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# The effect of calcium on rat lens permeability

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*In the absence of calcium, the penetration of sucrose and mannitol into the rat lens is increased, while that of inulin remains unchanged. The extracellular space of the lens, estimated with inulin, is 6 per cent of the wet weight of the lens. The active accumulation of potassium and rubidium is 65 per cent of normal in the absence of calcium, while the uptake of  $\alpha$ -aminoisobutyric acid (AIB) is only 20 per cent of normal. The loss of rubidium from the lens in the absence of calcium is considerably greater than that of AIB. The increased permeability of the lens shown with rubidium is sufficient to account for the change in concentration of potassium and sodium which takes place when the lens is incubated in calcium-deficient media. Sodium penetration into the lens is also calcium dependent, with the sodium space nearly equal to that of the lens water, in the absence of calcium.*

Interest in the effect of calcium on the lens stems from reports that lens opacities are occasionally observed in hypoparathyroidism. Studies of hypoparathyroid cataracts produced in animals have not yet been fruitful in elucidating the mechanism of this type of cataract. Perhaps more promising has been the *in vitro* approach, in which striking effects of calcium deficiency on the lens have been observed. In early experiments, opacities were observed in lenses incubated in medium deficient in calcium.<sup>1</sup> Later work showed a marked reduction in the usual high potassium-sodium ratio in lenses incubated in

calcium-free medium.<sup>2</sup> While the normal distribution of cations in the lens is altered during incubation in the absence of calcium, the rate of glycolysis remains normal, so it seems unlikely the change in cation levels is caused by a deficiency in biological energy.<sup>3</sup>

The work reported here is an extension of these *in vitro* studies and was undertaken to delineate more clearly the effect of calcium deficiency on lens permeability. The study showed that the absence of calcium in the medium affects the permeability of the lens in such a manner so as to alter the accumulation of certain carbohydrates, potassium, rubidium, sodium and  $\alpha$ -aminoisobutyric acid (AIB).

## Methods

Sprague-Dawley strain rats, weighing approximately 100 grams, were used throughout. Immediately after decapitation, the globes were excised and the lenses removed via a posterior approach. With forceps, the lenses were cleaned carefully of adherent retina and vitreous body and

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then transferred on a wire loop to modified Merriam-Kinsey tubes containing 4 ml. of medium.

The medium composition was 0.462 mM.  $MgSO_4$ , 0.241 mM.  $Na_2HPO_4$ , 0.252 mM.  $KH_2PO_4$ , 3.33 mM.  $KCl$ , 23.1 mM.  $NaHCO_3$ , 0.840 mM.  $KHCO_3$ , 5.0 mM. glucose, and additional  $NaCl$  to bring the tonicity to 295 mOsm. The  $^{14}C$ -labeled mannitol, sucrose, inulin, or AIB was added in tracer amounts. In some of the experiments, non-radioactive material to give a concentration of 10 mM was added. The radioactive isotopes used,  $^{42}K$ ,  $^{24}Na$ , and  $^{86}Rb$ , were obtained from the Iso-Serve Corp., Cambridge, Mass. When  $^{86}Rb$  was used, all potassium was omitted from the medium and replaced with 5 mM. of nonradioactive rubidium. Calcium was added to the medium to achieve a final concentration varying from 0 mg. per cent to 12 mg. per cent. No attempt was made to deplete the lenses of calcium before beginning the experiments. The pH of the medium was maintained at 7.45 by continual equilibration with a 5 per cent  $CO_2$ -95 per cent  $O_2$  atmosphere.

All incubations were carried out at  $37.5^\circ C$ . for a 5 hour period, unless noted otherwise. At the conclusion of the incubation period, the lenses were removed from the culture tubes, blotted on filter paper, and weighed. Lens water was taken as 61.1 per cent of the wet weight of the lens, as determined from wet weight/dry weight ratios obtained with a number of unincubated lenses. Those incubated lenses which were cloudy, or those which had gained more than 7 per cent of the weight of the second lens of the pair, were discarded. The rejection was independent of the calcium level of the medium. Even with no calcium in the medium there was no consistent change of lens weight during incubation. However, the absence of calcium did cause consistent weight gain when ouabain was present in the medium. For this reason it was necessary to include swollen lenses at 0 mg. per cent calcium in the experiments to determine rubidium loss from the lens.

The lenses were homogenized in 1 ml. of 10 per cent trichloroacetic acid and centrifuged. Aliquots of the deproteinized lens filtrate and the final medium were then taken for radioactive assay in a liquid scintillation counter.<sup>4</sup> With the relatively large volume of medium used for culture, the specific activity of substances in the medium did not decrease significantly, despite accumulation by the lens.

