Water Distribution Abnormalities in Hypothyroid Dogs Due to Acid–Base Disturbances[1] (34641)

M. B. Wolf,[2] E. C. DeLand, and James V. Maloney, Jr.
(Introduced by F. S. Grodins)

Departments of Surgery and Physiology, UCLA School of Medicine, Los Angeles, California 90024; and the RAND Corporation, Santa Monica, California

Previous work by Dörr *et al.* (1) in this laboratory has shown that a hypertonic $NaHCO_3$ infusion increased extracellular water (ECW), measured as the rapidly equilibrating sucrose space, by 25% in hypothyroid dogs and only by 17% in euthyroid dogs. He suggested that this difference could be caused by an accumulation of mucopolysaccharides in the extracellular space of hypothyroids.

Substantially increased amounts of the mucopolysaccharide, hyaluronic acid, have been found (2) in the extracellular space of humans and animals with myxedema (the chronic form of hypothyroidism). This material has been localized in the ground substance of connective tissues. It has been demonstrated (3) that its polyelectrolyte characteristics make hyaluronic acid a very influential substance in determining water and electrolyte distribution in the body. Engel (4), Engel *et al.* (5) and Joseph *et al.* (6) studied the ion and water binding characteristics of the ground substance of connective tissues, and showed that under hormonal influences the macromolecules comprising the tissue would depolymerize. Their work demonstrated that ion and water shifts between the various body fluids occurred as a result of this change in the physicochemical state of the ground substance.

It is the hypothesis of this paper that the physicochemical state of the connective tissue ground substance is altered by changes in the hydrogen ion concentration of its environment (the interstitial fluid), and that these changes of state are particularly significant in the hypothyroid. Since a base infusion increased the extracellular and interstitial space in the hypothyroid as compared to the euthyroid, an acid infusion should yield comparable changes in the opposite direction. In the present study, a series of experiments was performed to measure the changes in water and electrolyte distribution in euthyroid and hypothyroid dogs as a result of an infusion of HCl.

Materials and Methods. Fluid and electrolyte distributional studies were performed on two groups of dogs. The first group (9 dogs) were euthyroid controls. The second group (10 dogs) had been surgically thyroidectomized 6 weeks previously and then maintained on a low iodine diet until the time of the experiment. Radioiodine uptake, PBI, and serum cholesterol were measured on the dogs of the second group before thyroidectomy and immediately before the distributional studies to insure that the animals were clinically hypothyroid.

Following a 24-hr fast, the dogs of both groups were anesthetized with sodium pentobarbitol (30 mg/kg) and splenectomy and bilateral nephrectomy were then performed. A venous blood sample was taken for measurement of Na^+, K^+, and Cl^- concentrations and water content, and an arterial sample for hematocrit, pH and P_{CO_2}. Then 30 μCi of radiosucrose-(^{14}C) was injected. Arterial samples were subsequently taken at 100, 120, 140, and 160 min following injection time for use in radioactivity measurements. Isologous blood, tagged with 15 μCi of radiochro-

[1] This work was supported by grants-in-aid from the U.S. Public Health Service and from Frank Clark, Jr., Charities Inc. Dr. DeLands' work is supported by Air Force Project RAND.

[2] Present Address: Department of Electrical Engineering, University of Southern California, Los Angeles, California 90007.

mate-($Na_2{}^{51}CrO_4$), was injected at the 120-min point and an additional arterial sample was taken 40 min later for use in measurement of pH P_{CO_2} and red cell volume (RCV). 25 mg/kg of 0.2 N HCl were then infused over a 90-min period by a constant speed infusion pump. 10 min after the infusion, an arterial blood sample was withdrawn and then radiosucrose was injected again. Subsequent blood samples were taken at the same time intervals as in the preinfusion period to be used for the same measurements as previously.

Red cell volume (RCV) was measured as previously described (1), with red cell water (RCW) computed as the product of RCV and the water content per milliliter of erythrocytes. Plasma water (PW) was computed from RCV, plasma water content and a corrected large vessel hematocrit (1). The volume of water in the interstitial space (ISW) was taken to be the rapidly equilibrating sucrose space minus the PW volume. The β emitting isotope was counted in a liquid scintilation system at 4° in total darkness using an external standard quench correction. The γ emitting isotope was counted in a deep-well automatic gamma counting system. The water content in plasma and erythrocytes was determined by weighing 0.5 ml amounts, drying for 24 hr at 110°, and then reweighing. Na^+ and K^+ concentrations were determined by flame photometry and Cl^- by the gravimetric method of Van Slyke (7). P_{CO_2} and pH were measured using the Astrup method. All analyses were performed in duplicate.

Results. The significant quantities measured before and after acid infusion and the percentage change from the preinfusion measurements are shown for both groups of animals in Table I. Of all the data taken, only the PW and ISW volumes showed a significantly different change between the two groups of animals. The PW space decreased significantly more in the euthyroid groups than in the hypothyroid group (—6.08% vs. —0.51%; $p < .02$) while the ISW space increased significantly more in the euthyroid animals than in the hypothyroid animals (+5.91% vs. —4.81% ; $p < .05$).

We may contrast these results to those presented by Dörr (1) for $NaHCO_3$ infusion. Table II shows the comparison. The ISW space was computed from Dörr's experiments as the difference between his measured rapidly equilibrating sucrose space (ECW) and the PW space. Thus, there is an increase in the volume of ISW in the hypothyroids relative to the euthyroids after $NaHCO_3$ is administered and a decrease after the infusion of HCl.

