rise to a syndrome comparable to odoratism even when administered to rats in large doses (rats fed 1% methionine sulfoxide grew normally).§ A clue to the problem may lie in recent publications of Boström, Rodén, and Vestermark(14,15) who reported that glutamine, but not glutamic acid, appears to act as an accelerator of chondroitin sulfate synthesis and probably plays a part in biosynthesis of mammalian mucopolysaccharides. In this connection, it has been pointed out repeatedly that sweet pea lathyrism in the rat seems specifically to involve mesodermal tissues and that the metabolic defect appears to be localized in the ground substance, which is rich in mucopolysaccharides (*cf.*, for example, 4 and 16).

Conclusions. Of a wide variety of supplements tested for their protective action against development of skeletal deformities in rats fed sweet peas or β-aminopropionitrile, none gave complete protection. Only L-cysteine-HCl, L-glutamine, and proteins were partially protective. Of the proteins tried, casein, gelatin, lactalbumin and casein hydrolysate gave some protection; zein did not. The protective action of glutamine may be related to its probable role in chondroitin sulfate and mucopolysaccharide synthesis.

Note added in press. Experiments completed since this paper was submitted for publication indicate that the protection afforded by L-cysteine-HCl is prolonged if the diet is made up fresh weekly. Under these conditions the protection against development of skeletal changes was greater with cysteine than with glutamine. The decreased protective activity of L-cysteine in an aging diet is probably due to a gradual loss of cysteine because of the lability of its sulfhydryl group.

§ Unpublished data.

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Relation of Brain Stem to Renal Electrolyte Excretion.* (22326)

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Experimental evidence that a cerebral lesion might affect formation of urine was first presented by Claude Bernard. He produced polyuria without glycosuria in the experimental animal by puncturing the median eminence of the floor of the fourth ventricle

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slightly cephalad to the point where puncture caused polyuria and glycosuria(1). The author's interest in the relationship between the lower brain stem and renal excretion of electrolytes was aroused by study of a patient with probable "cerebral salt-wasting syndrome," due to tumor within the fourth ventricle of the brain. The syndrome consists of persistent loss of sodium and chloride in the urine, despite low concentration of these ions in the blood. In 4 of 6 cases in medical literature (excluding tuberculosis and tuberculous meningitis), patients had diffuse diseases of the central nervous system(2,3). One patient had glioma of the posterior thalamus, with an area of demyelination extending down the brain stem(4). In the sixth case, a brain tumor was present, involving the floor of the fourth ventricle just rostral to the obex(2).

These cases raise the question whether the lower brain stem may exert any control over electrolyte excretion. The following experiment was carried out in an attempt to clarify this problem.

Method. Ten adult female mongrel dogs were used. Anesthesia was induced and maintained by intravenous pentobarbital. An indwelling urethral catheter was left in place throughout each experiment. When intravenous fluids were administered, a continuous gravity drip was started immediately after catheterization. Using aseptic technic, the *cerebellar fossa was opened.* The lower portion of the cerebellar vermis and the medulla were exposed. The lower portion of the fourth ventricle was visualized by retraction or resection of the lower vermis (results were the same whether or not the vermis was resected). Stimulation of the caudal portion of the floor of the fourth ventricle just cephalad to the obex was then carried out by the Grass model S4B stimulator and bipolar wire electrodes. Parameters of stimulation generally were 0.5 volts, 5/sec. (monophasic), 1 millisec. duration. During stimulation, the voltage was momentarily increased several times. Generalized clonic movements usually appeared at 0.8 to 1 volt. *Urine collection* periods were generally 20 to 30 minutes during control and stimulation. All urine secreted during stimulation was collected as one specimen. Collection periods during operative procedure were generally 1 hour. Urine sodium and potassium content was determined with Baird flame photometer using lithium as internal standard. Chloride was determined by the method of Sanderson(5). The pH was measured on Beckman pH meter.

Results. Complete data from 3 representative experiments are given in Table I. Three

TABLE I. Effect of Stimulation of Caudal Fourth Ventricle Floor on Sodium, Potassium and Chloride Excretion and Urine Volume in Dogs (3 Representative Experiments).

Dog No.		cc/min.	Na	K	Cl	Plasma Na (mEq/l)
			(μEq/min.)			
17 No intrav. fluids	Operative 9:30-10:30	.10	12	9	10	
	Control 10:30-10:55	.08	10	7	3	
	Stim. 11:17-11:47 .5 v, 5/sec.	.40	90	18	20	(Vermis resected)
	Closure 11:55-12:25	.27	50	11	14	
200 Intrav. 5% NaCl	Operative 1:10-2:10	2.4	529	41	575	
	Stim. 2:40-3:10 .5 v, 5/sec.	4.4	1090	79	1160	
	After coagulation of floor of 4th ventricle 3:17-3:47	2.1	570	44	607	
144 Intrav. 5% dextrose in water						Preop. 144
	Operative 9:45-10:45	1.25	210	26	220	
	Stim. 11:20-11:45 .5 v, 5/sec.	4.52	537	44	551	Before stim. 137
						After stim. 134
	After stim. 12:00-12:30	.9	41	17	58	
	Close 12:30-1:00	.7	12	12	15	(Vermis resected)

dogs received no intravenous fluids. In one instance, sodium and chloride excretion increased from 10 μEq/min. and 3 μEq/min. respectively before stimulation to 90 μEq/min. and 20 μEq/min. during stimulation. In the second, the sodium excretion rose from 12 to 85 μEq/min. and chloride from 8 to 101 μEq/min. during stimulation. Potassium excretion increased, but not as much as sodium or chloride.‡ Urine volumes also rose. The effect tended to persist in the immediate post-stimulation period. In the third animal, the increase in sodium excretion was insignificant during stimulation with 0.5 volt, but increasing the parameters to 1 volt, 10 per second, resulted in a small *decrease* in sodium excretion and urine volume. During this latter period, there were continuous clonic movements of the suboccipital muscles.