To study the reversibility of the effect of calcium on permeability, lenses were incubated initially for 3 hours in the above medium, with either 0 mg. per cent or 3 mg. per cent calcium present. At the end of this time, the medium was withdrawn from each tube and replaced with fresh medium containing tracer and the appropriate amount of calcium. The experiment was

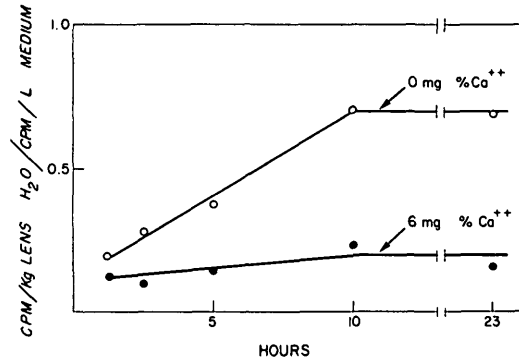


Fig. 1. The effect of calcium deficiency on the penetration of sucrose into the lens. The values represent the mean of at least five lenses.

terminated at the end of an additional 3 hours of incubation.

## Results

The penetration of substances into the lens is presented as the distribution ratio between the lens and medium, expressed as counts per minute (CPM) per kilogram of lens water, divided by CPM per liter of medium.

Fig. 1 shows the effect of calcium deficiency on the penetration of sucrose into the lens. The distribution ratio of sucrose for intervals of 75 minutes to 23 hours indicates that even prolonged incubation does not result in a sucrose "space" of more than 20 per cent of the lens water. The term "space" refers to a volume which would be occupied by a particular substance, if the concentration of the substance in that volume of lens water were equal to the concentration of the substance in the external medium, and is expressed as the per cent of the total lens water. The penetration of sucrose into lenses incubated in a calcium-free medium (discounting the small amount of calcium which leaches from the lens into the medium) increases linearly for the first 10 hours, until sucrose occupies about 70 per cent of the lens water.

Experiments similar to those presented in Fig. 1, but employing inulin, show that the inulin space at the end of 75 minutes is

10 per cent of the lens water, and that this space essentially remains unchanged throughout 5 hours of incubation. The inulin space is independent of the calcium concentration of the medium, as shown in Fig. 2, where the spaces occupied by in-

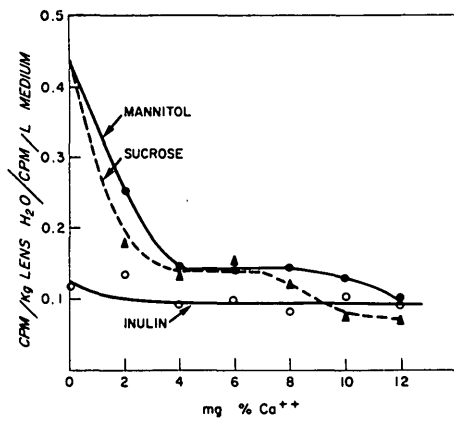


Fig. 2. The effect of calcium on the inulin, mannitol, and sucrose spaces of the lens. The values represent the mean of at least three lenses.

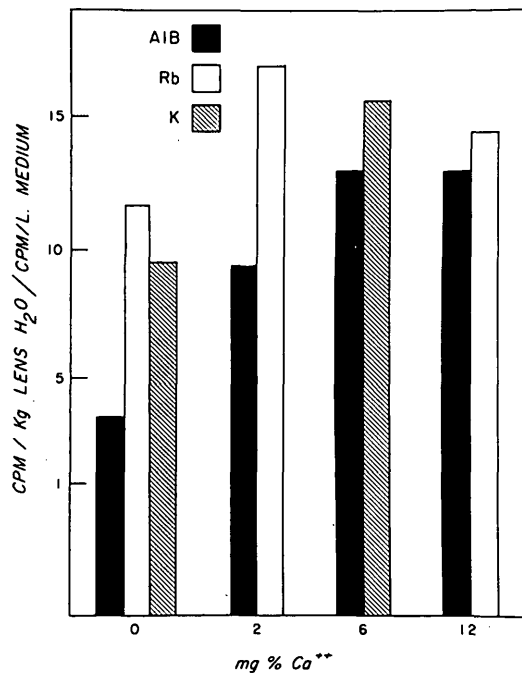


Fig. 3. The effect of calcium on the accumulation of rubidium, potassium, and AIB by the lens. The values shown represent the mean of at least five lenses.

ulin, mannitol, and sucrose after 5 hours of incubation are compared. On a wet weight basis, the extracellular space of the lens, estimated with inulin, is 6 per cent of the total lens. Mannitol penetration and sucrose penetration are quite dependent on the calcium concentration and are not distinguishable from each other. At 6 mg. per cent calcium, the space for mannitol and sucrose is slightly larger than that for inulin. At 12 mg. per cent, the space for these three carbohydrates is identical.