Discussion. The acid infusion experiments were performed under the assumption that following the infusion, the interstitial water volume (ISW) would, in the hypothyroid,

TABLE I. Volume of Body Spaces Before and After Infusion of HCl in Euthyroid and Hypothyroid Dogs.[a]

	Before infusion			Infusion of 25 ml/kg of 2 N HCl	
Exp.	Wt	PW	ISW	PW	ISW
Euthyroid					
1	24.0	0.763	1.93	0.709	2.14
2	24.5	0.692	3.15	0 688	3.20
3	19.9	0.645	2.71	0.576	2.61
4	25.4	1.02	3.00	0 929	3.16
5	22.7	0.861	2.54	0.781	3.03
6	19.0	0.625	2.30	0 661	2.35
7	14.5	0.524	2.23	0.465	2.06
8	20.6	0.994	2.76	0 941	3.23
9	16.2	0.529	1.84	0.506	2.00
	Change (%) from pre-infused period			—6.08	+5.91
	± standard deviation			±5.3	±9.0
Hypothyroid					
10	21.3	0.855	2.38	0.871	2.43
11	19.5	0.751	2.57	0.737	2.20
12	21 1	0.686	2.22	0.647	2.40
13	19.5	0.716	2.01	0.717	1.81
14	23.0	0.788	1.84	0.792	2.33
15	23.9	0 763	3.04	0.746	2.83
16	20.8	0.779	2.69	0.768	2.12
17	18.3	0.604	2.74	0.624	3.12
18	21 8	1.40	3.18	1.32	2.84
19	22.2	0.887	3.03	0.837	3.12
	Change (%) from pre-infusion period			—0.51[c]	—4.81[b]
	± standard deviation			±2.9	±12.

[a] Body fluid spaces are in liters; Wt: weight; PW: plasma water volume; ISW: interstitial water volume.

[b] $p < .02$; [c] $< .05$ vs. euthyroids.

TABLE II. Percentage change ± SD of Interstitial Fluid Volume After Infusion of Basic and Acidic Solutions.

	Base ($NaHCO_3$)	Acid (HCl)
Euthyroid	+15 ± 5	+5.9 ± 9
Hypothyroid	+25[a] ± 4	−4.8[b] ± 12

[a] $p < .01$; [b] $p < .05$.

show a lesser increase or an actual decrease when compared to the same results in euthyroid animals. The data shown in Table II confirm this assumption. This belief was based on evidence that water shifts occur between the connective tissue and other fluid compartments due to changes in the physicochemical state of the ground substance (4–6), coupled with the obervation that excessive amounts of this material exists in the hypothyroid (2).

There are a number of possible ways in which the hydrogen ion concentration [H+] of the body fluids can affect the physical state of the macromolecules comprising the connective tissue (CT). It is well known that the physical state of high molecular weight organic gels is a function of the [H+] of their environment (8). There could be an equilibrium state in the ground substance between a high molecular weight polymer chain formed from hyaluronic acid (HA) molecules and the depolymerized molecules themselves. The individual HA molecules would then also exist in solution in the IS fluid environment. The results of Table II can then be explained by a polymerization of the HA molecules as [H+] increases with water leaving the IS space and entering the CT space due to osmotic forces. Then a decrease in [H+] (basic infusion) causes the reverse set of circumstances to occur. Another explanation involves the water binding properties of high molecular weight, electrically charged colloids. Joseph (6) has shown that a relaxation of the physical state of the pubic symphysis of the guinea pig led to a decrease in the charge density of the CT and an uptake of water. If an increase in [H+] decrease the charge density of the colloid and decreases in [H+] caused an increase in charge density, then the subsequent water shifts would be in the relative directions shown in Table II. A combination of both of these hypotheses is most likely the real explanation.

There is also a possibility that the data reported in this paper can be explained by an altered distribution of solute and water in the intracellular space in hypothyroids. The volume of this space was measured in these experiments but was not included here since the changes were not significant. Even if they were significant, the controversies concerning the measurement of this volume would cast doubt upon the validity of our conclusions.

In the euthyroid animals the increase in the ISW was partly at the expense of PW with the result that the total ECW remained relatively constant after the infusion as compared to the preinfusion values. In contrast the ECW decreased in the hypothyroids after the infusion which indicates that the change of colloid charge density, or decrease in the number of HA molecules in the interstitial space affects the Donnan distribution and thus water distribution between the ISW and PW compartments in hypothyroidism. This would explain the significant difference in the PW changes between the two groups.

A mathematical approach (9) has been used to test these hypotheses. Qualitative agreement with our experimental results was obtained; however, the predicted quantitative changes due to acid infusion were somewhat different from our measured data.

1. Dörr, D., Wolf, M. B., Maloney, J. V., and DeLand, E. C., J. Surg. Res. 9, 8, 471–475 (1969).

2. Schiller, S., J. Chron. Dis. 16, 291–304 (1963).

3. Bland, J. H., "Clinical Metabolism of Body Water and Electrolytes," Chap. 16. Saunders, Philadelphia, (1963).

4. Engel, M. B., Oral Surg., Oral Med., Oral Pathol. 11, 724-735 (1958).

5. Engel, M. B., Joseph, N. R., and Catchpole, H. R., AMA Arch. Pathol. 58, 26-39 (1954).

6. Joseph, N. R., Engel, M. B., and Catchpole, H. R., Biochim. Biophys. Acta 8, 575-587 (1952).

7. Van Slyke, D. D., and Sendroy, J., J. Biol. Chem. 58, 223 (1923).

8. Glasstone, S., "Textbook of Physical Chemistry," 2nd ed., Van Nostrand, Princeton, N. J. (1946).

9. Wolf, M. B., DeLand, E. C., and Maloney, J. V., "A Mathematical Model of the Chemical Distribution in a Disease State: Hypothyroidism," The RAND Corporation, RM-5376-NIH, June 1969.

Received Oct. 17, 1969. P.S.E.B.M., 1970, Vol. 133.