changes associated with clumping. Furthermore, the magnitude of the net red cell charge is very small and was never reduced by more than about half when the red cells clumped. To show the smallness of the net charge magnitude one has only to assume a cell count of 5×10^6 cells/mm^3 and to calculate that if the excess cellular charges were distributed uniformly throughout the solution then they would have a concentration of only about 50 nanomoles/liter.

Although the ionic effects are thus of a minor magnitude compared to the observed energy requirements for separating sludged red cells, the roles of surface energy and viscous frictional losses remain to be considered. To test the possible role of surface energy changes the above energy and force data can be combined with Norris' observations of the surface energies of large nucleated red cells (about 1 erg/cm^2)(6), and with reasonable estimates of the surface area changes that accompany sludging. This consideration shows that surface energy changes could be large enough to account for major portions of at least some of our observed total energy expenditures. The surface energy of a given interface, however, is usually proportional to the area of that interface, while the observed energy consumptions did not appear to vary with pulling distance in a way that would be consistent with that proportionality.

Specifically, if we assume that the intracellular sludge strings remain cylindrical during stretching, then the surface area and surface energy should increase as the square root of the growing intercellular distance ($x_f - x_i$ in equation 2. Actually, though, the observed forces were all like those of Table I in increasing monotonically with that distance—so that the energy requirement always increased as either the first power, or a higher power, of that intercellular separating distance. Hence it follows that at least a major part of the observed energy requirements for separating sludged cells is still unexplained and may later be shown attributable to more complicated phenomena, such as those of viscous flow.

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In vivo Effects of Ouabain on Calcium Metabolism in Rabbit Hearts and Plasma.* (30061)

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There is considerable *in vitro* evidence suggesting that the cardiac glycosides produce their effects on cardiac muscle by altering calcium (Ca) metabolism(1). The present study was undertaken to determine if an effect of the cardiac glycosides (ouabain) on Ca metabolism of the heart could be demonstrated under *in vivo* conditions.

Methods. Rabbits weighing between 1 and 2 kg were used. Ca determinations were done as follows: The rabbits were anesthetized with pentobarbital and the ear vein

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cannulated. 1 ml ouabain (1 ml = 0.1 mg) was then infused every 20 minutes until the animal showed toxic changes on the electrocardiogram. The chest was then immediately opened and 10 cc of blood was taken from the heart in a heparinized syringe and the heart immediately removed. The blood was centrifuged at 5,000 rpm for approximately 10 minutes. The plasma was removed and recentrifuged for another 5 minutes. 0.5 ml plasma was then withdrawn and added to 6 volumes of 6.2% trichloroacetic acid (TCA) and centrifuged at 10,000 *g* for 10 minutes. One ml supernatant was removed and added to 4 ml of an organic diluting fluid as described by Geyer and Bowie(2) for determination of the total plasma Ca. To determine the ionized Ca concentration, the remainder of the plasma was put into a dialysis bag and centrifuged at 5,000 rpm for 1 hour. The dialysate was collected, added to 3 volumes of 6.2% TCA, and centrifuged at 10,000 *g* for 10 minutes. One ml of supernatant was then added to 4 ml of organic diluting fluid for Ca determinations.

The heart which had been removed was opened, blotted, and trimmed so that only the ventricles remained. The ventricles were weighed and homogenized in 9 volumes of 6.2% TCA and centrifuged at 10,000 *g* for 10 minutes. One ml of supernatant was taken and added to 4 ml of organic diluting fluid. The Ca contents of the heart, whole plasma, and non-protein bound plasma were then determined by the direct micro method using the flame photometer with photomultiplier attachment as described by Geyer and Bowie (2). For each ouabain treated rabbit, a control was used in which the infusion was carried out for an identical period of time without ouabain present.

For the ^{45}Ca experiments, the rabbit carotid on one side was cannulated and 0.5 ml of heparin infused. Each rabbit then received an injection of ^{45}Ca solution containing approximately 1 million counts per ml per minute (1 ml/kg). The ^{45}Ca was obtained from Oak Ridge National Laboratories as the chloride and diluted in distilled water. Ca disappearance rate was determined by withdrawing blood samples at periodic intervals from the carotid cannula in both control and ouabain treated animals. ^{45}Ca uptake experiments were performed as follows: After injection of ^{45}Ca solutions, blood samples were withdrawn every 20 minutes following a one-hour equilibration period. After the first sample was collected, 0.1 mg ouabain (0.1 mg = 1 ml) was infused every 20 minutes until ECG toxicity appeared. The heart was then removed, ground in 9 volumes of 6.2% TCA, and centrifuged at 10,000 *g* for 10 minutes. An aliquot of the supernatant was then counted for radioactivity. A control for each ouabain treated rabbit was run under identical conditions for exactly the same time interval (between 20-60 minutes) without the presence of ouabain.

