THE EXTENT TO WHICH VARIOUS METABOLITES REPLACE GLUCOSE AS ENERGY SOURCE FOR CATION TRANSPORT IN THE INTACT RABBIT LENS IN VITRO

by

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The intact lens like cells in general maintains a high concentration of potassium and a low concentration of sodium although bathed in a medium with high sodium and low potassium concentrations. The maintenance of this disparity between the intralenticular and extralenticular concentrations of cations must be accounted for either by the fact that the limiting barrier is impermeable to cations, or that an active "pump" is superimposed on a barrier which normally passes these positive ions. That the former is unlikely is evidenced by the fact that radioactive sodium and potassium have been shown to exchange with the cations of the lens substance.[1,2]

A large body of evidence to support the "pump" theory has accumulated from a variety of studies closely linking the metabolic activity and the cation content of the lens.[3-7] When normal metabolic intensity is reduced by any of a variety of means the concentrations of sodium and potassium in the lens approaches equilibrium with the surrounding medium. Such a true equilibrium is approached in vitro when the lens is maintained at a low temperature, subjected to anaerobic conditions, or bathed in a medium containing certain metabolic poisons such as cyanide, fluoride, or iodoacetate.[3] Hydration of the lens accompanies this shift in cations.[7] This probably is due to the osmotic pressure exerted when a system approaches the Donnan equilibrium. Parenthetically it might be mentioned that these changes mirror those which occur during cataract formation.[8] As a cataract develops the lens loses potassium and gains sodium and water. The oxygen consumption and glucose utilization of a cataractous lens is greatly reduced.[8]

Of great significance is the fact that this in vitro shift of cations which occurs when the metabolic intensity is reduced can be reversed if the metabolism is normalized. Such a reversible system is best achieved with temperature alterations. Thus in a suitable medium the loss of potassium and gain of sodium induced by refrigeration at 0° C. is reversed when the lens is subsequently incubated at 37° C.; potassium reentering the lens and sodium being excreted from it.[4] (Figure 1) This restoration of the cation distributions following refrigeration requires the movement of both cations against concentration gradients and therefore necessitates the expenditure of energy. This reversible shift has also been demonstrated in other cells or cellular structures such as the erythrocyte[9,10], and the retina[11].

The recovery to normal cation distributions following this cold-induced cation shift is altered by a variety of situations. For example, it is either reduced or abolished in the absence of glucose[5], anaerobic conditions[3], or when certain metabolic poisons such as cyanide, fluoride, or iodoacetic acid[5] are included in the incubating medium.

From these studies it has been concluded that the limiting barrier, or barriers, of the lens are permeable to the positive ions (and probably the diffusible anions as well) and that the concentration gradients normally encountered are maintained by the active transport of cations by an energy consuming "pump". Previous studies have shown that this movement of potassium and sodium is generally equivalent. Thus it has not been possible to determine whether separate pumps exist for sodium and potassium or whether, as has been suggested for other cells[12], sodium is actively

transported by the lens and potassium enters to maintain electrical neutrality.

The site, or sites, of the lenticular barriers are not known with certainty. Strong evidence suggests that the lens capsule or some closely adjacent structure may act as a cation-permeable membrane across which the cation "pump" functions. (The lens is an avascular structure, relatively acellular, with a high protein concentration. It is encompassed by a non-cellular, thin membrane, the capsule. This capsule like other cellular membranes contains a substantial amount of polysaccharide.[13]) First, when the capsule is nicked with a sharp blade, care being taken to avoid injury to the underlying lens, the cation concentration in the lens approaches that in the bathing medium and recovery of a cold-induced shift is virtually abolished.[7] Second, very gentle massage of the lens surface with a blunt instrument produces a marked reduction in the ability of the lens to concentrate cations even though no alteration in glucose utilization occurs.[7,14] Third, when calcium is deleted from the bathing medium a similar reduction is noted in the extent to which a return to the normal cation steady state can be achieved during incubation at 37° C. following refrigeration at 0° C. (It is well known that calcium in the external medium markedly influences the permeability of animal membranes; when this ion is absent permeability is increased, and upon its addition the permeability is decreased.)[15] The possibility that there is an additional barrier, e.g., the lens fiber membrane, cannot be excluded.

This resemblance of the lens capsule to a cell membrane suggests that the lens is physiologically analogous to a single cell rather than a tissue.

Further support of this analogy is easily found. The lens is avascular and derives its entire nutrition across the capsule. Likewise, like that of the cell, the metabolism of the lens is compartmentalized,[16] the most intensive metabolic activity occurring near the lens surface.

In many respects, then, the lens provides an excellent tool for the study of cation exchange across cellular barriers in general. The major advantages of the lens for such studies accrue from the facts that the lens can be removed for in vitro studies with little disturbance to its normal environment and that one lens generally suffices for an analytical situation.

Previous studies have indicated that the energy for active cation transport across the lenticular barriers generally derives from the metabolism of glucose and to an undetermined extent requires oxygen[8]. (It is difficult to prove by other means that any given substrate is essential to the lens. A normal metabolism is undoubtedly necessary for the continued growth and transparency of the lens. However, these functions are slow and their measurements in an acute experimental situation are not sufficiently sensitive to be of value.) It was the original purpose of these studies to determine whether various metabolites delivered to the lens in place of glucose could support the cation "pump". As the studies progressed it became apparent that the effect of lens age on the cation concentrating mechanism was of greater significance than had previously been recognized. It became necessary, therefore, to expand the thesis to include considerable data on the effect of lens size on cation transport under various conditions.

MATERIALS AND METHODS:

Fresh intact rabbit lenses were used throughout. Generally eyes were obtained from a rabbit meat company. They were enucleated immediately following death by decapitation, placed in ice-cold saline, and transported to the laboratory packed in ice. Lenses were removed within one hour of the death of the animal using aseptic precautions. Lens extraction was accomplished by incising and turning back the sclera, removing the vitreous with a lens loop, and carefully cutting the zonules with a sharp scissors. In this procedure particular care was taken to avoid touching the lens surface with the lens loop or scissors. The isolated lens, resting on its posterior surface, was placed in a sterile tube containing 3.0 ml. of the appropriate media. The tubes were flushed with a mixture of 95% oxygen and 5% carbon dioxide (95% nitrogen and 5% carbon dioxide when anaerobic conditions were imposed), tightly stoppered, and placed in an ice bath at 0° C. After refrigeration for 40-44 hours one lens of each pair was analyzed immediately, while the other was transferred to a water bath at 37° C. and incubated for an additional six hours before analysis. For the purposes of exposition this experimental procedure is listed as "recovery experiment". In certain instances the lenses were incubated at 37° C. directly for periods of 20-48 hours without prior refrigeration.

For analysis the lens was blotted lightly to remove surface moisture, weighed rapidly, and placed in a drying oven for 48 hours at 105° C. The dried material was weighed and dry ashed with the aid of a drop of concentrated sulphuric acid. For sodium and potassium determinations a Baird

associates flame photometer with an internal standard was employed. Fresh lenses were analyzed in the same manner.

Unless otherwise stated the medium consisted of a Tyrode's solution modified to contain 148 meq/liter sodium, 5 meq/liter potassium, 30 meq/liter bicarbonate, and 200 mg% glucose. The pH of the medium was adjusted to physiologic range (pH 7.32-7.48) and checked at the end of each procedure. Osmotic pressure of the medium was maintained constant at the equivalent of 315 milliosmoles as determined on a Fiske associates osmometer. The sodium and potassium concentrations of all media were checked by analysis.

Results of the recovery experiments are generally expressed as percent recovery (Figure 1) rather than as differences in cation concentrations between the refrigerated and subsequently incubated lens since the former figure provides a more adequate expression of the ability of the lens to return to normal, that is, to regain its original sodium and potassium concentrations. (The formula for the determination of cation recovery is: $\frac{(C^t - C^o)}{(C^i - C^o)} \times 100$ where: C^t, C^o, and C^i, respectively are the concentrations after refrigeration plus incubation, after refrigeration alone, and normally observed.) (Figure 1)

In the few instances that the penetration into the lens of isotopically marked metabolites was determined the isolated lenses were placed in 3.0 ml. of media containing known amounts of C^{14} labeled substrate, refrigerated at 0° C. for 24 hours and subsequently incubated at 37° C. for an additional six hours. At the end of this period the lenses were thoroughly rinsed in a similar medium devoid of isotope, weighed, frozen rapidly, and

divided equatorially. The capsules were removed from the separate sections, and transferred onto 1" planchets of aluminum construction for counting with an end window counter. The entire nucleus plus cortex was macerated, spread evenly over a 1" planchet and counted after drying for one hour at 105° C.

