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Effect of Intravenous Infusion of Isethionate on Rats[1] (38064)

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(Introduced by E. Mihich)

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Isethionate ($HOC_2H_4SO_3^-$), a metabolite of taurine in man (1) and perhaps in other species as well, is a commonly occurring organic anion. It has been isolated from squid axoplasm (2) and dog heart (3), and its conjugate acid has been extensively used as a solubilizing agent for diamidine compounds such as hydroxystilbamidine (4) and pentamidine (5). Bourke and coworkers (6) have postulated isethionate to be a potentially useful agent in the reduction of cerebral edema based upon the following observations (a) the swelling of intact primate cerebral cortex is dependent upon both the K^+ and the Cl^- content in the extracellular fluid of the cerebral cortex since a reduction in the concentration of either ion is associated with a concomitant reduction in cortex swelling (b) a reduction in brain K^+ and Cl^- can be brought about both *in vitro* and *in situ* by perfusion of the cortex with an isotonic solution of salts containing isethionate as the major anion. The possible systemic effects of isethionate administration are not known. This investigation reports the results of a study carried out in rat under conditions of possible therapeutic administration of isethionate salts.

Materials and Methods. Chemicals and glassware. All the chemicals used were of analytical grade from Fischer Scientific Co. Sodium and potassium salts of isethionate were provided by Dr. R. Bourke of this Institute. All the glassware used was made chloride-free by thoroughly rinsing with deionized distilled water.

Isotonic salt solutions for perfusion. Two isotonic salt solutions were used throughout this investigation. One (CLD) contained chloride and the other (IST) contained isethionate as the major anion. The solutions were prepared with the following compositions (in m*M*): Common to both CLD and IST were dextrose 1.1, sodium bicarbonate 25, calcium lactate 2.5, and magnesium sulfate 1.2. In the CLD, sodium chloride 115 and potassium chloride 4.0 were also added. In the IST, equimolar concentrations of sodium (115 m*M*) and potassium (4.0 m*M*) isethionate salts were used instead of the chloride salts. The salts were dissolved in chloride-free deionized water and these solutions were employed for intravenous infusion in rats.

Preparation of animals for infusion. Charles River female white rats weighing 100–200 gm were used. Twenty-four hours prior to the onset of infusion, food was withdrawn and the rats were kept on a 50% dextrose throughout the infusion period. The lateral tail vein was cannulated toward the proximal end with polyethylene tubing (Intramedic, P. E. 10, Clay-Adams, Inc.) filled with the appropriate fluid for iv infusion. In the initial experiments, while the rat was under light ether anesthesia the lateral tail vein was exposed by surgical

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incision and cannulation was carried out using standard techniques (7). Subsequently a simpler procedure for cannulation was adopted. This involved direct puncturing of the lateral tail vein through the skin of an unanesthetized rat in a restrainer cage (Carworth, N. Y.). With practice it was possible to make a proper puncture enabling direct insertion of the polyethylene tubing into the vein. In both procedures the other end of tubing was then attached to a glass syringe filled with the appropriate infusion fluid through a 27 gauge hypodermic needle. The syringe was fixed in a Harvard Dual Infusion pump (model 600-000). Successful insertion was always confirmed by a back flow of blood into the tubing under negative pressure. Infusion was begun immediately to prevent any possible clotting of blood in the tubing. The restrainer cage was then fixed on top of a glass metabolism cage (Delmar, Chicago, Ill.) for collection of urine. The tail of the animal was taped down to a piece of plywood for keeping the cannula in place during infusion. The animals were infused with CLD or IST over an average time of 96 hr at a rate of either 0.582 ml/hr or of 1.482 ml/hr.

Collection of fluids. Urine was collected and analyzed for Na^+, K^+, and Cl^- contents every 24 hr and also for any pathological changes. At the end of each experiment, rats were anesthetized and blood was obtained by means of ventricular heart puncture using an 18 gauge needle. It was allowed to clot for 5 min at room temperature, centrifuged in a refrigerated centrifuge at 900*g* for 5 min and serum was separated for analyses.

Preparation of Homogenates. After obtaining the terminal blood sample, the rats were sacrificed by exsanguination. All thoracic and visceral organs, brain, skin, and muscle were taken out for histopathological studies as described below. In addition, portions of brain, liver and kidney were weighed for homogenization. Homogenates were prepared in cold deionized distilled water using a Potter and Elvehjem homogenizer. For tissue Cl^- determination, aliquots from each homogenate were extracted with alkaline solution of sodium perborate (8). The remainder was centrifuged and the supernatant was analyzed for Na^+ and K^+.