The specific activity of the tissue (SA_T) and media (SA_M) and percentage exchange were determined as follows:

$$SA_T = \frac{^{45}Ca\ (epm)/g\ tissue}{Ca\ (meq)/g\ tissue}$$

$$SA_M = \frac{^{45}Ca\ (epm)/ml\ plasma}{Whole\ plasma\ Ca\ (meq)/ml\ plasma}$$

$$\%\ exchange = \frac{SA_T}{SA_M} \times 100.$$

Results. The mean half time of ^{45}Ca disappearance from the plasma was 66 minutes. There was no difference between controls and ouabain treated animals in this respect.

In Table I we present the effects of ouabain on myocardial Ca content, Ca plasma concentrations, and percentage Ca exchange. It should be noted that ouabain had no effect on myocardial Ca content or Ca plasma concentrations. There was also no significant difference in the non-protein bound Ca concentrations. However, there was a significant increase in the percentage of Ca exchange.

Discussion. There is now considerable *in vitro* data which indicate that Ca is intimately involved in the action of the cardiac glycosides on the heart. Lüllmann and Holland found that in perfused rabbit ventricles contracture was associated with a 9-fold increase in the exchangeable Ca fraction but no appreciable change in tissue Ca(1). Evidence is presented here which suggests that under *in vivo* conditions ouabain toxicity is also associated with a change in Ca metabolism of a similar nature. Thus, although there

TABLE I. Myocardial and Plasma Ca Concentrations.

Conditions	Ca myocardial content (meq/kg wet tissue)	% myocardial Ca exchange	Ca plasma concentration (meq/l of plasma)	
			Whole plasma	Non-protein bound
Control	6) 4.43 ± .18	5) 30.5 ± 2.4	6) 7.89 ± .33	6) 4.66 ± .21
	*p > .9	*p = .01–.001	*p > .9	*p > .5
Ouabain	6) 4.57 ± .48	5) 45.0 ± 8.0	6) 8.12 ± .43	6) 4.27 ± .51

Values = mean ± S.E.M. Figures preceding the mean indicate number of experiments.
* Probabilities of statistically significant differences calculated by the "chi-square" test.

was no significant change in tissue Ca concentrations in ouabain treated rabbits, there was a significant increase in Ca exchange (Table I). These studies lend support to the *in vitro* observations and indicate that cardiac glycoside (ouabain) toxicity is associated with a change in cardiac Ca metabolism in the intact animal.

It is possible that ouabain could affect Ca binding to plasma proteins and thus change the concentration of ionized Ca in the interstitial spaces. We were, however, unable to measure any significant affect of ouabain on the whole plasma and non-protein bound Ca concentrations (Table I).

It should be noted that the rabbit has a high plasma Ca concentration (7.89-8.12 meq/l) (Table I) as compared to other vertebrates including man(3). These species differences cannot be accounted for solely by the level of plasma proteins(3). Our values, although somewhat higher, are in the same range as those obtained by Cole *et al* of 7.68 meq/l (range 5.2 to 10 meq/l)(4). The ionized Ca concentration of rabbit plasma would also likely be high since 95% or more of the non-protein bound Ca of 4.27-4.66 meq/l (Table I) is probably ionized(5). This suggests that in the rabbit the extracellular ionized Ca concentration *in vivo* may be considerably higher than *in vitro* when artificial solutions are used. The ionized Ca concentration of modified artificial solutions varies considerably depending on the electrolyte and buffer content and pH. Many solutions used contain much less than 3 meq/l of ionized Ca. These differences may be important when comparing *in vivo* and *in vitro* Ca experiments in the same or different species.

Summary. The *in vivo* effects of ouabain on rabbit myocardial and plasma Ca metabolism were determined. It was found that ouabain toxicity was associated with a significant increase in myocardial Ca exchange but no significant change in myocardial Ca content. The whole plasma Ca and non-protein bound Ca concentrations were high as compared to other vertebrates. Ouabain had no significant effect on these parameters. It was concluded that the *in vivo* effects of ouabain were similar to that seen *in vitro* using artificial solutions and that a change in Ca metabolism may be the basic cause of *in vivo* cardiac glycoside toxicity. In addition, it would appear that the concentration of extracellular ionized Ca of the rabbit *in vivo* is considerably higher than that which occurs when using many artificial solutions.

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