6. Reiser, R., *J. Nutr.*, 1950, v42, 325.
7. Bieri, J. G., Briggs, G. M., Spivey Fox, M. R., Pollard, C. J., Ortiz, L. O., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 237.
8. Marion, J. E., Edwards, H. M., Jr., *J. Nutr.*, 1962, v77, 23.
9. Ames, S. R., Risley, H. A., Harris, P. L., *Analyt. Chem.*, 1954, v26, 1378.
10. Ross, E., Adamson, L., *J. Nutr.*, 1961, v74, 329.
11. Gridley, M. F., *Am. J. Clin. Pathol.*, 1953, v23, 303.
12. Jungherr, E. L., Luginbuhl, R. E., Jacobs, R. E., *Proc. Am. Vet. Med. Assn.*, 1953, p303.
13. Nagai, H., Sudo, M., Akaishi, K., *Annales Paediatrici Japonici*, 1961, v7, 36.

Received June 27, 1963. P.S.E.B.M., 1963, v114.

Effects of Acid-Base Alterations on Cerebrospinal Fluid Production. (28593)

W. W. OPPELT, T. H. MAREN, E. S. OWENS AND D. P. RALL

Clinical Pharmacology and Eperimental Therapeutics Service, National Cancer Institute, National Institutes of Health, Bethesda, Md., and Departments of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville

Since the introduction of ventriculo-cisternal perfusion with inulin-containing buffer as a technique to measure cerebrospinal fluid (CSF) production rates(1) it has become possible accurately to determine the effect of various drugs and metabolic manipulations on CSF formation rate. Using this technique we have previously reported on the effects of parenteral carbonic anhydrase inhibitors and parenteral and intrathecal cardiac glycosides on this rate(2). In the dog, carbonic anhydrase inhibitors were found to decrease CSF formation rate by 40-50%, whereas ouabain had no effect(2). In the cat, however, a significant decrease of CSF production was noted with intrathecal ouabain(3). To investigate further the factors that control CSF production rates the effects of metabolic acidosis and alkalosis, and respiratory acidosis and alkalosis on CSF production were studied in dogs.

Methods. Mongrel dogs weighing from 8-14 kg were lightly anesthetized with intravenous pentobarbital. Their heads were fixed in a stereotaxic apparatus, and a 22-gauge needle was introduced into the right lateral ventricle. A 19-gauge needle was placed into the cisterna magna. Ventriculo-cisternal perfusion was then begun with a buffer* containing inulin-C^{14}, 1 μc/100 ml. Perfusion rates were kept constant for each experiment at 0.123 ml/minute. CSF production rates were calculated by the following formula: production $= r\ (C_i - C_o)/C_o$, where C_i and C_o refer to inulin concentration in the inflow and the outflow and r refers to the inflow rate in ml/minute. It can thus be seen that CSF formation rates were calculated as a factor of the dilution of inulin as it passes from the lateral ventricle to the cistern. Studies of Pappenheimer and Halsey(1) and of Rall *et al.*(4) have shown that less than 1% of such perfused inulin passes either into the blood or the extracellular space of brain. Therefore, the assumption is valid that the dilution of inulin occurring as it passes through the ventricular system is due almost exclusively to newly formed CSF. This method allows for relatively exact determination of CSF production rates and is independent of brain vascular volume.

Metabolic acidosis was produced by intravenous infusion of 0.1-0.3 N HCl at a rate of 3-5 meq/kg/hr. Metabolic alkalosis was caused by intravenous 0.5 N $NaHCO_3$ infused at a rate of about 5 meq/kg/hr. The dogs were put into respiratory acidosis by allowing them to breathe a 5-10% CO_2/oxygen mixture. A Jefferson ventilator was used to hyperventilate the dogs with air to produce respiratory alkalosis. Each experiment consisted of a 2-hour control period followed by a 4-5

* NaCl – 123.5 meq/l; KCl – 3 meq/l $CaCl_2$ – 1.3 meq/l; $MgCl_2$ – .8 meq/l; $NaHCO_3$ – 25 meq/l; NaH_2PO_4 – .5 meq/l; Na_2HPO_4 – .5 meq/l.

