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Influence of Induced Changes in Blood Plasma Osmotic Activity on Intestinal Absorption.

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Since isotonic salt solutions become hypotonic within a short time after introduction into ileal segments in dogs,[1] it appeared to be of interest to ascertain what might be the effect of altering the osmotic activity of the blood plasma upon the degree of hypotonicity so developed. It is not possible to predict *a priori* whether the gut epithelium will tend to maintain a certain absolute osmotic activity or a certain difference in osmotic activity between blood plasma and intestinal fluid. Experiments designed to answer this question also throw light on the mechanism of absorption of salt and water. Only two experiments of this type have been performed, but they have statistical validity because of the large number of control observations which have already been reported.

Methods. Adjacent ileal segments were prepared in nembutalized dogs, as previously described,[2] and measured volumes of isotonic solutions of equiosmotic proportions of sodium chloride and sodium sulfate were introduced at the times indicated. Immediately and at intervals thereafter, small measured samples were removed for analysis, and the residues removed and their volume and composition determined 50 min after insertion. Osmotic activity,[3] chloride[4] and sulfate[5] concentrations were determined by the methods indicated. In each experiment one ileal segment was studied without altering the animal osmotically, to serve as a control. Immediately thereafter 100 cc of 5% sodium chloride solution was injected intravenously and within a few minutes the

1 Roepke, R. R., and Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 500.

2 Ingraham, R. C., and Visscher, M. B., *Am. J. Physiol.*, 1936, **114**, 676.

3 Baldes, E. J., *J. Sc. Instruments*, 1934, **11**, 223.

4 Van Slyke, D. D., *J. Biol. Chem.*, 1923, **58**, 523.

5 Visscher and Smith, *Experimental Physiology*, Philadelphia, 1934.

second ileal segment was filled with fluid and studied. Samples of femoral arterial blood were drawn at appropriate intervals for osmotic activity and chloride concentration determinations.

Results and Discussion. It may be seen in Table I that when the osmotic activity of the blood plasma is increased by intravenous injection of hypertonic salt solution the osmotic activity of salt solutions placed in ileal segments rises to a level greatly above that found before such a change. In these experiments the greatest lowering of osmotic activity in ileal solutions below plasma levels was at about 20 min after introduction. Comparisons will therefore be made at such times. The figures to be given are interpolations obtained from graphs constructed from the data of Table I.

In the experiments 1a and 2a, while plasma

TABLE I.
Osmotic Activity. Composition and Volume Data from Experiments in Which Plasma Osmotic Activity Was Increased by Hypertonic Salt Solution Injection.

			Plasma		Ileal Fluid			
Experiment No.	Time, min.	Volume, cc	O.A.‡ mM/kg H_2O	$[\overset{-}{Cl}]$ mE/L	O.A.‡ mM/kg H_2O	$[\overset{-}{Cl}]$ mE/L	$[\overset{=}{SO_4}]$ mM/L	Remarks
1a	0	42.1*			159.5	78.6	66.0	Upper segment
	10	2.2†			158.8	61.6	75.0	
	21	2.0†			157.1	38.2	86.4	
	29		161.6	99.6				
	36	2.0†			158.7	16.4	102.6	
	51	9.6†			161.2	4.8	120.6	
	56							Inj. 100 cc 5% NaCl
1b	60	48.0*			161.1	80.4	63.6	Lower segment
	68		173.0	126.8				
	71	2.0†			163.5	66.2	67.0	
	84	2.0†			162.7	41.2	83.4	
	98	2.0†			164.5	22.8	99.0	
	105		170.3	120.7				
	110	13.6*			165.0	12.4	108.6	
2a	0	48.0*			159.5	77.8		Upper segment
	10	2.0†			159.5	67.8		
	25	2.0†			156.3	49.4		
	30		158.8	102.0				
	36	2.0†			156.3	32.8		
	50	32.0†			157.4	24.8		
	62							Inj. 100 cc 5% NaCl
2b	66	48.0*			161.7	78.4	60.0	Lower segment
	72		172.6	108.6				
	79	2.0†			168.0	72.6	41.0	
	92	2.0†			167.0	65.6	37.6	
	104	2.0†			165.5	59.6	38.4	
	109		168.4	112.3				
	116	68.0†			164.5	53.8	36.4	

* Inserted.
† Removed.
‡ Osmotic activity expressed in equivalent concentrations of NaCl.

osmotic activities were normal, the differences between plasma and gut osmotic activities at 20 min were —4.4 and —1.6 mM/kg H_2O. In experiments 1b and 2b, after the plasma osmotic activity had been increased, the corresponding differences 20 min after introduction of fluid were —9.0 and —3.2 mM/kg H_2O. Thus in both instances the osmotic pressure difference maintained across the intestinal epithelium was about twice as great after the hypertonic salt injection as before.

Nevertheless, as will be seen from Table I, the absolute osmotic activity in the gut solution is considerably greater after the hypertonic salt solution injection than before. At 20 min in experiment 1a the value 157.2 is to be compared with 162.8 in experiment 1b. Thus with an increase of about 10 mM/kg H_2O in plasma osmotic activity, the ileal fluid value rose 5.6 mM/kg H_2O. In experiments 2a and 2b the corresponding values are 157.6 and 167.2 mM/kg H_2O at 20 min, the difference being 9.6 mM/kg H_2O. The increase in plasma osmotic activity was 11.2 mM/kg H_2O for the same times. In both instances the increase in plasma osmotic activity was greater than the change in the gut fluid values.