Table I shows that, although the penetration of sucrose into the lens is dependent on the concentration of the molecule in the medium, the distribution ratio and the space are independent of the external sucrose concentration.

The reversible nature of the effect of calcium deficiency on lens permeability is shown in Table II. The lenses were incubated without <sup>14</sup>C-labeled sucrose for 3 hours, with calcium present or absent, as indicated. Labeled sucrose then was added as the calcium concentration in the medium was changed. The concentration of the radioactive molecule achieved during the final 3 hour incubation period shows the restoration of relative impermeability by calcium added at the same time as the tracer.

The effect of calcium on the accumulation of rubidium, potassium, and AIB is shown in Fig. 3. The uptake of rubidium, both in the presence and absence of cal-

Table I. The effect of calcium on sucrose penetration into the lens

Mg. % calcium	0.015 mM. sucrose	10.0 mM. sucrose
0	0.369 ± 0.119 (5)	0.439 ± 0.115 (4)
2	0.169 ± 0.015 (3)	0.180 ± 0.060 (3)
4	0.169 ± 0.041 (4)	0.134 ± 0.032 (3)
6	0.122 ± 0.030 (3)	0.158 ± 0.072 (3)
8	0.104 ± 0.013 (3)	0.121 ± 0.011 (3)
10	0.141 ± 0.051 (4)	0.077 ± 0.018 (3)
12	0.095 ± 0.010 (5)	0.096 ± 0.008 (3)

Values shown are the ratios of CPM/Kg. lens water divided by CPM/L. of medium, plus or minus their standard deviations. The number of lenses is shown in parentheses.

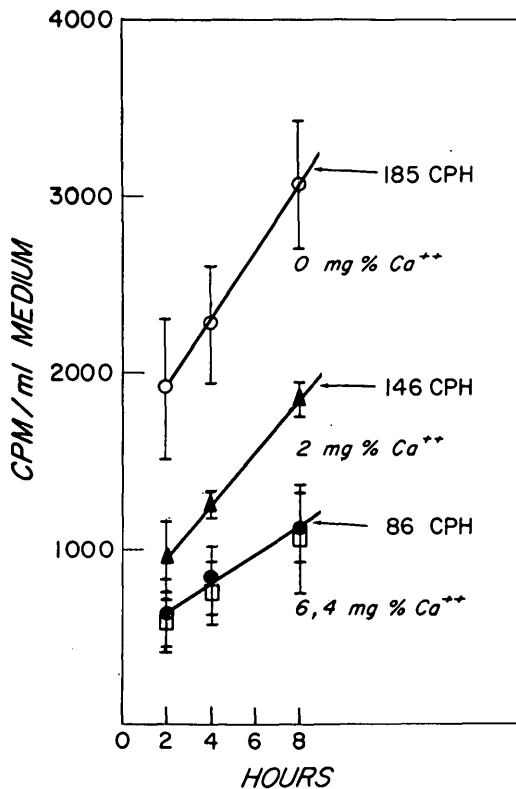


Fig. 4. The effect of calcium on rubidium loss from the lens. Lenses were preloaded with <sup>86</sup>Rb for 2 hours and then incubated in rubidium-free medium as described in the text. The values represent the concentration of radioactivity in the medium at the end of each time interval, and are bracketed by their standard deviations. The differences between the concentrations achieved with 4, 2, and 0 mg. per cent calcium are significant at  $p < 0.01$  for the 8 hour interval. The rate of loss is expressed in terms of counts per hour per milliliter of medium (CPH).

Table II. The reversal of lens permeability by calcium

Initial calcium	Final calcium (mg. %)	Ratio L./M
No initial incubation	0	$0.296 \pm 0.052$ (5)
0 mg. %	0	$0.268 \pm 0.056$ (6)
3 mg. %	3	$0.100 \pm 0.011$ (6)
0 mg. %	3	$0.125 \pm 0.022$ (4)

Lenses were incubated for 3 hours with the calcium concentration indicated, followed by a final 3 hour incubation with the calcium level changed as shown. Tracer sucrose was added at the beginning of the final incubation period. The distribution ratios are CPM/Kg. lens water divided by CPM/L. of medium (L./M.), plus or minus their standard deviations. The number of lenses is shown in parentheses.

