8. Udenfriend, S., Bogdanski, D. F., Weissbach, H., *Science*, 1955, v122, 972.

9. Sjoerdsma, A., Smith, T. E., Stevenson, T. D., Udenfriend, S., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 36.

10. Udenfriend, S., Weissbach, H., Bogdanski, D. F., *J Pharmacol. Exp. Therap.*, 1957, v120, 255.

11. Tabor, H., *Pharmacol. Rev.*, 1954, v6, 299.

12. Fouts, J. R., Blanksma, L. A., Carbon, J. A., Zeller, E. A., *J. Biol. Chem.*, 1943, v147, 415.

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Fluid and Electrolyte Movement Across Intestinal Wall of Bullfrog. (24487)

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While normal movement of fluid and electrolyte across intestinal wall is one of net absorption, experimental circumstances, both *in vitro* as well as *in vivo*, frequently do not result in absorption. The following results indicate that sacs of bullfrog intestine do absorb saline and establish concomitant gradients of chloride and bicarbonate ion.

Methods. Large bullfrogs (*Rana catesbiana*) were maintained in a state of pseudahibernation at 12°C. Sacs of mid-small intestine (jejunum) were formed by taking segments 2-3 cm in length from the pithed frog and tying each end. The large intestine was tied at the ends to form a sac. In half of the experiments, normal orientation of the sac wall was retained with mucosal surface bounding the lumen. The remainder of the sacs were formed after first everting the intestinal tube. The lumen of the everted sacs was thus bounded by the serosal surface. The sacs were incubated at room temperature (24°C) in 5 ml of a salt solution, pH 7.4, containing 87 mM NaCl, 20 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM $CaCl_2$, 0.5 mM $MgSO_4$, 1.33 mM Na_2HPO_4, 0.33 mM NaH_2PO_4 and 5 mM glucose/liter. About 0.3-1.3 ml of the same solution was placed inside each sac. 95% O_2 and 5% CO_2 was bubbled through Dubnoff shaker during experimental period of 4-6 hours. Water movement was measured by weighing the sacs at time "zero" (after 10-15 minute equilibration period) and at end of experiment, 4-6 hours later. Adherent water was removed from the sacs prior to weighing by rolling them on a dry glass surface. This resulted in a uniform stable weight. "Drying" the intestinal sacs by blotting them on a variety of papers did not result in a reproducible weight. Successive drying on filter paper was accompanied by small progressive decrease in weight. In early experiments, radio-inulin (C^{14}) was incorporated in the solution placed inside the sacs. Radio-inulin concentration within the sacs as well as in the bathing solution was determined(1) at end of experiment. The change of concentration of radio-inulin in the solution within the lumen of the normally oriented sacs indicated that weight change did measure net movement of water across intestinal wall. The bicarbonate concentration of inner contents of sacs was measured by back-titration, with NaOH, of an added volume of a standard HCl solution. Chloride concentrations of inner contents were determined by coulometric - amperometric method of Cotlove, *et al.*(2).

Results. The tabulated results indicate that there is a net movement, corresponding to absorption, of both water and electrolyte across wall of the normally oriented and everted sacs of small and large intestine.

Use of the everted sac has been favored in the study of isolated mammalian intestine because of the presumed better oxygenation of the epithelial surface(3). In our experiments, there was a significantly greater movement of water across the wall of the everted sac than across that of normally oriented sacs. However, while there was no movement of radio-

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TABLE 1.

	Net movement*	Conc. change, meq/l	Net movement*	Conc. change, meq/l
	Small intestine			
	Normal sacs (12)		Everted sacs (9)	
H_2O	−8.0 ±2.0		+32.0 ±5.0	
Cl^-	−1.9 ± .4	−20.1	+ 1.4 ± .5	+6.5
HCO_3^-	+1.3 ±1.8	+ 4.8		
	Large intestine			
	Normal sacs (10)		Everted sacs (10)	
H_2O	− .4 ± .1		+ 5.0 ±1.0	
Cl^-	−1.1 ± .2	−22.0	+ .5 ± .06	+6.6
HCO_3^-	− .5 ± .04	− 5.0	+ .9 ± .2	+4.2

* Net movement into or out of the sac for H_2O in μl and for ions in μeq/hr/100 mg wet wt of tissue.

± signifies stand. error, and figure in parenthesis No. of experiments.

inulin out of the normal sacs, there was a decided loss of radio-inulin from the everted sacs of small intestine. In 8 experiments, mean internal radio-inulin content decreased due to diffusion by 15.6% and 1.1% in the everted sacs of small and large intestine, respectively. Thus, a rapid transfer of water occurred in spite of an unusual permeability to a relatively large molecule, radio-inulin. It is not known whether the greater transfer of water resulted from the more efficient oxygenation of epithelial cells or from a more rapid hydraulic flow through larger pores.

Presumably the escape of inulin out of the everted sacs is an experimental artifact which could have resulted from either excessive stretching during eversion or from the physical trauma associated with rocking the small sacs within 10 ml breakers. In the mammalian small intestine impermeability to radio-inulin has been clearly demonstrated.

The difference between everted and normal sacs is also evident in concentration changes developed. These were less in the everted sacs.

It is noteworthy that in 6 experiments the transfer of water was not affected by absence of glucose from the solution within and without the sacs. This is in agreement with the finding of Lifson and Parsons(5) that glucose transport was not a necessary prerequisite for water transport by the isolated rat intestine, even though exogenous glucose utilization was necessary.

