N. Y. Acad. Sci., 1960, v23, 148.
6. Bruckmann, G., Westheimer, E., *Brit. J. Exp. Path.*, 1945, v26, 217.
7. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 292.
8. ———, *J. Immunol.*, 1948, v59, 173.
9. ———, PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 656.
10. Hyman, G. A., *Blood*, 1954, v9, 911.
11. Ponder, E., Nesmith, J., *Cancer Res.*, 1952, v12, 104.
12. Ponder, E., Ponder, R. V., *Rev. Hematol.*, 1954, v9, 562.
13. Weil, R., *J. Med. Res.*, 1908, v19, 281.

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Effect of Hypochloremia on Cerebrospinal Fluid Chloride Concentration In a Patient with Anorexia Nervosa and in Dogs. (28803)

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There has been some question as to whether the cerebrospinal fluid (CSF) chloride concentration is the result of a passive transport process, or whether an active mechanism accounts for the normal CSF/plasma chloride ratio of approximately 1.15. In the dogfish (1) and the cat(2), an active mechanism has been postulated, though data reported by Held, Fencl and Pappenheimer(3) indicate that, normally, the gradient in dogs and goats can be accounted for by the existing potential difference between the CSF and blood.

Studies in a patient with chronic hypochloremia associated with anorexia nervosa demonstrated that the plasma chloride concentration was an important determinant of chloride concentration in CSF from the lumbar sac, but that CSF removed from higher in the neural axis was less influenced by the low plasma concentration of chloride. Preliminary work in dogs (Dogs 1-5) also indicated a tendency to maintain cisternal CSF chloride concentration over a wide range of plasma chloride concentrations.

Further studies with dogs (Dogs 6-8) demonstrated that changes in blood pH, which alter the potential difference between CSF and blood(3), could not account for the observed high CSF/plasma chloride ratio.

Materials and methods. Clinical studies. A 27-year-old woman† with anorexia nervosa (4) developed hypochloremia secondary to frequent self-induced vomiting. Extensive diagnostic studies failed to reveal evidence of an organic lesion involving the brain or meninges. On each of 2 occasions, after a blood specimen was obtained, a lumbar puncture was performed. The cerebrospinal fluid was obtained in the following manner. After collection of the first 2 ml, approximately 14 ml was removed over a 5-minute period. Then a third sample of 2 ml was obtained; the first and third specimens were analyzed by the clinical laboratory for chloride and potassium. Considering that the normal intraspinal (thoracic and lumbar) volume of cerebrospinal fluid is approximately 30 ml in adults of average size(5), the third specimen obtained from this 33 kg patient was assumed to be from the high thoracic area.

Dog studies. Hypochloremia of varying degrees was induced in 8 dogs of mixed breed, weighing from 12 to 15 kg, by surgically creating a gastric fistula. In Dogs 1-2,

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† Patient was under the care of Dr. Jacob Fishman, Adult Psychiatry Branch, Nat. Inst. Mental Health.

TABLE I. Laboratory Data on Patient with Anorexia Nervosa.

		CSF—Lumbar region		CSF—Thoracic region	
	Plasma concentration*	Conc	CSF/plasma	Conc	CSF/plasma
		Depleted state			
Cl	71	81	1.14	102	1.44
K	2.2	2.3	1.04	3.1	1.41
pH	7.66	7.67		—	
	Na—139 CO_2—51 TP—6.6 g† Ca—9.0 Mg—2.5 Creatinine—1.1 mg % Hgb—12.9 g %	TP—56 mg % Mg—3.1 Glucose—62 mg %		CO_2—38	
		After replacement therapy			
Cl	101	114	1.13	129	1.28
K	4.7	3.6	.76	3.4	.72
pH	7.54	7.49		7.48	
	Na—140 CO_2—32 Hgb—9.4 g %	CO_2—30		CO_2—27	

* All electrolyte concentrations in meq/l. † TP = Serum total protein concentration.

