Iron Content of Intestinal Lymph of Rats. (18441)

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Studies on the iron content of thoracic duct lymph, Moore *et al.*(1) and Endicott *et al.* (2), were acute experiments, lasting less than 24 hours. This work has been repeated using the technic of Bollman *et al.*(3), which allowed drainage of lymph exclusively from the intestinal bed and which permitted continued observation over a period of days.

Methods. Lymph was obtained by continuous drainage through cannulae of polyethylene tubing secured in the lacteal draining the intestine of Sprague-Dawley strain rats weighing about 200 g. Following intubation of the lymphatic under ether anesthesia, the animals were maintained in plastic cages(4) and allowed free access to either 0.4 or 0.9% saline as drinking water and commercial rations. No attempt was made to regulate the iron content of the diet in this group of animals. Specimens, collected over 12-hour intervals in iron-free tubes containing 1.5 ml of 2% potassium oxalate solution were measured and analyzed within 24 hours, by the method of Kitzes *et al.*(5). Strict precautions were taken to avoid contamination of reagents or glassware by iron. Dialyses were carried out in cellophane bags rendered iron-free by soaking in 0.1 normal hydrochloric acid for 24 hours followed by thorough washing with iron-free water. Specimens were dialyzed against 8-10 volumes of iron-free 0.9% saline containing acetate buffer at pH 7 and 4.5 or hydrochloric acid at pH 1.5 for 24 hours.

Results. 1. Lymph volume was found to be about one ml per hour in animals receiving 0.4% saline. (Table I.) When 0.9% saline was given, however, lymph volume increased 3- to 4-fold. Iron content of 12-hour specimens of intestinal lymph from 7 rats varied slightly from animal to animal, but was reasonably constant in the same animal for 7 days. When lymph volume was increased by giving 0.9% saline, the concentration of iron varied inversely to the volume. The corresponding output of iron in gammas per hour was slightly, but significantly, increased.

TABLE I. Iron Output from Intestinal Lymph Fistulae of 7 Rats for Periods Up to 7 Days.

Drinking water, % saline	Lymph vol. (ml/hr)	Iron output (γ/hr)	Conc. (γ/100 ml)
.4%	.94 ± 0.48*	.47 ± .28	53.74 ± 18.62
.9	2.84 ± 1.74	.88 ± .52	35.43 ± 15.68

* —Mean ± stand. dev.

TABLE II. Dialysis of Lymph.

Aliquot	pH	Lymph iron content	
		Control	After dialysis
1	7.0	35.8	34.8
2	4.7	32.6	32.6
3	1.5	28.2	9.6

TABLE III. Dialysis of Lymph Plus Added Iron.

Aliquot	Added iron γ/100 ml	Lymph iron content	
		Control	After dialysis
1	150	35.8	48.6
2	150	38.3	47.2
3	200	38.3	47.2
4	250	38.3	47.2

2. Aliquots of pooled lymph were dialyzed against buffered saline at pH 7.0, 4.7, and 1.5. As shown in Table II, the iron was not removed from the lymph samples at pH 7.0 and 4.7 but freely dialyzed at pH 1.5. From Table III it is apparent that added iron, up to 48 γ per hundred ml of lymph, is not dialyzed under these conditions at pH 7.0.

Discussion. There are 3 possible sources of

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the iron in intestinal lymph: (a) absorbed iron, (b) iron stores in intestine including lymph nodes and intestinal mucosa, (c) plasma iron. Following the administration of iron orally to dogs, Moore(1) found a greater increase of iron in the blood than in the lymph. Similarly, Endicott(2) recovered negligible amounts of orally administered radioactive iron in the lymph. These studies certainly seem to relegate the lymphatics to a minor role in iron absorption. It is conceivable that the iron stores of the reticuloendothelial system and the intestine(6) are in equilibrium with both the blood and interstitial fluid, in this case, lymph. The relative constancy of iron concentration in the lymph studied in these experiments is compatible with such an equilibrium as is the failure of iron concentration to keep pace with the rise in lymph volume produced by administration of 0.9% saline.

Blood plasma as the third possible source of lymph iron seems the most likely(1). The dialysis experiments point out a striking similarity between the adsorbed iron of lymph and that of plasma. From the work of Barkan(7), Laurell(8), Schade(9) and many others has evolved the concept that iron is present in plasma in combination with a specific globulin fraction, recently isolated and identified by Cohn(10). Because of this combination, serum iron is non-dialyzable at the pH of blood, but becomes dialyzable in more acid solutions(11).

Apparently a similar iron-protein linkage is present in lymph; and the protein resembles that of plasma in the range of pH over which it binds the iron firmly enough to prevent dialysis. Under the conditions of the experiments the protein is only about 60% saturated with iron.

Summary. The iron content of intestinal lymph of rats maintained on a normal diet is relatively constant over periods as long as seven days. At lymph outputs of one ml per hour, there is an hourly output of 0.5 γ of iron. With a 3- to 4-fold increase of lymph volume, iron concentration falls off and total output remains less than 1.0 γ per hour. The iron present in lymph is non-dialyzable at pH 7.0 to 4.7, but is dialyzable at pH 1.5. Added iron, up to concentrations of 48 γ per 100 ml in 2 specimens of pooled lymph, was non-dialyzable at pH 7, but all added iron in excess of this concentration was dialyzable.

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'Benemid' p-(Di-n-Propylsulfamyl)-Benzoic Acid: Lack of Effect of Aureomycin, Chloromycetin, Streptomycin and Terramycin.* (18442)

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Para-(Di-n-propylsulfamyl)-benzoic acid, 'Benemid',† increases by two to four times the penicillemia resulting from the administration of penicillin(1,2) and enhances the

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† Sharp & Dohme's trade-mark for p-(di-n-propylsulfamyl)-benzoic acid. The drug has been tentatively given the generic designation "probenecid".

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