TABLE I. Blood Acid-Base Changes and CSF Production Rates During Metabolic and Respiratory Acidosis.

	CSF production, ml/min			Blood								
				pH			CO_2, mM			pCO_2, mm Hg		
Dog	Control	Exp	Acet	Control	Exp	Acet	Control	Exp	Acet	Control	Exp	Acet
Metabolic												
324	.066	.053	.044	7.39	7.19	7.14	27.7	19.2	22.5	45	49	64
326	.047	.054	.020	7.36	7.26	7.19	24.6	15.9	17.5	43	36	45
330	.063	.040	.033	7.55	7.42	7.24	21.0	14.6	16.1	26	23	40
337	.047	.048	.040	7.38	7.34	7.27	26.0	19.2	19.1	42	35	42
346	.064	.058		7.42	7.13		23.0	16.8		35	54	
362	.076	.073		7.38	7.31		25.0	15.0		44	31	
377	.058	.056		7.37	7.29		25.0	14.0		44	33	
369	.037	.031		7.42	7.38		28.5	13.4		45	23	
Respiratory												
334	.080	.065		7.47	7.23		27.4	28.7		41	74	
336	.056	.071		7.42	7.25		27.9	32.6		46	78	

All values are avg of ½ hr periods for each experiment. Control periods lasted 2 hr, experimental periods 4-5 hr. Acetazolamide was given, in doses of 50 mg/kg 2 hr after the end of control period. *Exp* refers to periods of acidosis, while *Acet* refers to periods of acidosis and acetazolamide effect.

hour period of each metabolic alteration. In some of the dogs, 50 mg/kg acetazolamide was given intravenously 2 hours after initiation of acid-base alteration.

During each experiment blood pH was determined at hourly intervals with a Beckman expanded scale pH meter on heparinized arterial blood collected under oil. Total plasma CO_2 was obtained at the same time on plasma that had been immediately separated by centrifugation under oil. A Natelson Microgasometer was used to determine total CO_2. Plasma CO_2 tensions were read from a nomogram based on the Henderson-Hasselbalch equation.

Results. Acidosis: Eight dogs received intravenous infusion of 0.15-0.3 N HCl after a 2-hour control period. In some of the dogs a moderate increase in CSF production was noted, while a decrease occurred in others (Table I). The net rseult was no significant change in CSF formation rate during metabolic acidosis. Four dogs, while in metabolic acidosis, received 50 mg/kg acetazolamide intravenously. In all 4 dogs a decrease in CSF production, averaging 31% from production rates during acidosis alone, was noted (Table I).

A 5-10% CO_2/oxygen mixture was inhaled by 7 dogs. In 2 of these dogs blood pH and total CO_2 were determined, while in the other 5, done in the same manner, these values were not obtained. There was no consistent change in CSF production rates in the 2 dogs shown in Table I. The 5 other dogs had control CSF formation rates of 0.053 ± 0.003 ml/minute,† while the rate during respiratory acidosis was 0.58 ± 0.004 ml/minute. Thus, here too, no significant change in CSF production occurred.

Alkalosis: Six dogs were made alkalotic by infusion of 0.5 N $NaHCO_3$ after a 2-hour control period. A consistent decrease in CSF production rate averaging 23% occurred (Table II). Three dogs, while alkalotic, received 50 mg/kg acetazolamide intravenously. A 45% decrease in CSF production as compared to the rate during alkalosis alone occurred (Table II). In 6 dogs significant respiratory alkalosis was produced by hyperventilation. CSF production consistently decreased an average of 46% (Table II). Two dogs received intravenous acetazolamide, 50 mg/kg, while their respiratory alkalosis was maintained. Again, a 62% decrease in CSF production occurred as compared to production during alkalosis alone (Table II). This represents an overall decrease of 74.7% from control production.

A summary of the effects of acid-base alterations on CSF production is seen in Table III.

Discussion. It appears that alteration of body acid-base balance in the dog has a definite effect on rate of formation of CSF. During metabolic and respiratory acidosis no

† Data express ± one standard error of mean.