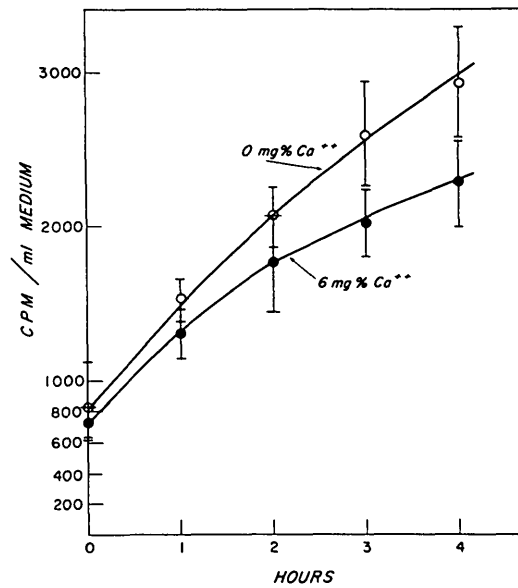


Fig. 5. The effect of calcium on AIB loss from the lens. Lenses were preloaded with AIB for 90 minutes and then incubated in AIB-free medium containing 0 and 6 mg. per cent calcium. The values represent the concentration of radioactivity in the medium at the end of each time interval, and are bracketed by their standard deviations. The difference between the concentrations achieved with 0 and 6 mg. per cent calcium at the end of 4 hours is significant to  $p < 0.05$ .

cium, is virtually the same as that of potassium ion. The distribution ratio of potassium and rubidium achieved in the absence of calcium is 65 per cent of that observed in the presence of the divalent ion. On the other hand, the uptake of AIB in the absence of calcium is only 20 per cent of the control.

The loss of rubidium and AIB from the lens is dependent also upon calcium, as shown in Figs. 4 and 5. To demonstrate rubidium loss, lenses were incubated in a medium containing tracer rubidium, 5 mM. nonradioactive rubidium, and 6 mg. per cent calcium. At the end of 2 hours the medium was replaced with one containing no rubidium, 5 mM. potassium,  $10^{-5}$  M ouabain, and variable concentrations of calcium. The ouabain was added to this medium to stop the active uptake of rubidium by the lens, so that accumulation of

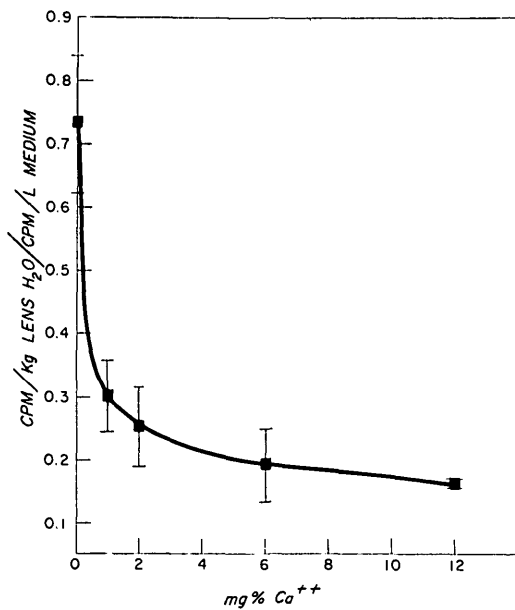


Fig. 6. The effect of calcium on the penetration of sodium into the lens. The values are bracketed by their standard deviations.

radioactivity in the medium would not be influenced by active transport of the ion into the lens for a second time.<sup>5</sup> The rate of rubidium loss into the medium, as shown in Fig. 4, is nearly doubled by lowering the external calcium concentration from 6 mg. per cent to 2 mg. per cent. It is further increased when calcium is absent from the medium.

A similar but less striking result is obtained with AIB, and is shown in Fig. 5. In this case, the initial incubation medium contained a tracer level of AIB. After 90 minutes a medium containing no AIB, but 10 mM. methionine, was substituted. Methionine at this concentration effectively blocks AIB uptake under the conditions of the experiment.<sup>6</sup> The rate of appearance of AIB in the medium in the absence of calcium is significantly higher than that at 6 mg. per cent calcium.

The accumulation of sodium is greatly enhanced by the absence of calcium, as is shown in Fig. 6. The sodium space is over 70 per cent of the lens water in the absence of calcium and is restricted to 20 per cent

when the medium contains 6 mg. per cent of calcium.