While ionic absorption by the canine jejunum has been considered to be a relatively inefficient process(6), absorption from the normally oriented bullfrog jejunal sacs resulted in an appreciable concentration gradient of chloride. Under comparable conditions the electrical potential across the small intestine is close to zero(unpublished). The accumulation of bicarbonate within the lumen of normally oriented small intestine sacs of the frog is similar to the transfer occurring in the hamster(7). In contrast, bicarbonate is depleted at the mucosal surface in normal man, intact dog(8) and the isolated rat small intestine(7).

The transfer of chloride by the normally oriented large intestine sacs of the frog created a striking concentration gradient for chloride. In the case of bullfrog large intestine there was also a depletion of bicarbonate at the mucosal surface. While this would be expected in view of the electrical potential difference developed by active sodium transport across the isolated bullfrog large intestine mucosa(9), it contrasts sharply with bicarbonate accumulation that occurs in large volumes of saline placed in the colon of man and dog(10). In the one instance where sufficiently fluid contents could be aspirated from the colon of a freshly killed bullfrog, the pH was 7.41.

Summary. 1) Isolated sacs of small and large intestine of the bullfrog incubated at room temperature, transfer water and electrolyte from mucosa to serosa. The transfer of chloride results not only in a net movement but also in creation of concentration gradients which are pronounced in the case of both small and large intestine. There is an increase in bicarbonate concentratiion at the mucosal surface of the small intestine and a decrease in bicarbonate concentration at the mucosal surface of the large intestine. 2) Eversion of the intestines resulted in sacs that were significantly permeable to radio-inulin. Associated with this abnormal permeability, there is a greater transfer of water and smaller ionic concentration gradients develop.

1. Cotlove, E., *Fed. Proc.*, 1955, v14, 32.

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

3. Wilson, T. H., Wiseman, G., *J. Physiol.*, 1954, v123, 116.

4. Schanker, L. S., Tocco, D. J., Brodie, B. B., Hegben, C. A. M., *J. Pharm. Exp. Therap.*, 1958, v123, 81.

5. Lifson, N., Parsons, D. S., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 532.

6. Visscher, M. B., *et al.*, *Am. J. Physiol.*, 1944, v141, 488.

7. Wilson, T. H., Kazyak, L., *Biochim. Biophys. Acta*, 1957, v24, 124.

8. Bucher, C. R., Anderson, C. E., Robinson, C. S., *Am. J. Physiol.*, 1950, v163, 1.

9. Cooperstein, I. L., Hogben, C. A. M., *J. Gen. Physiol.*

10. D'Agostino, A., Leadbetter, W. F., Schwartz, W. B., *J. Clin. Invest.*, 1953, v32, 444.

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"Dopa Oxidase" in Melanocytes of X-Irradiated Quiescent (*Telogen*) Hair Follicles. (24488)

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Melanocytes within growing hair follicles of pigmented mice contain melanin granules and active "dopa oxidase." In the presence of dihydroxyphenylalanine (dopa) *in vitro*, they form abundant dopa-melanin(1). On the other hand, melanocytes of quiescent (telogen) hair follicles in pigmented mice generally are unpigmented and are dopa negative (1). Thus, it has not been possible to identify melanocytes with certainty at resting stage of hair growth cycle. Although X-radiation has been shown, under certain conditions, to stimulate melanogenic activity within latent melanocytes of the epidermis, there is no report of a similar effect on latent melanocytes within quiescent hair follicles. Previous studies have shown that repeated partial-body exposure of mice to 200 r week of X-radiation is particularly effective in eliciting epidermal hyperpigmentation(2). Utilizing similar technics in the present study, mice with quiescent hair follicles were exposed weekly to X-radiation. The purpose was 2-fold; first, to determine whether "dopa oxidase" activity could be demonstrated in latent follicular melanocytes following X-irradiation; second, to characterize more fully the distribution of melanocytes within telogen hair follicles.

Materials and methods. Eight C3H mice (4 males, 4 females), 4 to 8 months of age, were shielded with lead, except for small rectangular area (1.5 x 2.0 cm) on the dorsum, and exposed to 200 r of X-rays once weekly for 3 or 4 weeks. The radiation factors were: 100 kv, 5 ma, 0.25-mm Al, HVL-0.55 mm Al, target distance, 15 cm, and dose rate, 580 r minute. The design of the lead shield ensured irradiation of essentially the same area of skin each week. At start of irradiation, hair follicles of all mice were quiescent (telogen stage) as indicated by light pink color and thinness of skin(3,4). Subsequently, gross examinations of skin of each mouse were made at frequent intervals to detect alterations in hair growth status. The mice were killed by cervical dislocation 24 hours after last exposure to radiation. Specimens of skin were removed from both irradiated and non-irradiated regions of the dorsum and tested for "dopa oxidase" activity according to the method of Becker(5). The specimens were then dehydrated in dioxane and imbedded in paraffin; sections were cut at 8 μ and stained with Mayer's alcoholic carmine. Skin samples from 4 non-irradiated C3H mice with quiescent hair were tested for "dopa oxidase" activity in a similar manner.

Results. Due to lack of pigmentation, melanocytes could not be identified in quiescent hair follicles of non-irradiated mice, although pigmentary debris was frequently observed. "Dopa oxidase" activity was also absent in melanocytes of non-irradiated telogen