TABLE II. Blood Acid-Base Changes and CSF Production Rates During Metabolic and Respiratory Alkalosis.

	CSF production, ml/min			Blood								
				pH			CO_2, mM			pCO_2, mm Hg		
Dog	Control	Exp	Acet	Control	Exp	Acet	Control	Exp	Acet	Control	Exp	Acet
Metabolic												
327	.051	.036	.016	7.28	7.47	7.42	24.7	36.7	33.6	51	52	53
328	.076	.071	.039	7.49	7.52	7.44	24.4	28.8	26.3	34	36	39
331	.063	.047	.029	7.42	7.57	7.62	23.6	33.1	34.3	38	39	35
354	.043	.031		7.42	7.62		24.0	39.8		38	40	
364	.040	.036		7.42	7.60		26.1	36.4		40	38	
383	.046	.029		7.39	7.53		22.0	35.6		35	43	
Respiratory												
536	.051	.024	.004	7.42	7.77	7.47	26.2	15.9	19.7	44	11	29
538	.049	.034	.021	7.41	7.78	7.52	25.4	12.8	18.4	44	10	24
535	.071	.036		7.44	7.65		20.9	13.2		33	13	
534	.063	.045		7.40	7.53		18.2	8.3		32	9	
530	.055	.022		7.42	7.71		16.8	11.4		28	9	
529	.053	.024		7.46	7.82		21.0	12.8		32	8	

All values are avg of ½ hr periods for each experiment. Control periods lasted 2 hr, experimental periods 4-5 hr. Acetazolamide was given, in doses of 50 mg/kg 2 hr after the end of control period. *Exp* refers to periods of alkalosis, while *Acet* refers to periods of alkalosis and acetazolamide effect.

significant change in this rate occurred, whereas moderate decreases in CSF production were noted during metabolic alkalosis, and greater decreases during respiratory alkalosis.

To attempt to explain these results one might first consider the mechanism of CSF production. It is generally agreed that CSF is a secretory product of the choroid plexus. The best evidence for this was probably presented by de Rougemont *et al.*(5) who devised a technique for collecting fluid directly as it leaves the choroid plexus. They found that the electrolyte concentration of such CSF as well as fluid collected further along its course of circulation could only be explained on the basis of active secretion. The factors controlling this secretion are unclear. Several authors have shown(2,6,7) that carbonic anhydrase inhibitors such as acetazolamide decrease the rate of formation of CSF. Quantitation of this effect, however, has shown that at presumably maximal inhibition of tissue carbonic anhydrase only a 50% decrease in CSF production occurs. Thus, mechanisms other than those dependent on carbonic anhydrase must be involved in CSF secretion. Na-K activated ATP'ase, thought to be involved with active sodium transport has been demonstrated in the choroid plexus(8). Inhibition of its activity by intrathecal administration of cardiac glycosides has given variable results, in that in the dog no effect was observed(2) whereas in the cat there was a dramatic decrease in CSF formation rate(3).

TABLE III. Summary of the Effects of Acid-Base Changes on CSF Production.

	Blood			
	pH	CO_2, mM	pCO_2, mm Hg	% change CSF production
Metabolic acidosis alone	7.29	16.0	36	NC
" " with acetazolamide	7.21	18.8	48	↓31
Respiratory acidosis	7.24	30.6	76	NC
Metabolic alkalosis alone	7.55	35.1	41	↓23
" " with acetazolamide	7.49	31.4	42	↓45
Respiratory alkalosis alone	7.71	12.4	10	↓46
" " with acetazolamide	7.50	19.1	27	↓62

Values are avg of data in Tables I and II.

NC refers to no significant change in CSF production rates. Decreases noted with acetazolamide are percentage decreases of CSF production rates during acid-base alteration alone.