Standard curves were obtained by spreading decapsulated lenses on planchets in the aforementioned manner, adding known amounts of C^{14} labeled substrate, thoroughly mixing, and counting as previously described. Standard curves for capsules were not obtained, but the assumption was made that the counting efficiency was in the same range as that of the macerated lens. Counting efficiency was determined by comparison with end-window counting of infinite thickness barium carbonate.

RESULTS:

Variation of cation concentration of fresh lenses with lens age (size):

Analyses of forty-five fresh lenses of varying size were made for their sodium and potassium concentrations. Figure 2 demonstrates that no appreciable difference in cation concentration in fresh lenses was found in the size range studied.

Lens size is here recorded in terms of dry weight. The lens continues to grow through life and the relationship of lens size to the age of the animal has been well established[17a]. Using this data it has been computed that the age range here analyzed was from four weeks to twelve months.

The effect of lens age (size) on cation recovery in vitro:

Lenses of various sizes were incubated in the modified Tyrode's solution. The extent to which the lens recovered original cation concentrations during incubation at 37° C. following refrigeration at 0° C. decreased as the size of the lens increased. Both sodium and potassium transport appeared to be equally affected.

Thus lenses obtained from 4-6 week old albino rabbits recovered completely the original cation concentrations, whereas, lenses obtained from 4-12 month old albino rabbits recovered approximately 50% of the original cation concentrations. (Figure 3)

The effect of lens age (size) on cation transport in vitro in the absence of glucose:

Lenses of various sizes were incubated in the usual manner in a media devoid of glucose. Figure 4 shows that young lenses (obtained from 4-6

week-old albino rabbits) recovered their original cation concentrations to a certain extent during incubation at 37° C. following refrigeration at 0° C. in the absence of added glucose. Lenses (obtained from 4-12 month-old albino rabbits) were unable to recover their original cation concentrations to any significant degree.

Although cation recovery was observed in the absence of glucose it resulted in a dissociation in the recovery of sodium and potassium concentrations. In the absence of glucose sodium recovery was reduced in each lens size, but potassium recovery was influenced to a lesser degree.

The effect of continuous incubation at 37° C.:

An analogous dissociation in cation transport was observed during the course of continuous incubation at 37° C. In these experiments lenses were incubated at 37° C. directly without previous refrigeration in the absence of added glucose. Figure 5 demonstrates that in the absence of added glucose as incubation was continued the concentration of sodium increased. When glucose was present in adequate concentration (200 mg. %) the concentration of sodium remained relatively constant during 40 hours of continuous incubation. The concentrations of potassium in both instances tended to rise but was essentially the same whether glucose was present or absent during the same period of incubation. (Table 1)

The effect of lens age on cation recovery when incubated in the presence of high glucose concentrations (500 mg. %):

Previous studies have shown a deleterious effect of high glucose concentrations on cation recovery using unselected lenses. Young lenses have been demonstrated to be more susceptible to diabetic cataracts than

old lenses.[17,18] In addition, there is some evidence to indicate that the development of a diabetic cataract is due simply to a high blood (and presumably aqueous) sugar level.[19] Possibly the deleterious effect on cation transport previously observed would prove to be more marked in young lenses.

Lenses of various sizes were subjected to the temperature changes in the usual manner in a medium containing 500 mg. % glucose. Figure 6 indicates that there is no exact correlation between the effect of high glucose concentrations and the age of the lens, although, cation recovery was altered in each size lens studied. The recovery of potassium concentrations was uniformly reduced, but the recovery of sodium reached final concentrations less than normal controls. The movement of sodium and potassium again were seen to be dissociated.

Cation recovery in the absence of oxygen and glucose:

Since cation transport is supported in young lenses in the absence of glucose under the experimental situation, it seemed reasonable to assume that the required energy was derived from the aerobic oxidation of some metabolite, perhaps lactate, contained in the lens. To test this hypothesis the cation recovery of lenses of various sizes was measured under anaerobic conditions bathed in a medium devoid of glucose or any other source of energy. Cation recovery was observed to be abolished in all lens sizes studied as demonstrated in figure 7. Sodium and potassium recovery were equally affected.

This observation supports the hypothesis that under the conditions of the experiment and particularly in young lenses that cation transport

to an undetermined extent is dependent upon energy derived from terminal aerobic oxidative pathways.

The effect on cation recovery of metabolites which may be metabolized via the citric-acid cycle:

Lenses of such a size as to require added glucose were refrigerated and subsequently incubated at 37° C. in the usual manner. Glucose was replaced by various metabolites (in 0.01M. concentration) which may be metabolized via the citric-acid cycle. Pyruvate, acetate, lactate, alpha-keto-glutarate, citrate, and oxaloacetate were studied.

With none of the above compounds was cation recovery observed to approach that obtained when glucose was added. (Figures 8 and 9) Indeed, citrate appeared to exert a deleterious effect on cation recovery. (Figure 10) When added to produce a concentration of 0.01 M. cation recovery was virtually abolished. A marked hydration of the lens was observed. Citrate is known to effectively bind calcium and the observed effect is probably similar to that seen when calcium is deleted from the medium.[4]

The effect on cation recovery of substituting for glucose metabolites which may be metabolized via the hexose-monophosphate shunt:

Two compounds, d-ribose, and gluconate, were substituted for glucose in the modified Tyrode's solution and cation recovery measured in the usual manner. Lenses of such a size as to require added glucose were utilized throughout.

Neither ribose, nor gluconate, supported cation recovery so effectively as did glucose. (Figure 11)

Incorporation of labeled substrates into the lens during incubation at 37° C.:

The aforementioned results indicated quite clearly that the major metabolites of glucose when delivered to the lens were incapable of supporting lens function as have been measured. Since it is known that glucose is preferentially moved across some lens surface[14] it became imperative to determine whether the various metabolites moved into the lens with the same facility as did glucose. To this end the movement of isotopically marked glucose into the lens was compared with that of the smallest metabolite tested, the two carbon residue, acetate, also isotopically marked.

Intact rabbit lenses were incubated at 37° C. for six hours following refrigeration for 24 hours in the modified Tyrode's solution to which known amounts of labeled substrate in tracer quantity had been added.

Approximately 4.5-6 % of the labeled acetate was recovered in the lens substance. Table 2 shows that the majority of the activity was found in the lens substance but significant activity was recovered in the capsule. The anterior capsule in each instance was more active by a factor of 2-4 than the posterior capsule. Per unit weight the activity of the capsule far exceeded that of the lens substance.

Table 3 demonstrates that approximately 8.5-9.5% of the glucose was incorporated into the lens at the end of the six hours of incubation at 37° C. following refrigeration at 0° C. for 24 hours. Again the activity of the anterior capsule exceeded that of the posterior capsule and per unit weight the activity of the capsule was far greater than that of the lens substance.

The finding that glucose enters the lens more readily than acetate (on the basis of molecular size one would anticipate the opposite) supports the above mentioned hypothesis that glucose enters the lens by metabolic mediation. The failure of the various metabolites to support cation transport as well as glucose must, in the light of these experiments, be considered to be due largely to insufficient penetration of these metabolites into the lens.***

***I wish to express my thanks to Dr. John T. Van Bruggen and Miss Jean Scott for the valuable assistance given me during the course of this experiment.

DISCUSSION:

The inverse relationship of cation recovery to lens age is of more than passing interest. The most obvious explanation of this finding is that the rates of metabolic activity in young lenses is greater than that of the older. There is a good deal of evidence that this may be true. Glycolytic activity has been shown to be greater in young lenses.[20,21] The ability of aqueous extracts of lens homogenates to metabolize hexose-phosphate to triose phosphate is reduced in older lenses.[22] The concentration of acid-soluble phosphates decreases as the lens ages.[23] It has recently been shown that young lenses incorporate a greater amount of radioactive phosphorus from the incubating medium than old lenses.[24] This increase is reflected in a greater incorporation of P^{32} into the inorganic phosphate and ATP fractions as demonstrated by electrophoretic studies.[25] In the final analysis, the immediate energy for cation transport must derive from the high energy phosphates. However, no exact relationship of the high energy phosphate compounds to cation transport has been established. In a recent communication the relationship between cation transport, and the concentrations of the various phosphate fractions in the lens was studied.[26] No exact correlation between cation transport and the concentrations of the various phosphate fractions was observed.