Thus it is evident that the intestinal epithelial mechanism controlling the osmotic activity of the gut fluid is certainly sensitive to changes in plasma osmotic activity. But it seems unlikely that it is absolutely dependent upon it because if it were one would not expect greater differences in osmotic activity between plasma and gut fluid to occur when the former is raised.

The rates of water absorption in relation to osmotic activity differences are of interest. In experiment 1a a slightly larger fraction of the water was absorbed in 50 min than in experiment 1b. This is in spite of the fact that in the latter case the osmotic gradient between gut and blood over the whole period was 2 or more times as great as in the former. In experiment 2a about 10 cc of water were absorbed while in 2b, after hypertonic salt injection the intestinal fluid increased in volume by about 20 cc. The latter occurred in spite of the osmotic gradient of 3.2 mM/kg H_2O which should have moved water in the opposite direction if normal osmosis were the determining process.

In both experiments 1 and 2 the rate of decline in chloride concentration in the intestinal fluid was less after hypertonic salt solution injection than before. The difference in experiment 1 was not great indicating that an increase of 20+ mE/L in plasma chloride does not have a large effect upon the chloride impoverishment mechanism. The plasma chloride increase in experiment 2 was smaller but the effect on chloride impoverishment was greater.

The sulfate concentration figures in experiments 1a and 1b show the usual increase that occurs when there is a net volume decrease in sulfate-chloride solutions[6] resulting from the fact that the absorption of the sulfate ion is small. No analyses for sulfate were made in experiment 2a, but in 2b the decline in sulfate concentration is a result of the volume increase. It may be noted that the measured volume increase accounts for nearly the entire observed decline in sulfate concentration. There is no obvious reason to account for the fact that in one instance hypertonic salt injection by vein was associated with an intestinal volume increase, and in the other was not. It is possible that there was no causal connection whatever because we[7] have observed such volume increases in isotonic sodium sulfate-chloride solutions without hypertonic salt injection or other alteration. It may be noted that in experiment 2b the osmotic activity of the gut solution at the end is not accounted for by the measured electrolyte. Assuming the presence of sodium chloride and sodium sulfate an osmotic activity of only about 100 mM NaCl/kg H_2O are accounted for. Unfortunately other components were not analyzed. It is possible that bicarbonate might make up the difference because under certain circumstances[7,8] there is a large increase in acid-labile carbon dioxide in gut fluids.

These two experiments are reported primarily because they show something which we have never seen in the absence of poisons in over a hundred control studies,[7,8] namely that the osmotic activity in ileal segment fluids, originally isosmotic with the plasma, rises to values unmistakably higher than the original plasma values. The fact that this occurs when the plasma osmotic activity is raised by hypertonic salt solution injections

[6] Ingraham, R. C., and Visscher, M. B., *Am. J. Physiol.*, 1938, **121**, 771.

[7] Visscher, M. B., and Roepke, R. R., *Am. J. Physiol.*, 1945, **144**, 468.

[8] Visscher, M. B., Roepke, R. R., and Lifson, N., *Am. J. Physiol.*, 1945, **144**, 457.

is highly significant. The results of the two experiments, by themselves, might not have statistical significance, but in the light of the large number of other experiments which can serve as legitimate controls we are convinced of the causal connection between the increase in plasma osmotic activity and the increase in gut fluid osmotic activity. The likelihood of the results occurring fortuitously is less than one chance in ten thousand, in other words, practically negligible.

It seems likely that the activity of the intestinal epithelium in the movement of water across it is influenced greatly by the osmotic activity on both the blood and lumen sides. In neither experiment reported did the increased osmotic gradient across the intestinal epithelium increase the net rate of water absorption. In one case, as noted, the hypertonic salt injection was associated with a negative net water absorption. Nevertheless the fact that gut fluid osmotic activities were changed by the procedure proves that the intestinal epithelium does not act independently of blood osmotic activity, unless the salt concentration itself has a specific effect, which seems to us to be unlikely, though possible. Comparable experiments with non-electrolytes are essential to a settlement of this question.

Summary and Conclusions. Experiments are reported in which the volume, osmotic activity, chloride and sulfate contents of plasma and ileal loop segments are measured, before and after raising the plasma osmotic activity by hypertonic sodium chloride solution injection by vein. The osmotic activity of originally isotonic sodium chloride-sodium sulfate solutions placed in the ileum rises above the pre-injection plasma osmotic activity after 100 cc of 5% sodium chloride solution was injected intravenously. In view of the fact that in more than a hundred control experiments with equiosmotic mixtures of sodium chloride and sodium sulfate the gut fluid osmotic activity has never exceeded the plasma osmotic activity except when poisons were present, the results of these experiments are believed to constitute satisfactory proof that the effect observed was due to the hypertonic salt solution injection.

After the elevation of the plasma osmotic activity the gut fluid values did not approach the former as closely as occurred before the elevation was produced. The net water absorption was not increased in proportion to the increase in osmotic gradient. In fact in one case the direction of net movement was reversed in spite of the larger osmotic gradient. These data add to previous evidence that although normal osmotic forces are probably contributing factors in the transport of water across the intestinal epithelium they are not the primary driving forces determining the direction and magnitude of net water transfer.

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Effect of Feeding Glucoascorbic Acid to White Rats, Chicks and Guinea Pigs.*

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Woolley and Krampitz[1] showed that cotton rats and mice failed to grow, developed severe diarrhea and showed hemorrhages in various parts of the body when fed a synthetic diet containing 10% glucoascorbic acid. The condition was prevented when plant ma-

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1 Woolley, D. W., and Krampitz, L. O., *J. Exp. Med.*, 1943, **78**, 333.