Thus the ATP'ase system, in the cat, at least, also may be important in regulating CSF production. The role of carbonic anhydrase in controlling these secretory systems is the speeding of the equilibrium, $H_2O + CO_2 \leftrightarrows H_2CO_3$(9). One would thus expect that in a metabolic situation where the substrate for this reaction, gaseous CO_2, is decreased, the reaction should slow. In our experiments, in which the greatest reduction in pCO_2 occurred, namely during respiratory alkalosis, a significant 45% decrease in CSF production was noted. During metabolic acidosis, where there was only slight decrease in pCO_2 no consistent change in CSF production occurred. There also appears to be a pH effect, for during metabolic alkalosis, when pH was increased but the substrate CO_2 was present in plentiful supply, a decrease in CSF production was also noted.

Other carbonic anhydrase dependent systems have been investigated during alterations of acid-base balance. In the kidney, metabolic and respiratory alkalosis decreases acid secretion(10). In the nasal salt gland of the sea gull metabolic alkalosis had no effect on Cl^- secretion, whereas severe respiratory alkalosis and metabolic acidosis resulted in a decrease in this gland's Cl^-secretory rate(9). In the stomach metabolic acidosis increases secretion of H^+, and metabolic alkalosis reduces it(11). The situation in the pancreas(12) and the eye (13), two other systems controlled by carbonic anhydrase, is similar to that found in the nasal salt gland of the sea gull. It appears, therefore, that the CSF system does not exactly correspond to any of the other systems cited. This may be due to the rather complex nature and control of these various secretory processes. However, the effects of acid-base alterations on CSF formation seem to be roughly similar to those in the kidney, salt gland, pancreas and eye in that in all cases lowering of pCO_2 diminishes secretion.

In studying the response of the eye, pancreas and the gull salt gland to acetazolamide, it was noted that severe metabolic alkalosis "blocked" the effect of this carbonic anhydrase inhibitor(13,12,9). In our experiments acetazolamide was still active in reducing CSF formation during metabolic alkalosis, acidosis and respiratory alkalosis. Thus we were not able to observe this "blocking effect" of metabolic alkalosis. This may be due to the fact that even though the degree of alkalosis produced in our experiments was similar, the pCO_2 was considerably higher in the salt gland, pancreas and eye experiments.

Summary. Cerebrospinal (CSF) fluid formation rates were measured by ventriculocisternal perfusion with an inulin containing buffer during metabolic acidosis and alkalosis and respiratory acidosis and alkalosis in dogs. Some of the animals also received acetazolamide, 50 mg/kg, intravenously during the acid-base alteration. It was found that metabolic and respiratory acidosis had no constant effect on CSF production rates. Metabolic alkalosis reduced CSF production 23% and respiratory alkalosis reduced it 46%. The addition of acetazolamide during metabolic acidosis and alkalosis as well as during respiratory alkalosis caused a significant decrease in CSF production rates as compared to production during the periods of acid-base alterations alone.

1. Pappenheimer, J. R., Heisey, S. R., *Drugs and Membranes,* 1963, v4, 95.

2. Oppelt, W. W., Patlak, C. S., Rall, D. P., *Proc. Int. Union Physiol. Scien.,* 1962, v11, 618.

3. Vates, T. S., Jr., Bonting, S. L., *Fed. Proc.,* 1963, v22, 213.

4. Rall, D. P., Oppelt, W. W., Patlak, C. S., *Life Sciences No. 2,* 1962, 43.

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

6. Kister, S. J., *J. Pharm. Exp. Ther.,* 1956, v117, 402.

7. Tschirgi, R. H., Frost, R. W., Taylor, J. L., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 373.

8. Bonting, S. L., Simon, D. A., Hawkins, N. M., *Arch. Biochem. and Biophys.,* 1961, v95, 416.

9. Nechay, B. R., Larimer, J. L., Maren, T. H., *J. Pharm. Exp. Ther.,* 1960, v130, 401.

10. Smith, H. W., *The Kidney;* Oxford Univ. Press, New York, 1951, p633.

11. Byers, F. M., Jordan, P. H., Maren, T. H., *Am. J. Physiol.,* 1962, v202, 429.

12. Rawls, J. A., Wistrand, P., Maren, T. H., *Am. J. Physiol.,* in press.

13. Wistrand, P., Maren, T. H., *Am. J. Ophthal.,* 1960, v50, 291.

Received July 1, 1963. P.S.E.B.M., 1963, v114.