The thought that reduced cation recovery in older lenses is due to a reduced metabolic level is not free of objections. Major among them is the finding that the concentration of cations is not altered as age advances as would be anticipated. The most inclusive statement that can be made from the vantage of our present knowledge is that the older lenses are

not able to withstand the stress imposed by cold. The ability of the younger lenses to recover from this stress may result from an availability of alternate metabolic pathways. The results obtained when glucose was deleted from the medium are compatible with that view.

The pathways in the lens by which glucose is metabolized and in which oxygen participates are still not known with certainty. The glucose consumption of the lens is known to be small.[5] Its oxygen consumption is so low that measurement by usual Warburg techniques is extremely difficult and subjected to high error. Indeed, some[27] have suggested that oxygen consumption is non-enzymatic but this does not appear to be true.[6] Present knowledge of these pathways in the lens is best summarized as follows:

First, the studies of Green and his associates have demonstrated that the classical anaerobic pathway, the Embden-Meyerhof cycle appears to be followed in lens homogenates.[28,29,30]

Second, there is suggestive, although not very strong evidence that the cytochrome system of enzymes serves the same function in the lens as in other tissues.[31]

Third, the participation of the citric-acid cycle in the lens is not certain. Bovine lens homogenates have been found to oxidize certain intermediates of the citric-acid cycle.[32] Dehydrogenase activity of the members of the citric-acid cycle have been demonstrated in lens homogenates.[32] However, the intact lens does not appear to oxidize isotopically labeled pyruvate to carbon dioxide to any appreciable extent. Rather, the labeled pyruvate appears in significant amounts as lactate in the lens substance.[33]

Fourth, the hexose-monophosphate shunt* may be utilized by lens homogenates.[33] Thus it has been shown that more isotopically marked carbon dioxide is recovered when glucose is labeled in the one position than the six** and that certain supposed intermediates, i.e. glucose-6-phosphate and 6-phosphogluconate are utilized by lens homogenates.[33]

Most of this data has of necessity been obtained from studies of lens homogenates. To what extent it can be quantitatively applied to the intact structure is not certain. Indeed measureable changes in metabolic activity attend even slight alteration of structure of the lens. For example, when the lens capsule is nicked, the rate of oxygen consumption immediately rises.[34] For this reason any postulated mechanism must ultimately be proved to occur in the intact structure. The observations here reported indicate that these metabolites delivered to the lens do not support lens function as herein measured possibly because they do not penetrate to the

*By this mechanism glucose is aerobically oxidized with the subsequent formation of utilizable energy in the form of high energy phosphates. It involves the two-step oxidation of glucose 6-phosphate with the formation of pentose phosphate; the first step resulting in the formation of 6-phospho-gluconic acid. These reactions are mediated through their respective dehydrogenases, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. Two pentose-phosphate molecules undergo condensation to form sedoheptulose phosphate and a triose phosphate catalyzed by the enzyme transketolase. The intermediates react further to reform hexose-phosphate. This pathway appears to be as efficient in the production of ATP as the citric-acid cycle.[35]

**The shunt mechanism is thought to preferentially cleave carbon # 1 of glucose. When glucose is glycolized then oxidized via the citric-acid cycle all carbons of glucose should be recovered approximately equally as carbon dioxide. The ratio of labeled carbon dioxide derived from isotopically labeled glucose in the 1, and 6 positions is thought to provide a measure of the quantity of glucose metabolized via each pathway. A ratio of unity would indicate that the shunt mechanism is inoperative.

site of utilization in sufficient quantity. The ability of glucose to serve as a substrate is due in a certain measure to the fact that it probably enters the lens by metabolic mediation. No definite conclusion concerning the specific pathways of glucose degredation can be drawn from these studies.

One of the most intriguing results here reported has been the clear cut observation that a separate sodium and potassium pump exists. Heretofore it has been suggested from studies of the lens[4-6], erythrocyte[36], and frog muscle[37], among others that the movement of sodium and potassium is equivalent and that the active transport of sodium alone is required. It is apparent, however, from the present studies that the two may be sharply dissociated and that independent secretory movement of the two ions may exist.

Recent studies of cells have permitted essentially the same conclusion. In particular, separate independent mechanisms regulating sodium and potassium distributions in marine algae both dependent upon cellular metabolism have been reported.[38] More recent examination of sodium and potassium movements in red cells[39], and nerve axons[40] have indicated that although there is evidence for a coupling between the movements of sodium and potassium their movements are not rigidly linked and that a portion of the movement of each is independent of the other.

Present studies would suggest that a similar situation prevails in the lens under the conditions of the experiments. A portion of the movement of the two appears to be linked and the remainder independent. It is

particularly interesting that the unlinked portion of potassium movement may not require glucose in younger lenses whereas, the independent movement of sodium is definitely dependent upon an exogenous glucose supply.

SUMMARY OF RESULTS:

1. The concentration of sodium and potassium in the lens did not vary appreciably with lens age.

2. The recovery of the cold-induced cation shift varied inversely to the age of the lens in vitro.

3. In the absence of glucose young lenses recovered to a certain degree their original cation concentrations following a cold-induced cation shift, whereas, old lenses did not recover their original cation concentrations to any significant degree. Sodium recovery was affected more by the absence of glucose than was potassium recovery.

4. Continuous incubation in the absence of glucose resulted in an analogous dissociation of cation movement. The concentration of sodium tended to rise when glucose was absent but remained essentially the same when glucose was present during 40 hours of incubation. The concentration of potassium rose but was essentially the same whether glucose was present or absent during the same period of incubation.

5. Cation recovery following the cold-induced cation shift was abolished in all lenses when incubated in the absence of glucose and oxygen.

6. Compounds which may be metabolized via the citric-acid cycle did not support cation recovery following the cold-induced cation shift as well as does glucose.

7. Compounds which may be metabolized via the hexose-monophosphate shunt did not support cation recovery following the cold-induced cation shift as well as does glucose.

8. Glucose was incorporated into the intact lens in vitro to a greater degree during incubation at 37° C. for six hours than was acetate.

9. The significance of these findings are discussed.

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Figure 1. Schematic representation of the changes in cation concentrations during refrigeration at 0° C. and subsequent incubation at 37° C.

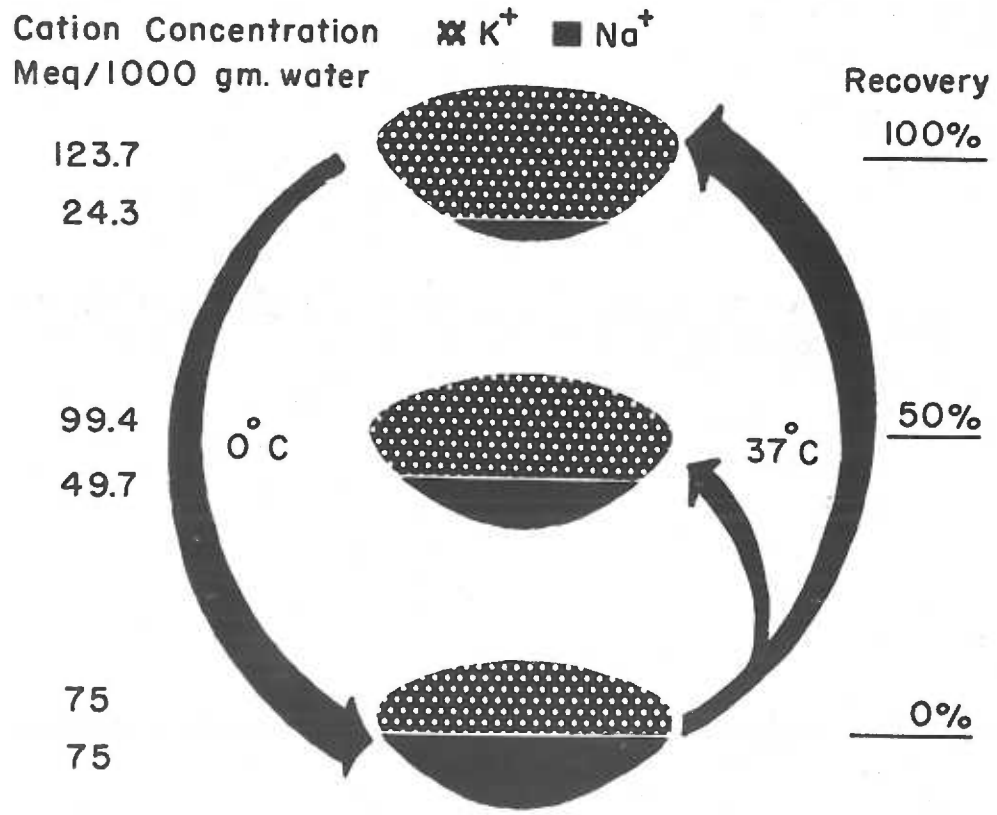

Cation Concentration
Meq/1000 gm. water
K+
Na+
Recovery
100%
123.7
24.3
99.4
49.7
0° C
37° C
50%
75
75
0%

Figure 2. Effect of lens age (size) on the concentrations of sodium and potassium. (Each figure represents a minimum of eight analyses.)