Tissue water. A portion of brain, liver, kidney, abdominal muscle and skin were obtained during autopsy. These were rapidly weighed and dried to a constant weight in a vacuum oven at 50° for 72 hr. The loss in weight was considered to measure the total water content.

Analytical methods. Standard methods in clinical biochemistry were used to determine the concentrations of sodium (9), potassium (9), and chloride (10) in tissue extracts and fluids and of protein (11), glucose (12), urea (13) in blood. The activity of the serum enzymes, namely lactic acid dehydrogenase (LDH) (14), serum glutamic-oxaloacetic transaminase (SGOT) (15), and alkaline phosphatase (AP) (16) were also measured. Total counts of red (RBC) and white cells (WBC) and the differential WBC counts were carried out manually by standard hematological methods.

Histopathological studies. A total of 7 rats from each group were autopsied at the end of the experiment and examined for gross morphological changes in major organs and tissues. Tissue sections were then removed, fixed in buffered formalin (10%) solution, embedded in paraffin, cut in 5–10-μm-thick sections and stained with hematoxylin-eosin using standard techniques. The results obtained were further compared with those from tissue sections taken from 6 noninfused control rats.

Results. Comparison of 24 hr collections of urine samples from rats receiving either CLD or IST infusion showed that within the first 24 hr a significant ($p < .01$) decrease in Cl^- occurred in the group of rats receiving IST infusion; an increased excretion of Na^+ and K^+ also occurred within 48 hr (Fig. 1). No occult blood, protein, or reducing sugars could be detected. The average urinary pH, as determined by pH indicator paper, was 8.4 for the group receiving CLD infusion and 8.0 for that receiving IST infusion.

Serum analyses. In comparison with CLD group, a significant decrease ($p < .05$) occurred in the concentrations of K^+ and Cl^-

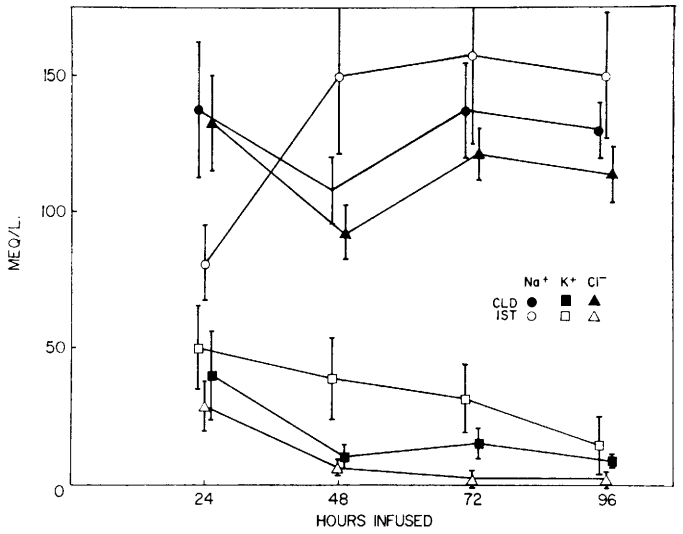

FIG. 1. Comparative effects of CLD and IST infusions on the urine electrolytes in rats. Rats were infused with either CLD or IST for approximately 96 hr. Twenty-four hour collections of urine samples were made and Na^+, K^+, and Cl^- contents were determined. Each point represents an average of 6 determinations, and the bar represents its standard deviation. Concentration of Cl^- was significantly lower in the IST group ($p \leq 0.01$) at all the time points of infusion.

in the terminal serum samples from the IST group infused at the rate of 1.482 ml/hr. A similar decrease in K^+ ($p < .05$) and Cl^- ($p < .1$) also occurred in the IST group in comparison with the noninfused control. Regardless of treatment, no change occurred in the serum Na^+ level which was comparable to that from the noninfused group of animals (Table I). A slight elevation occurred in the glucose level in the CLD and more so in the IST group (Table I). The activities of SGOT and AP were found to increase with the rate of infusion in both groups. The activity of LDH was, however, increased in the CLD group while it decreased somewhat in the IST group (Table I). The urea nitrogen decreased in both the groups and the total protein did not show any noticeable change. Qualitatively similar data were obtained when IST was infused at the rate of 0.582 ml/min (data not shown).

