that DMPC acts by expanding the cell membrane outer monolayer, as in the bilayer couple mechanism proposed by

Sheetz and Singer [15]. Similarly, Fujii et al. [7] suggested that indomethacin induces cell crenation by preferential distribution into the membrane outer monolayer. To test how DMPC and indomethacin influence the shape of goose red cells, the following experiments were carried out. DMPC (5 mg/ml) was added to high-Na^+ buffer and sonicated in Branson B-220 sonicating bath until a clear suspension was obtained. Red cells were suspended in this buffer at a hematocrit count of 20, and the suspension incubated at 40 °C for 2 h. Samples were taken for microscopic examination after 5, 15, 30, 60 and 120 min. Another batch of cells was treated with indomethacin (concentrations ranging from 10^{-6} to $2 \cdot 10^{-3}$ M) in high-Na^+ buffer (at hematocrit 20) for 15 min at 40 °C. Samples were fixed and examined microscopically as above. Neither of these treatments caused any alterations in the gross morphology of goose erythrocytes: the cells retained their ellopsoidal symmetry, and did not form corrugations. However, as shown by Fig. 1c, small irregularities were observed on the surface of the DMPC-treated cells after 2 h incubation.

These data indicate that corrugation and cell sphering in goose red cells apparently are not caused by simple expansion of the outer monolayer of the cell membrane. Also, it is doubtful that the dissolution of marginal bundles of microtubules, which disappear during calcium loading [1], as such causes the shape change, since other studies on nucleated cells have shown that the marginal bundles can be 'dissolved' without affecting the cell shape [16,17]. Further, 1 h cold treatment, which causes the disassembly of the marginal bands of microtubules in dogfish red cells, has no effect on goose cell shape in the present study (not shown). Therefore, it is possible that the cytoskeletal system (composed mostly of actin and spectrin or spectrin-like proteins in nucleated red cells [17,18]), plays a role in the retention of ellipsoidal shape. Thomas and coworkers [9] have shown that alfa-spectrin, goblin and microtubule-associated proteins undergo a limited proteolysis during calcium and A23187 treatment and consecutive morphological changes. Also, the flattened ellipsoidal symmetry of anucleate camel red cells is lost only upon complete spectrin extraction [19]. Other cell proteins might also participate; Khodadad and Weinstein [20] have suggested that the high concentration of band 3 in ellipsoidal llama red cells makes them more resistant to shape changes than human red cells.

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