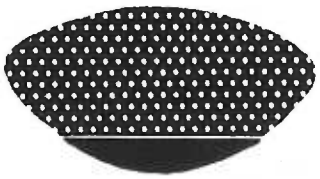
Lens Size Mgm. Dry Wt.	K^+ / Na^+	Cation Concentration Meq/1000 gm. of water
65.0 – 85.0		K^+ 122.5 ± 4.9 Na^+ 19.7 ± 3.7
85.1 – 105.0		K^+ 122.7 ± 1.9 Na^+ 23.5 4.2
105.1 – 125.0		K^+ 123.2 ± 2.4 Na^+ 26 ± 4.2
> 125.0		K^+ 121.2 ± 3.6 Na^+ 23.2 ± 2.2

Figure 3. Effect of lens age (size) on cation recovery during incubation at 37° C. following a cold-induced cation shift. (Each bar represents the average findings using at least ten pair of lenses.)

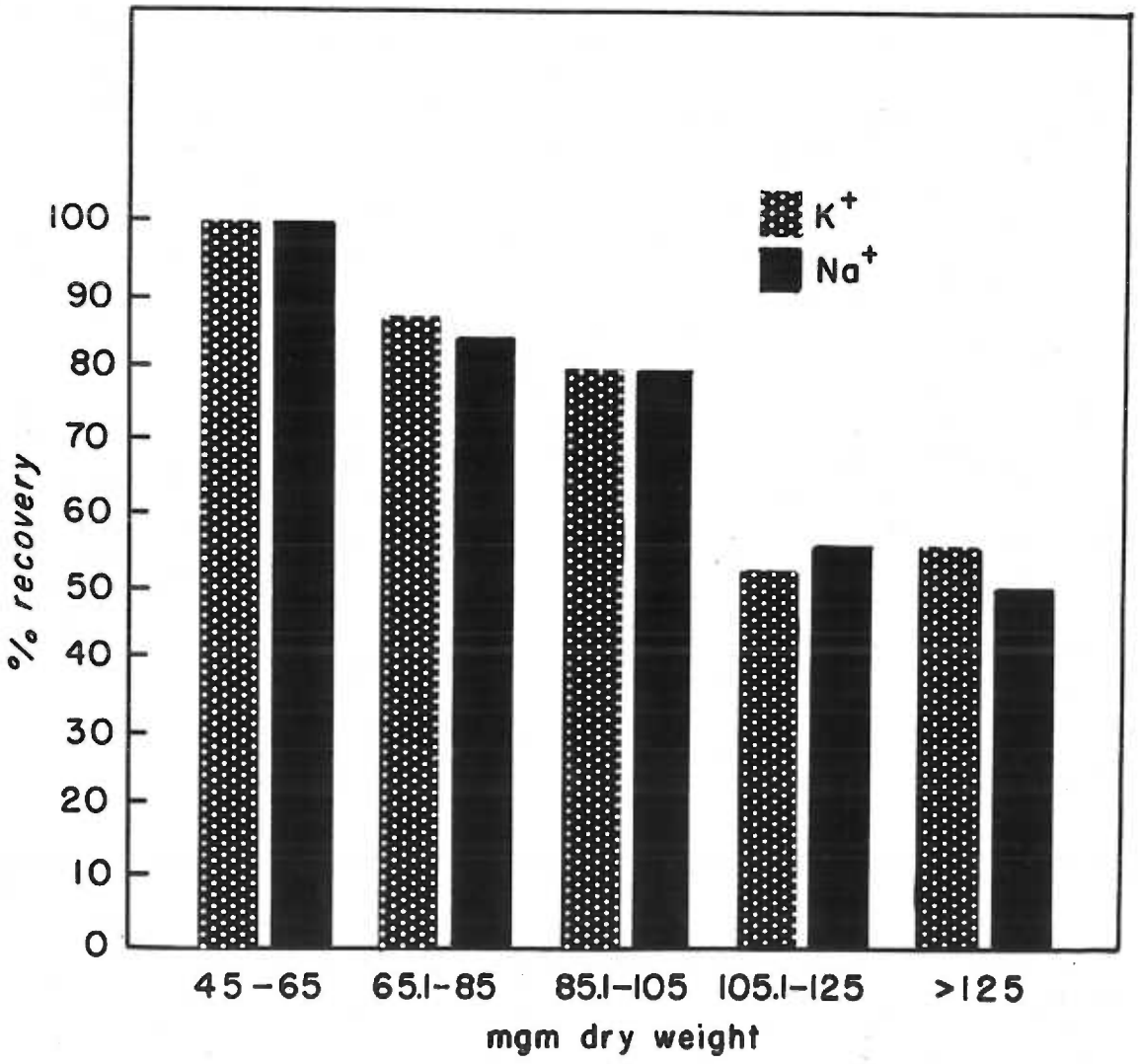

100
90
80
70
60
50
40
30
20
10
0
% recovery
K+
Na+
45-65
65.1-85
85.1-105
105.1-125
>125
mgm dry weight

Figure 4. Effect of lens age (size) on cation recovery during incubation at 37° C. following a cold-induced cation shift in the absence of glucose. (Each bar represents the average findings of at least ten pair of lenses.)