One animal received a continuous intravenous infusion of 5% sodium chloride. The output of sodium, potassium and chloride doubled during stimulation of the floor of the fourth ventricle, and urine volume almost doubled (Table I). Following stimulation, the superficial caudal portion of the floor of the fourth ventricle was destroyed with electrocautery. During the next 20 minutes, excretion rates were very close to pre-stimulation values.

Continuous intravenous infusions of 5% glucose in distilled water were administered by gravity to 6 dogs. In two animals, stimulation resulted in marked increase in sodium excretion (59 to 234 μEq/min. and 210 to 537 μEq/min.) and chloride excretion (58 to 196 μEq/min. and 220 to 551 μEq/min.). There was a rise in urine volume, and very little change in potassium excretion.§ In a third animal, sodium and chloride excretion increased slightly with stimulation at 1.5 volts, 10/sec.

In the remaining 3 dogs, the results varied. In Dog No. 101, sodium and chloride excretion increased with the electrodes in place, without electrical stimulation. The pressure of the electrodes may have acted as a stimulus. Stimulation at 0.2 volt resulted in a decrease in electrolyte excretion. There was little change at 0.5 volt, but when the stimulus was increased to 1 volt, a definite decrease in sodium and chloride output occurred. In Dogs No. 149 and 151, stimulation was accompanied by a decrease in sodium and chloride excretion, with an increase following stimulation in No. 151.

Two additional experiments were carried out, but are not listed. In 2 dogs, the orbital surface and the convexity of the frontal lobe were stimulated at varying voltages. One dog received 5% dextrose in distilled water, the other 5% sodium chloride. No definite change in urine volume or electrolyte excretion was noted.

Discussion. The bipolar method of stimulation used in these studies probably results in stimulation of a relatively large cross-sectional area of the brain stem at the level stimulated, depending on voltage. Thus at 0.7 to 1 volt, clonic movements of the extremities occurred, presumably from stimulation of the pyramidal tracts. It was noted, however, that voltage threshold for clonic movements varied from one animal to the next, suggesting that at the voltage selected (0.5 volt), the spread of the stimulus varied somewhat among the animals used. This may explain in part the variability of the results.

The data obtained, however, suggest that an influence may be exerted by the brain stem on renal excretion of electrolytes, particularly sodium and chloride. In 5 dogs, stimulation of the caudal portion of the floor of the fourth ventricle at 0.5 volt, 5 cycles per second, resulted in increased excretion of sodium and chloride. A smaller increase in potassium excretion also occurred in these dogs. Slight rises of questionable significance occurred in 3 dogs, and there was some decrease in excretion rate in 2 dogs. Increasing the stimulus strength to 1 volt (and thereby presumably increasing the spread of the stimulus) in 2 dogs resulted in a decrease in excretion rates.

Previous studies of the effects of brain stimulation(6), increased intracranial pres-

‡ Concentration of sodium and chloride in urine (mEq./l.) also increased during stimulation, while concentration of potassium decreased.

§ In Dog No. 144, see Table, the serum sodium had fallen from 144 mEq./l. to 137 mEq./l. prior to stimulation.

sure(7,8) and splanchnicotomy(9-11) suggest that the nervous system may influence renal electrolyte excretion. The author is not aware of studies similar to the present one.

These results are preliminary. Further studies are planned.

Summary. In anesthetized dogs, bipolar stimulation (0.5 volt, 5/sec.) of the caudal portion of the floor of the fourth ventricle resulted in increased renal excretion of sodium and chloride, and a smaller increase in potassium excretion in 5 instances, slight changes in 3, and decreased excretion in 2. In 2 dogs, increasing the stimulus to 1 volt resulted in a decreased excretion rate of these electrolytes.

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Identification of Cystine Calculi in Mink. (22327)

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Leoschke, Zikria and Elvehjem(1) in quantitative analyses of 31 urinary calculi from mink revealed the main constituent to be magnesium ammonium phosphate, accompanied by small amounts of calcium phosphate and magnesium phosphate. Sompolinsky(2), on the basis of qualitative analyses of 26 stones classified 12 as pure magnesium ammonium phosphate, 13 as magnesium ammonium phosphate plus calcium and 1 as magnesium ammonium phosphate plus calcium and urate.

Observations on bladder stones from mink submitted to the Veterinary Diagnostic Laboratory at Oregon State College have generally indicated an inorganic-type structure. Recently, however, a male mink was presented whose bladder contained 140 small creamy-white colored, smooth, irregularly-shaped calculi and these when submitted to normal ashing procedures proved to be wholly organic matter. Total oven dry weight of the calculi was 1.023 g. The appearance and size of these calculi are indicated in Fig. 1.

Observations. Qualitative analyses of these stones were carried out in the sequence proposed by Hawk, Oser and Summerson(3). These tests indicated strongly the presence of cystine.

Further identification was made by means of Sullivan's reaction which is reported to be specific for cystine(4). A few mg of powdered calculus were taken up in 5 ml of approximately 0.1 N HCl and 1 ml each of 1% NaCN in 0.8 N NaOH and 0.5% aqueous 1, 2-napthoquinone-4-sodium sulfonate were added. These were followed by the addition of 5 ml 15% Na_2SO_3 in 0.5 N NaOH and the mixture was allowed to stand 30 minutes. On