Tissue electrolytes and water. A significant ($p < .05$) decrease in the chloride level occurred in liver and kidney but not in brain from rats receiving IST infusion as compared to that in organs from the non-infused group and the group receiving CLD infusion (Table II). Very little change occurred in the K^+ content of the tissues from the IST group, whereas a slight increase was detected in those from the CLD group in comparison with those from the noninfused control animals (Table II). Levels of Na^+, however, differed very little regardless of treatment. Qualitatively similar results were obtained with IST and CLD infusions given at the rate of 0.582 ml/min (data not shown).

No differences were seen in the water content of liver, brain, kidney, skin or muscle from rats receiving either CLD or IST infusion.

Hematology. Periodic hematological examination throughout the infusion revealed a slight increase in white cells in both the CLD and the IST groups with time of infusion, namely, from 13,000 cells/mm^3 at 0 hr–20,000 cells/mm^3 at 120 hr. No significant changes in total RBC and differential WBC counts occurred, however, as a result of treatment. A detectable lymphoid depletion was observed in spleen, thymus and small intestine from both of the infused

TABLE I. The Effects of CLD and IST Infusions on the Serum Constituents in Rats.

Group	Infusion rate ml/hr	Na^+ (meq/l)	K^+ (meq/l)	Cl^- (meq/l)	Total protein (mg%)	Glucose (mg%)	BUN (mg%)	SGOT (mu/ml)	AP (mu/ml)	LDH (mu/ml)
Non[a] infused	—	128 ± 23	3.1 ± 0.7	97 ± 10	5.63 ± 1.0	112 ± 31	17.2 ± 2.6	125 ± 56	148 ± 55	627 ± 221
CLD[b]	1.482	146 ± 5	3.9 ± 0.6	100 ± 6	5.8	153 ± 49	10 ± 3.5	287 ± 50	216 ± 119	722 ± 260
IST[b]	1.482	143 ± 9	2.08 ± 0.4[+]	92 ± 3[+]	5.0	184 ± 49	12.7 ± 5.6	339 ± 61	235 ± 60	483 ± 209

Groups of rats were infused with CLD or IST for approximately 96 hr. Terminal blood samples were obtained by ventricular heart puncture. BUN: Blood urea nitrogen; SGOT: Serum glutamic–oxalacetate transaminase; AP: Alkaline phosphatase; LDH: Lactic dehydrogenase; mu = Milli-unit (International).

Values in the row.

[a] Represent averages of six determinations ± S.D.

[b] Represent four determinations except for Total Protein which is based on one determination ± S.D.

[+] Values significantly different from those of CLD group at $p \leqslant 0.05$ level.

groups. No histopathological changes were found in the other tissues examined. No changes specific to IST treatment were detected in any of the tissue studied.

Discussion. Decreases in the concentrations of K^+ and Cl^-, although of small magnitude, have occurred in edematous cat brain slices *in vitro* in the presence of isethionate fluid (17) and in edematous primate brain tissue by perfusing *in situ* with isethionate fluid (6). Such decreases in brain chloride concentration may have a potential use in the management of cerebral edema, which seems to be associated with a considerable rise in K^+ and Cl^- content resulting in a corresponding fluid accumulation (6). The increase in the Cl^- appears to be mediated by a K^+ dependent carrier transport (18) and not due to alteration in the electrochemical potential difference between cerebrospinal fluid and plasma (19). In the study reported herein, infusion of isethionate ions in normal rats resulted in a decrease in the concentration of K^+ and Cl^- in serum and of Cl^- in liver and kidney but resulted in no such decreases in the brain. Even under more drastic conditions, namely, extracorporeal hemodialysis with isethionate in normal cats, brain chloride levels did not change although the plasma chloride levels decreased to about 10–20% of their initial value (20). This inability of isethionate to decrease brain chloride levels may be due to its inability to compete for the carrier site/s and/or to interfere with the potent homeostatic mechanism for chloride transport in normal animals (20). The possibility that isethionate ions may not be able to interfere with homeostasis of Cl^- in the brain due to their possible non-diffusibility into the intracellular sites remains to be explored. Existence of altered permeability of the blood-brain barrier towards isethionate anion is still another question.