### Discussion

The penetration of sucrose and mannitol into the lens is dependent upon the calcium level of the external medium, and is similar to the results observed in kidney tissue slices.<sup>7</sup> The penetration of inulin, however, is calcium independent. The difference may be related to the size of the three molecules. While the molecular weight of sucrose is higher than that of mannitol, their molecular radii are estimated to be nearly equal, at about 4.5 Å.<sup>7, 8</sup> This similarity in molecular size is reflected in their equal penetration into the lens. The inulin molecule weighs much more, however, and is probably somewhat larger. If the entrance of these nonlipid soluble, non-metabolized molecules into the lens is through water-filled channels whose radius is near that of mannitol and sucrose, a small change in the radius brought about by a variation in the calcium concentration would have considerable effect on the penetration of these two molecules, but might not alter the penetration of inulin. Depending upon the tissue studied, and the method used, pore size in membranes has been estimated to be very similar to the size of the mannitol and sucrose molecules.<sup>8</sup> It has been suggested that the diameter of such pores may be determined in large part by calcium ions clinging to their inner surfaces.<sup>9</sup>

The calcium-responsive barrier to these molecules probably is located at the surfaces of epithelial cells and individual lens fibers, and not in the capsule. It has been shown by Friedenwald<sup>10</sup> that the diffusion of sucrose across the capsule is only slightly slower than the very rapid diffusion of dextrose. While there was a marked reduction in the rate of passage of gentian violet across the capsule when calcium was added to the solution,<sup>11</sup> there was almost no reduction in the high rate of dextrose transfer under the same conditions. He concluded that the capsule offers a perme-

ability barrier to colloid, but not solute, molecules. In the present experiments, 6 to 8 per cent of the lens is the minimum amount of space occupied by any test substance. This volume probably represents all of the lens water external to the cytoplasm of the epithelial cells and the individual fibers, or the "extracellular space." When there is no change in water, a space larger than this value may represent penetration into the cells and fibers themselves. The extracellular space obtained with inulin is somewhat smaller than the "available space" of 13.5 per cent obtained with sucrose in rabbit lens.<sup>12</sup> Unless a calcium level of 10 to 12 mg. per cent is present in the medium, however, a sucrose space of about this size is found also in rat lens.

The work of Harris<sup>2</sup> showed a marked decrease in potassium concentration when lenses were incubated without calcium. This effect was shown to be reversible,<sup>13</sup> as is the change in permeability shown in the present experiments. One would expect the reduction in net accumulation of radioactive potassium and rubidium to be the result of the same circumstances which lead to a decrease in the level of potassium in the lens. This observed decrease in uptake of potassium and rubidium can take place in one of three ways: by a decrease in the energy-requiring inward flux; by an increased outward flux, probably caused by increased permeability; or by a combination of the two processes. Flux, expressed as the quantity of substance transferred per unit area per unit time, could not be measured appropriately in our experiments. However, a qualitative measure of the rate of loss of rubidium from the lens shows it to be more than doubled as the calcium concentration approaches 0 mg. per cent. The net accumulation of the cation, on the other hand, is somewhat greater than would be expected with such a significant leak from the membranes. Becker<sup>14</sup> found the uptake of rubidium in rabbit lens incubated in the absence of calcium to be only 20 per cent of normal, a value more in keeping with

the greatly increased permeability found in the present experiments with rat lens. If, however, the "cation pump" can increase its rate of uptake to compensate for the increased leakiness of the membranes, a smaller decrease in net accumulation would be apparent. There is evidence to suggest that calcium deficiency does not interfere with the action of the cation pump.<sup>15</sup> The present work indicates that the increased permeability of the rat lens in calcium deficiency is sufficient to account for the decrease in potassium concentration and increase in sodium concentration which occur in such lenses.

Although the uptake of AIB is quite dependent upon calcium concentration, the rate of loss of AIB is only slightly increased in the absence of calcium. The results suggest that the amino acid pump may be inhibited directly by calcium deficiency.

The increased permeability to sodium found in calcium deficiency is somewhat larger than would be expected from a change in the potassium level alone. This phenomenon has been found in frog muscle also.<sup>16</sup> The sodium space found at higher levels of calcium is similar to that found in the rabbit lens.<sup>17</sup> Since this amount of sodium is larger than that which can be accounted for by extracellular water, the remainder must be present within the epithelial cells and fibers. Under normal circumstances, about one half of the sodium in the lens can be assigned to the extracellular space.

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