through a right upper quadrant incision, a #16 Bardex catheter was positioned into the distal stomach, held in place with purse-string sutures, and brought out through the abdominal wall *via* a stab wound. Gastroduodenal continuity was not interrupted, and moderate chloride depletion was accomplished over a 10-12 day period by frequent tap water lavage *via* the catheter, with gastric secretion stimulated by subcutaneous histamine administration. In Dogs 3-8, employing a similar right upper abdominal paramedian incision, the distal stomach and proximal duodenum were freed by ligation and division of blood vessels, and the duodenum was transected just distal to the pylorus. The duodenum was then closed with 3 layers of stitches, and a #16 Bardex rubber catheter with multiple side holes was inserted into the open end of the distal stomach, held in place with 2 purse-string sutures, and brought out through the abdominal wall by means of a stab wound in the right upper abdomen. The distal stomach was fixed to the peritoneum and anterior abdominal wall by interrupted sutures, allowing free drainage of all gastric secretions through the catheter. These dogs received daily 500 cc of fluid subcutaneously, either 5% dextrose in water alone or supplemented by a protein hydrolysate (Amino-Plus, Pitman-Moore Co.), while rapid and severe chloride depletion was accomplished over a 4-day period.

Cerebrospinal fluid was obtained from the dogs before and after chloride depletion by entering the cisterna magna with a 22-gauge needle. Simultaneously a venous blood sample was obtained with a heparinized syringe. Prior to sacrifice, the dogs were anesthetized with sodium nembutal, and in Dogs 2, 3 and 4 cerebrospinal fluid was obtained from the lateral ventricle with the aid of a Horsely-Clark Stereotaxic apparatus. Chloride concentrations in plasma and cerebrospinal fluid were determined, in duplicate, by the method of Cotlove(6), and sodium and potassium determinations were performed with a Perkin-Elmer flame photometer using an internal lithium-standard. All pH measurements were made on heparinized whole blood and CSF at 37°C.‡

Results. Patient studies. Initially, chloride concentration of cerebrospinal fluid from the lumbar sac in the patient was 81 meq/l, as compared to plasma level of 71 meq/l, resulting in a normal chloride CSF/plasma ratio of 1.14(7) (Table I). In contrast,

‡ pH meter manufactured by Instrumentation Laboratories, Inc., Boston, Mass.

TABLE II. Laboratory Data on Dogs 1-5.

Dog	Days of depletion	Control						Following depletion					
		Plasma*			Cist CSF		Chloride CSF/plasma	Plasma			Cist CSF		Chloride CSF/plasma
		Cl	Na	K	Cl	Na	K		Cl	Na	K	Cl	Na	K	
1	10	115	147	4.6	128	145	2.9	1.11	88	137	3.1	102	128	2.8	1.16
2	12	111	141	—	133	—	—	1.20	85	—	—	112	—	—	1.32
3	4	—	—	—	—	—	—	—	77	—	—	123	—	—	1.60
4	4	118	145	3.9	134	152	3.1	1.14	77	148	4.2	119	143	2.4	1.55
5	4	116	144	5.0	134	144	3.2	1.16	63	121	3.5	103	126	2.6	1.63

* All concentrations in meq/l.

chloride concentration in the CSF that had surrounded the thoracic region of the cord was 102 meq/l, with a CSF/plasma ratio of 1.44. A difference in potassium concentration was also noted between the 2 cerebrospinal fluid samples, with the potassium level in the fluid from the thoracic region being higher than the plasma concentration; this is a reversal of the normal CSF/plasma ratio for potassium(7).

Two weeks later, after intravenous replacement therapy, including administration of chloride, chloride concentration in the lumbar sac was 114 meq/l and 101 meq/l in the plasma, giving a normal lumbar CSF/plasma ratio of 1.13. Fluid from the thoracic region again had a higher concentration of chloride, 129 meq/l and a CSF/plasma ratio of 128; a ratio higher than expected under normal conditions as a difference between the chloride concentration of ventricular and spinal fluid has not been noted by others(8).