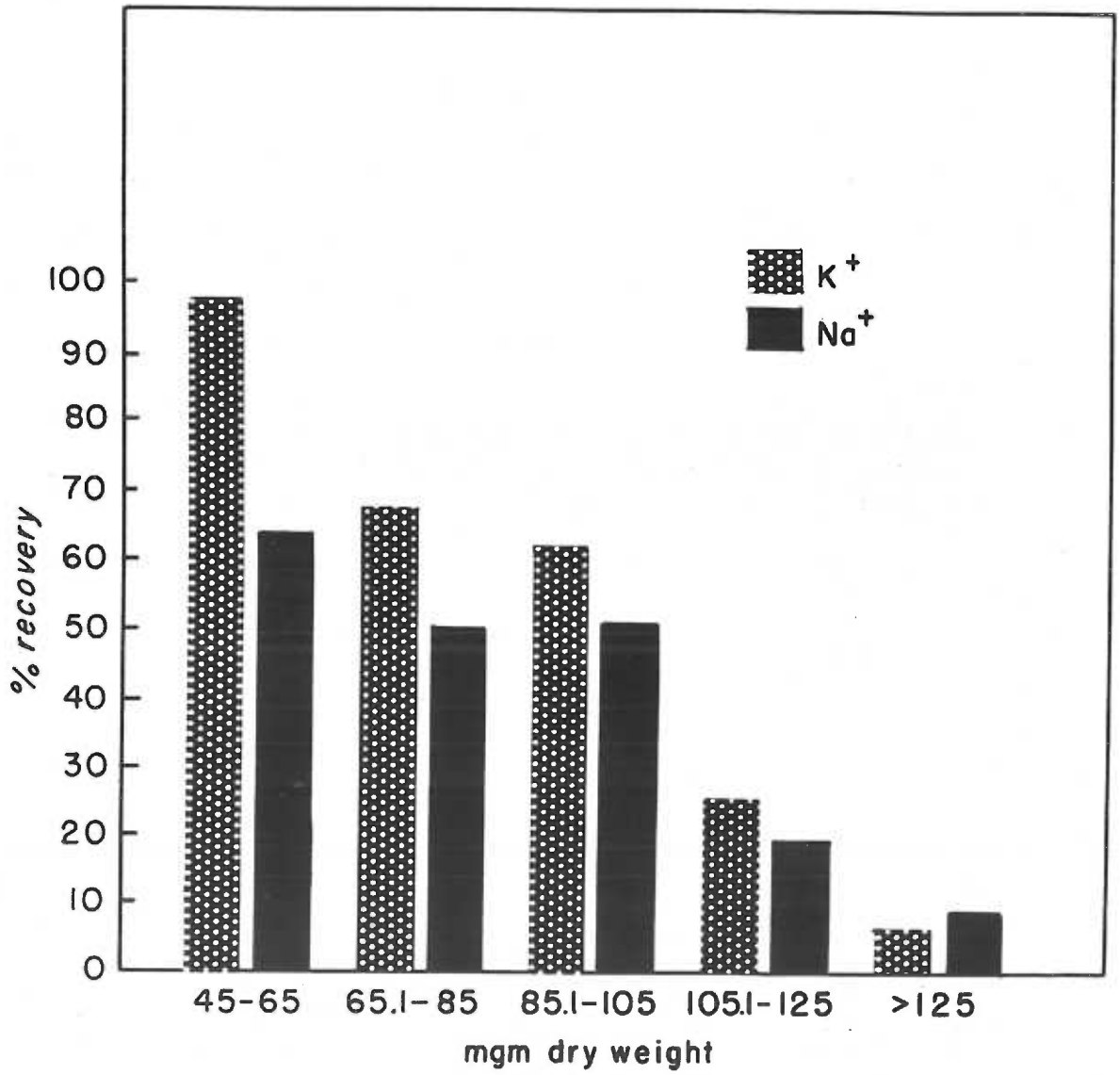

100
90
80
70
60
50
40
30
20
10
0
% recovery
K+
Na+
45-65
65.1-85
85.1-105
105.1-125
>125
mgm dry weight

Figure 5. Effect on continuous incubation at 37° C. on the concentration of sodium when bathed in a medium containing glucose and when glucose has been deleted. (Each point represents the average findings using at least ten lenses.)

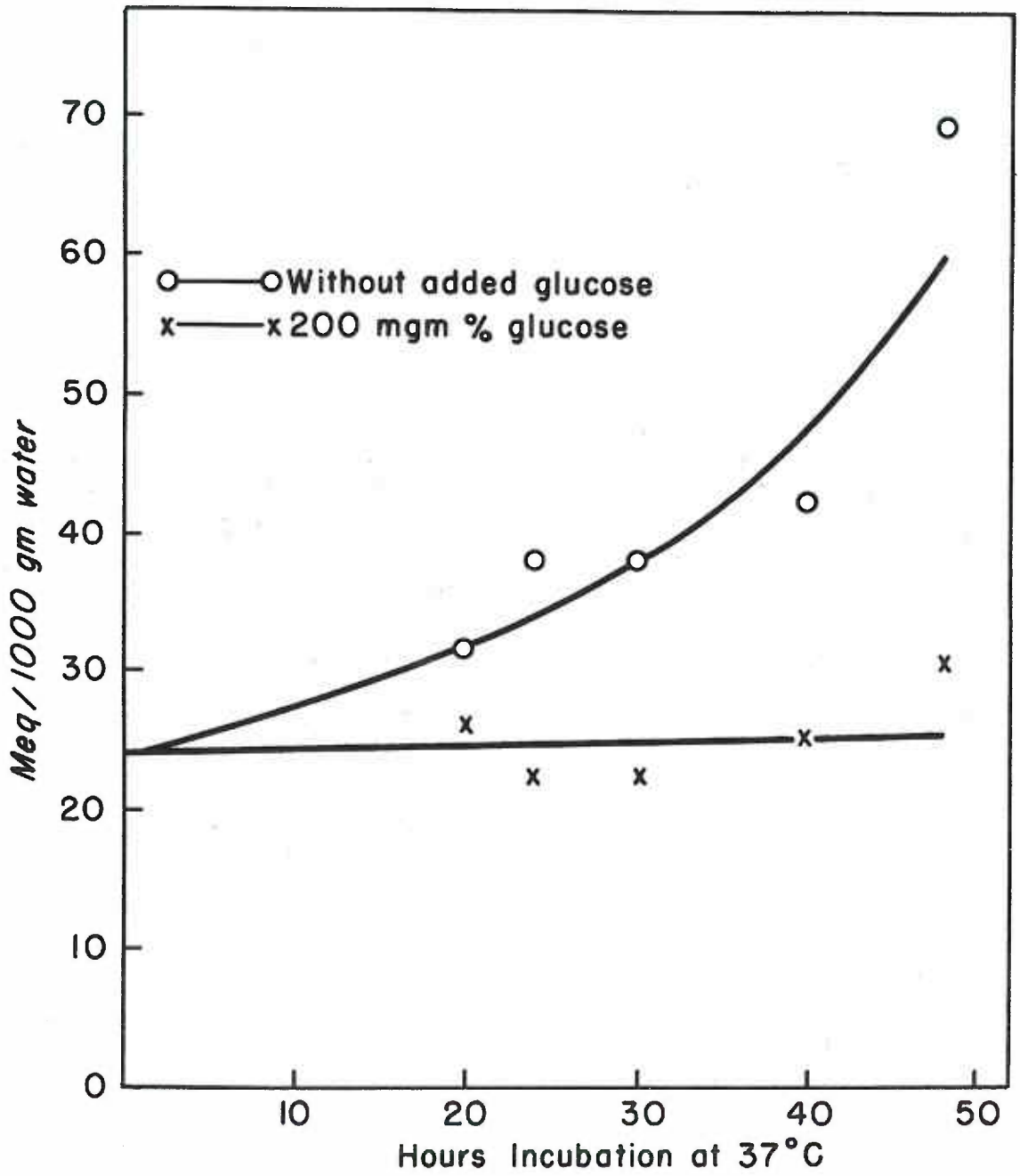

70
60
50
40
30
20
10
0
Meq/1000 gm water
Without added glucose
x 200 mgm % glucose
10
20
30
40
50
Hours Incubation at 37°C

Figure 6. Effect of lens age (size) on cation recovery during incubation at 37° C. following a cold-induced cation shift in the presence of high glucose concentrations (500 mg. %). (Each bar represents the average figure of at least ten pairs of lenses.)