The urinary excretion of K^+ ions was somewhat greater in IST receiving rats than in those receiving CLD infusion. This is consistent with the relatively lower potassium concentrations in serum and to some extent in tissues of IST receiving rats. Since high concentrations of a relatively imper-

TABLE II. The Comparative Effects of CLD and IST Infusions on Tissue Electrolytes in Rats.

Group	Infusion rate ml/hr	Liver		Kidney		Brain	
		K^+	Cl^-	K^+	Cl^-	K^+	Cl^-
		meq/kg		meq/kg		meq/kg	
Non[a] infused	—	78 ± 6	55 ± 7	73 ± 12	80 ± 9	90 ± 15	40 ± 7
CLD[b]	1.482	99 ± 18	51 ± 9	83 ± 10	71 ± 5	97 ± 7	50 ± 6
IST[b]	1.482	79 ± 16	40 ± 5+	65 ± 4	53 ± 5+	95 ± 2	44 ± 7

Groups of rats were infused with CLD or IST for approximately 96 hr. A portion of liver, kidney and brain was taken out, homogenized in chloride-free distilled water and analyzed for the electrolyte content as described in the text. Values for electrolytes in the row.

[a] Represent averages of six determinations ± S.D.

[b] Represent averages of two determinations ± 0.5 Range for K^+ and of four determinations ± S.D. for Cl^-.

+ Values significantly different from those of CLD group at $p \lesssim 0.05$ level.

meable anion in the renal tubules are known to decrease reabsorption of cations (21), these results suggest that a prevention of reabsorption of K^+ by IST presumably occurred due to relative nondiffusibility of isethionate anion (17). Since isethionate is known to have equal affinity for both Na^+ and K^+ (22), the proportionally greater increase in the excretion of K^+ ions is noteworthy. This selective action may be a result of activation of the sodium–potassium exchange pump in the distal tubule (21).

The rapid decrease in the excretion of the urinary Cl^- in the IST treated rats indicates that, of the portion of Cl^- displaced from tissues and serum and filtered into the tubules, a significant amount is being preserved by reabsorption. Hence, infusion of isethionate salts, although the most convenient technique for rapid administration, did not result in an efficient replacement of plasma chlorides as did a technique such as hemodialysis (18) which allows exchange of anions through a nonselective and perhaps more porous membrane than renal tubules. The lack of competition between isethionate and chloride anions or reabsorption through renal tubules is similar to the one observed in brain. Existence of a carrier-mediated transport for Cl^- reabsorption in renal tubules is, however, not established as yet.

The lymphoid depletion seen in histopathological specimens, the leukocytosis (23) and the hyperglycemia may be the result of pituitary-adrenal mediated stress reactions due to infusion as well as immobilization. The elevation of serum enzymes seems to support this contention (23, 24). The lack of change in the LDH values in the IST group is however, unclear at the moment and needs further investigation.

In conclusion, the chronic iv infusion of isethionate salts in a chloride free isotonic solution can be accomplished in rats without overt toxicity specifically attributable to isethionate. Changes in the electrolyte content of tissues, serum, and urine were observed in IST infused rats, however, when compared to CLD infused and non-infused rats. Indications of stress were seen; these stress reactions were not drug related since they were also seen in CLD infused rats. In spite of the relatively nontoxic nature of isethionate, its therapeutic usefulness for the reduction of brain edema is questionable, at least upon the results obtained in the rat model studied herein.

Summary. A technique was developed to infuse rats for a period of several days. The alterations in electrolytes produced by infusion with an iso-osmotic solution of isethionate salts were studied. In comparison with iso-osmotic chloride salts infused controls, the amount of Cl^- decreased and that of K^+ increased in the urine while both Cl^- and K^+ levels decreased in the serum of rats infused with iso-osmotic isethionate

salts. Concentration of Cl^- in liver and kidney also decreased but no change occurred in brain chloride level. Potassium levels did not change in any of these tissues. In comparison with noninfused control, increases in serum glutamic–oxalacetic transaminase and alkaline phosphatase were noted in both isethionate and chloride receiving rats, while a decrease in lactic dehydrogenase was seen in isethionate receiving rats. Except for occasional lymphoid depletion in both groups, no other histopathological changes were seen. Similarly no hematological or urinary changes of pathological significance were noted.

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