Dog studies. The data suggest an increasing chloride cisternal CSF/plasma ratio as chloride depletion becomes more severe (Table II). Dogs 1 and 2 were only slightly depleted, and the chloride CSF/plasma ratio went from a control value of 1.11 to 1.16 after depletion in Dog 1 and from 1.20 to 1.32 in Dog 2. Control data were not available for Dog 3, but when the plasma chloride concentration fell to 77 meq/l, the CSF concentration was 123 meq/l, with CSF/plasma ratio of 1.60. Dogs 4 and 5 had normal control chloride CSF/plasma ratios of 1.14 and 1.16, and were then depleted to a plasma chloride concentration of 77 meq/l and 63 meq/l, respectively. After depletion, Dog 4 had a chloride CSF/plasma ratio of 1.55, and Dog 5 a ratio of 1.63. The concentration of chloride in the CSF obtained from entering the cisterna magna in chloride de-

TABLE III. Comparison of Chloride Concentration in CSF from Cisterna Magna and Lateral Ventricle.

Dog	CSF chloride, meq/l	
	Cisterna magna	Lateral ventricle
2	112	110
3	123	125
4	119	127

TABLE IV. Laboratory Data in Dogs 6-8.

Dog	Days of depletion	Plasma* Cl	Na	K	CO_2	pH	Cisternal CSF Cl	Na	K	CO_2	pH	CSF/plasma ratio Cl	Na	K	CO_2
							Control								
6	0	114	144	4.2	21	7.47	131	154	3.0	20	7.44	1.15	1.07	.71	.95
7	0	110	145	4.6	23	7.36	128	149	4.7	24	7.42	1.16	1.03	1.02	1.04
8	0	114	149	4.4	22	7.42	135	154	5.0	23	7.43	1.18	1.03	1.14	1.05
							After chloride depletion								
6	4	49	122	1.9	49	7.67	81	124	2.3	39	7.51	1.65	1.02	1.21	.80
7	4	63	126	2.1	37	7.64	97	130	2.5	35	7.51	1.54	1.03	1.19	.94
8	4	46	132	4.4	47	7.65	84	134	2.3	46	7.52	1.83	1.02	.52	.98

* All electrolytes expressed in meq/l.

pleted dogs was approximately equal to the chloride levels in fluid removed directly from the lateral ventricle (Table III).

More detailed studies involving Dogs 6–8 documented that a metabolic alkalosis was resulting from the depletion procedure (Table IV). Dog 6 had a control chloride CSF/plasma ratio of 1.15 and a ratio of 1.65 at the time the plasma chloride concentration was 49 meq/l. Corresponding values for Dog 7 and 8 were control CSF/plasma ratios of 1.16 and 1.18, and after depletion when plasma chloride levels were 63 and 46 meq/l respectively, 1.54 and 1.83. Hemoglobin values of 38, 51, and 45 g/100 ml for Dogs 6, 7 and 8 respectively, were consistent with the clinical evaluation that dehydration was minimal at the time the final blood and CSF samples were obtained.

The sodium CSF/plasma ratios did not change during the experimental period in Dogs 6–8, and there was only a slight decrease in the CO_2 CSF/plasma ratios. The potassium CSF/plasma ratios appeared variable in these 3 dogs, a result that might reflect hemolysis in some of the blood samples.

Discussion. Data from the patient with hypochloremia demonstrated that the chloride concentration in CSF removed from the lumbar sac was substantially less than chloride levels in the fluid assumed to have surrounded the thoracic cord. The existence of a relatively high CSF to plasma chloride gradient suggested the active transport of chloride into the CSF, with progressive equilibration with plasma as the fluid moved away from the site of production and into the lumbar sac.

The high chloride CSF/plasma ratios observed in dogs depleted of chloride were also consistent with the concept of an active mechanism for transport of chloride into the cerebrospinal fluid. It has been reported recently(3) that the potential difference in mammals between CSF and blood is dependent upon the blood pH; at a normal blood pH, it is 3–5 mv (CSF positive). It is 12 mv (CSF positive) at pH 7.0, and 3 mv (CSF negative) at pH 7.6. Normally, this potential difference (3–5 mv) can account for the concentration gradient of chloride. However, in the patient studied and in the chloride depleted dogs with alkalosis, the potential difference would not be adequate to explain the observed chloride CSF/plasma ratios and an active mechanism must be considered.