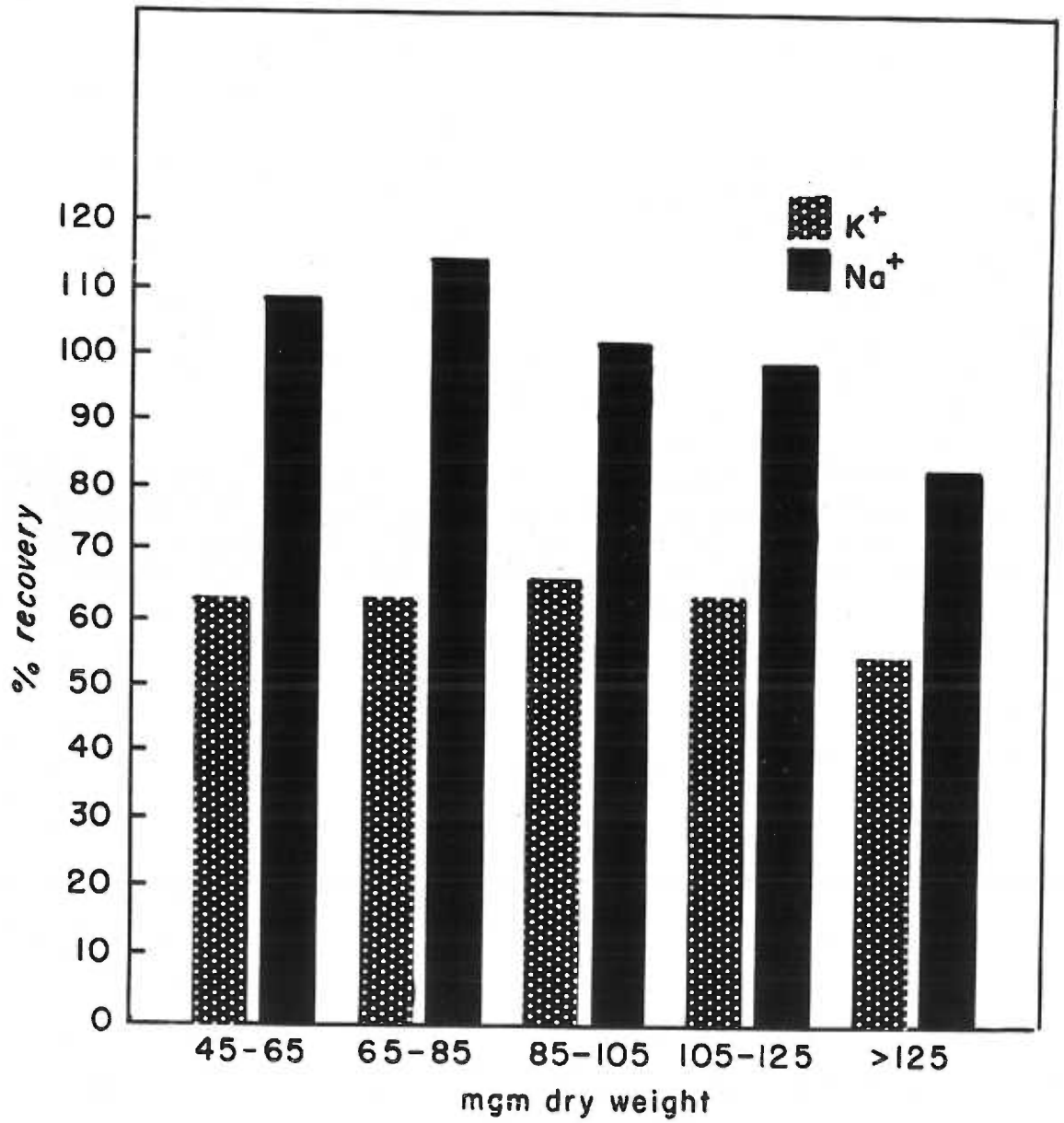

120
110
100
90
80
70
60
50
40
30
20
10
0
% recovery
K+
Na+
45-65
65-85
85-105
105-125
>125
mgm dry weight

Figure 7. Effect of lens age (size) on cation recovery during incubation at 37° C. following a cold-induced cation shift in the absence of glucose and oxygen. (Each bar representates the average figure of at least five pair of lenses.)

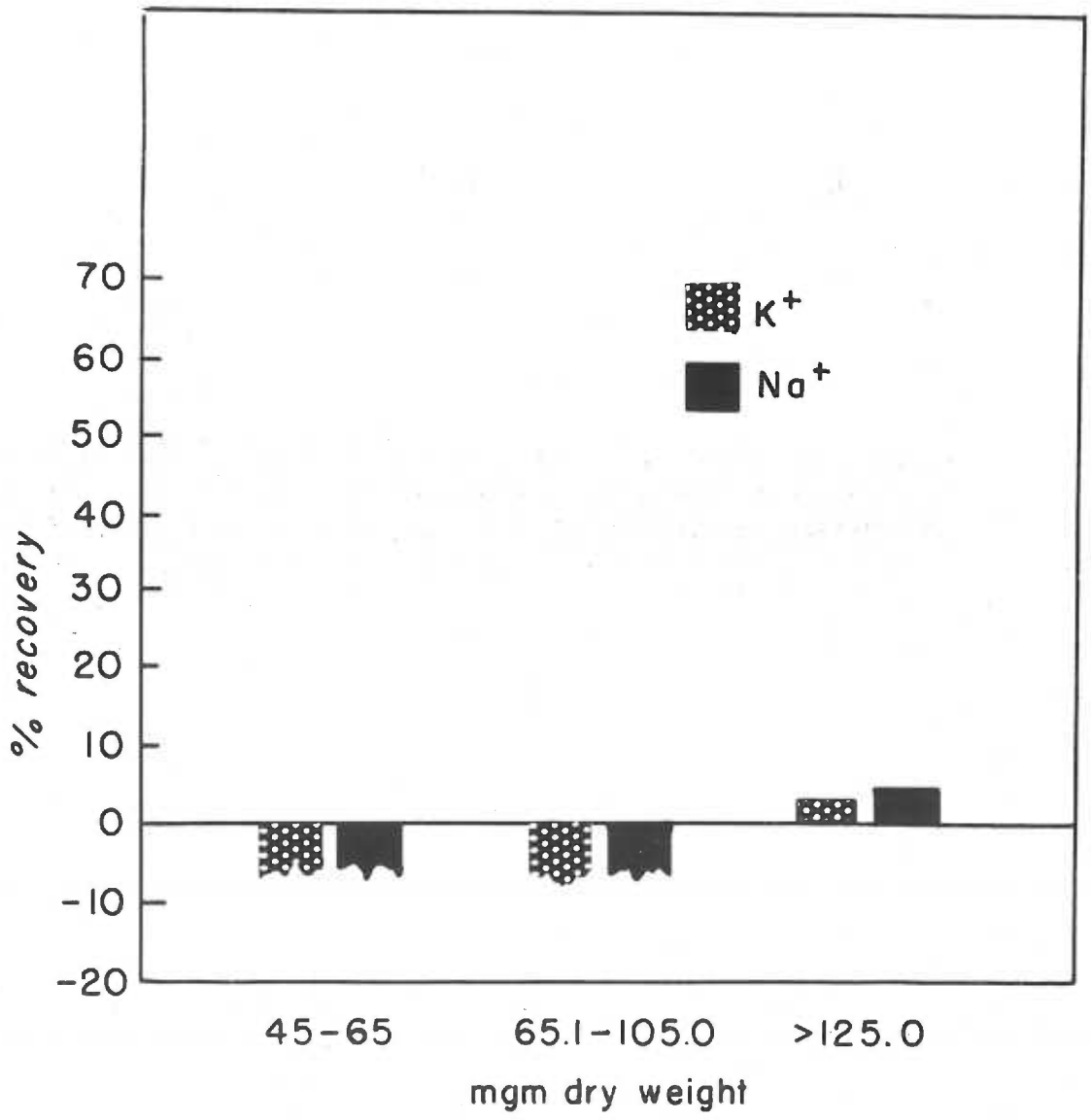

70
60
50
40
30
20
10
0
-10
-20
% recovery
K+
Na+
45-65
65.1-105.0
>125.0
mgm dry weight

Figure 8. Effect of replacement of glucose by lactate, pyruvate, or acetate on cation recovery during incubation at 37° C. following a cold-induced cation shift. (Each bar represents the average figure of at least ten pair of lenses.)

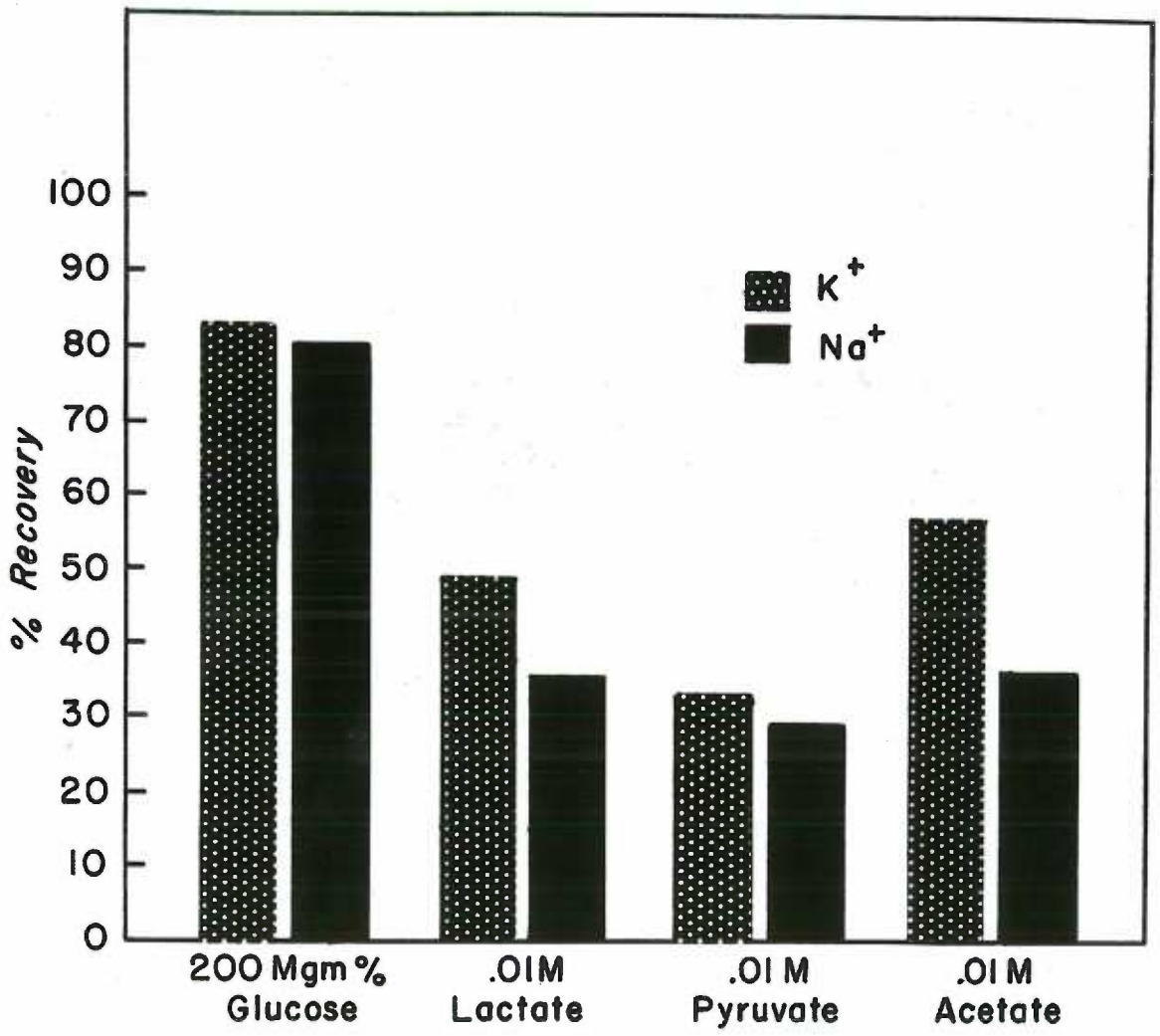

100
90
80
70
60
50
40
30
20
10
0
% Recovery
K+
Na+
200 Mgm %
Glucose
.01M
Lactate
.01 M
Pyruvate
.01 M
Acetate

Figure 9. Effect of replacement of glucose by alpha-keto-glutarate, or oxaloacetate on cation recovery during incubation at 37° C. following a cold-induced cation shift. (Each bar represents the average of at least ten pair of lenses.)

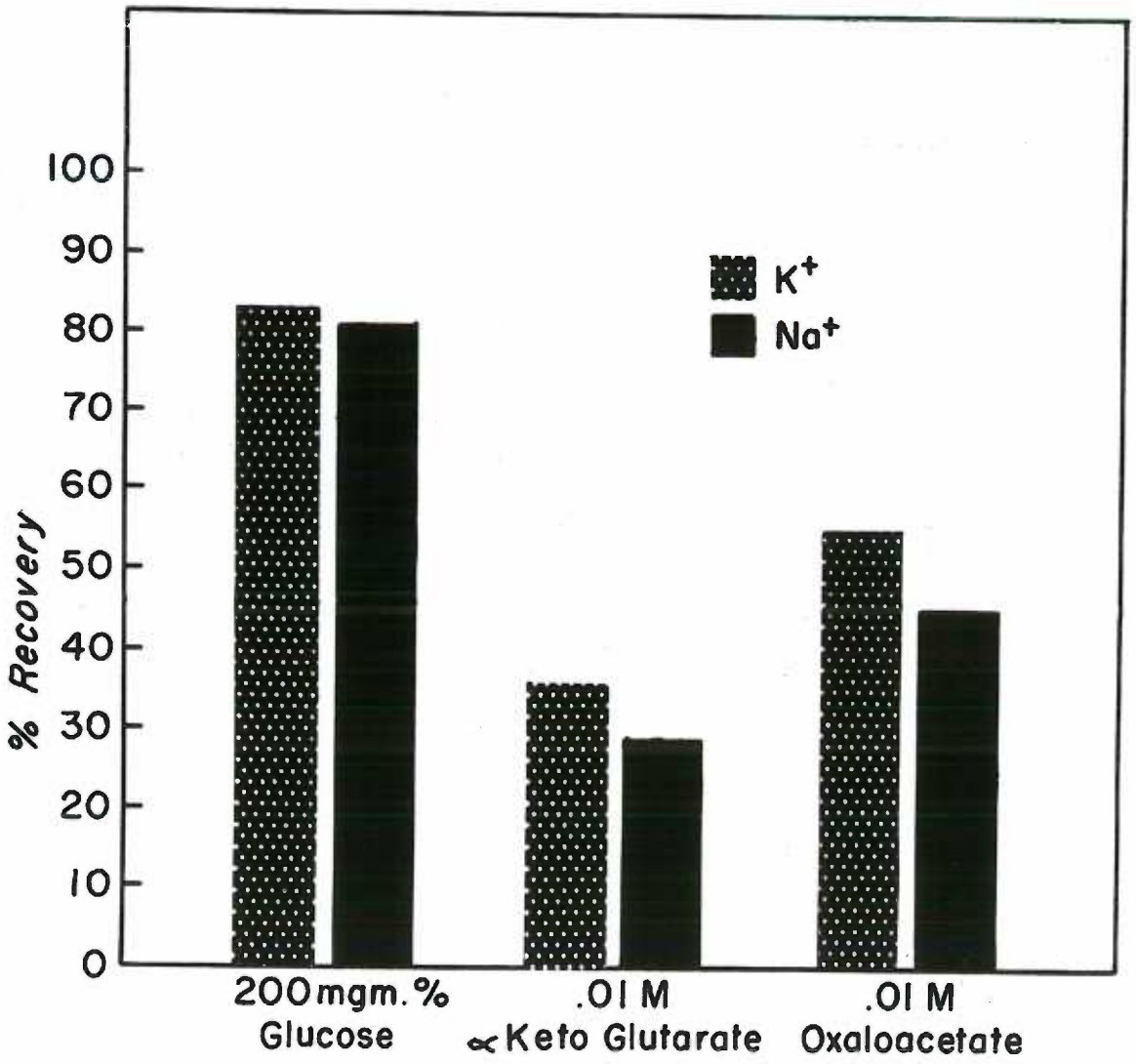

100
90
80
70
60
50
40
30
20
10
0
% Recovery
K+
Na+
200mgm.%
Glucose
.01 M
∝Keto Glutarate
.01 M
Oxaloacetate

Figure 10. Effect of replacement of glucose by nitrate on cation recovery during incubation at 37° C. following a cold-induced cation shift. (Each bar represents the average of at least ten pair of lenses.)