Summary. The human and animal data presented suggest that chloride may be actively transported into the cerebrospinal fluid from plasma against a concentration gradient. The data obtained from the patient further suggest that in states of hypochloremia the CSF chloride concentration decreases as the cerebrospinal fluid moves away from the site of production, presumably secondary to equilibration with the low plasma chloride levels.

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1. Hogben, C. A. M., Wistrand, P., Maren, T. H., *Am. J. Physiol.*, 1960, v199, 124.

2. deRougemont, J., Ames, A., III, Nesbett, F. B., Hofmann, H. F., *J. Neurophysiol.*, 1960, v23, 485.

3. Held, D., Fencl, V., Pappenheimer, J., *Fed. Proc.*, 1963, v22.

4. Bliss, E. L., Branch, C. H., *Anorexia Nervosa: Its History, Psychology and Biology,* Hoeber, New York, 1960.

5. Weston, P. G., *Arch. Neurol. and Psychiat.*, 1921, v5, 58.

6. Cotlove, E., Trantham, H. V., Bowman, R. L., *J. Lab and Clin. Med.*, 1958, v51, 461.

7. Rall, D. P., Zubrod, C. G., *Ann. Rev., Pharmacol.*, 1962, v2, 109.

8. Davson, H., *Physiology of the Ocular and Cerebrospinal Fluids,* Little, Brown and Co., Boston, 1956, p210.

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Influence of Fatty Acids on Appearance of Bacterial Phospholipase D in Incubated Plasma. (28804)

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In addition to clostridia, a number of bacilli are known to elaborate phospholipase D, degrading phospholipids to diglyceride (Dg) and phosphate ester(1). Since some of these bacilli are commonly present in air, water, and dust, incubations of biologic material with phospholipids under nonsterile conditions may lead to destruction of the phospholipids from phospholipase D of bacterial origin. Thus Gollub *et al*(2) found thromboplastin was destroyed by an enzyme elaborated by *B. cerus - B. megatherium.* Kushner and Feldman(3) showed that this bacillus elaborated phospholipase D.

During a study of a post heparin phospholipase A(4), phospholipase D of bacterial origin appeared in serum or plasma after incubation intervals of 8 to 10 hours. Fatty acids (FA) were required for the appearance of this enzyme. While the growth of some bacteria is enhanced by FA(5), an effect of FA on the elaboration of phospholipase D by bacteria has not previously been reported (1).

Methods. The collection of post heparin plasma, the use of Armour #2293 fraction V bovine albumin, and the preparation of egg phosphatidylethanolamine (PE) and lecithin (Le), have been described(4). Globulin was bovine fraction IV of Nutritional Biochemicals Corp. The oleic acid and palmitic acid added to incubation systems were the highest purity products of the Hormel Foundation and Nutritional Biochemicals Corp., respectively. A suitable aliquot of the FA in ethyl ether was introduced into the incubation tube and the ether removed with a current of nitrogen while the tube was warmed to 40°C. The μmoles of FA added to incubation systems are expressed as a ratio to the μmoles of phospholipid present (FA/PL). The optimal value of this ratio varies with the amount of protein present since the available fatty acid is that which exceeds the binding sites on proteins. Alteration of the albumin content of the system required a corresponding change in the amount of fatty acids required for diglyceride formation.

Bacterial contaminant. From incubation systems containing phospholipase D activity, a gram positive, aerobic, spore forming bacillus was isolated. Its morphology and colonial growth suggested *B. subtilis.* This was the organism first reported by Gollub *et al*(2) as the bacterium involved in destruction of thromboplastin. Further specification of the contaminant encountered was not attempted.

Results. Demonstration of diglyceride and phosphoethanolamine from the degradation of PE by bacterial phospholipase D. The two incubations of Fig. 1 differed in that on the left FA were derived from the degradation of PE to LyPE and FA by post heparin plasma(4), while on the right with pre hepa-