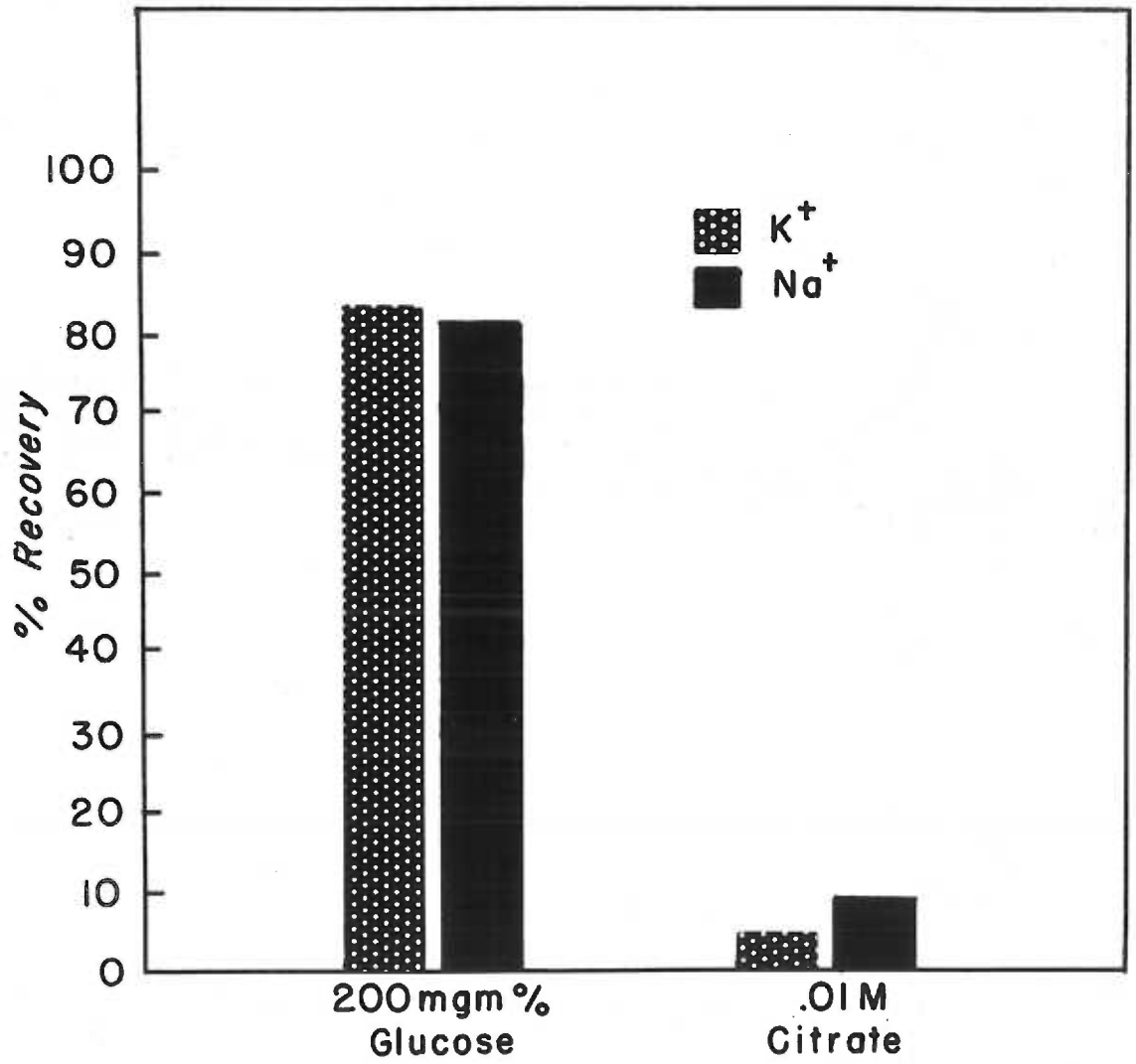

100
90
80
70
60
50
40
30
20
10
0
% Recovery
K+
Na+
200mgm%
Glucose
.01M
Citrate

Figure 11. Effect of replacement of glucose by ribose, or gluconate on cation recovery during incubation at 37° following a cold-induced cation shift. (Each bar represents the average of at least ten pair of lenses.)

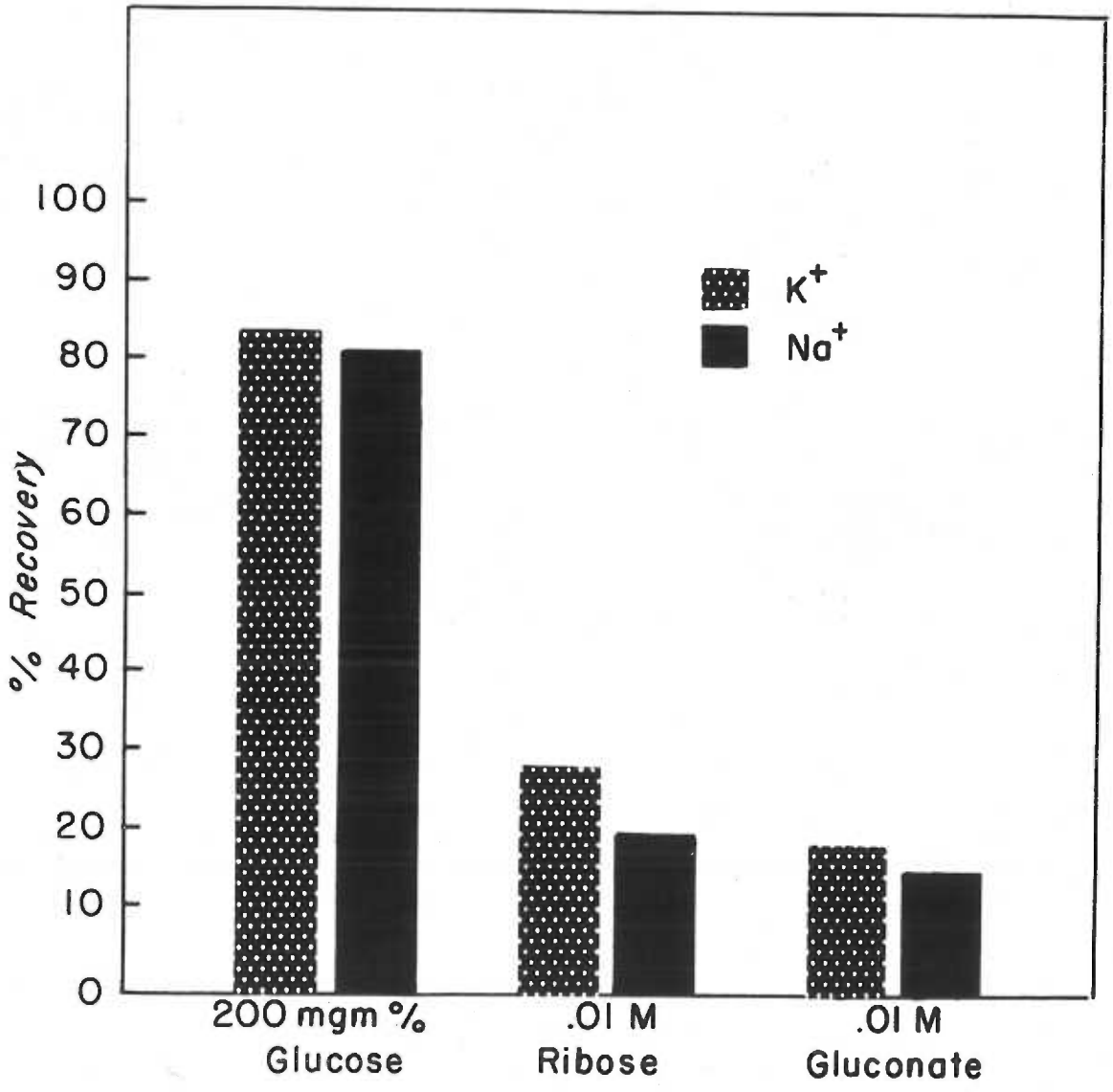

100
90
80
70
60
50
40
30
20
10
0
% Recovery
K+
Na+
200 mgm %
Glucose
.01 M
Ribose
.01 M
Gluconate

Table 1. Effect of continuous incubation at 37° C. in the presence of, or in the absence of added glucose on the concentration of potassium. (Each figure represents an average of at least ten analyses.)

Hours Incubation	Potassium meq / 1000 gm Water	
	200 mgm % Glucose	Without Added Glucose
20 hr.	127.0	138.7
24 hr.	133.1	130.0
30 hr.	128.7	127.2
40 hr.	144.0	141.9
48 hr.	132.4	99.5

Table 2

(Experiment)	(uC labeled substrate added)	(uC recovered) lens substance	ant. caps.	post. caps.	(% Incorporation)
1.	0.2	0.0076	0.0040	0.0008	6.2
2.	1.0	0.0392	0.0090	0.0040	5.3
3.	1.5	0.0564	0.0121	0.0024	4.7
4.	2.0	0.0833	0.0258	0.0068	5.8

Table 3

(Experiment)	(uC labeled glucose added)	(uC recovered) lens substance	ant. caps.	post. caps.	(% Incorporation)
1.	0.4	0.0295	0.0051	0.0034	9.5
2.	1.0	0.0695	0.0115	0.0050	8.6

Table 2. Incorporation of labeled acetate into the lens during incubation at 37° C. for six hours following refrigeration at 0° C. for 24 hours.

Table 3. Incorporation of uniformly labeled glucose into the lens during incubation at 37° C. for six hours following refrigeration at 0° C. for 24 hours.