Lymphatics in Intestinal Absorption of Vitamin B_{12} and Iron.* (26064)

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Intestinal malabsorption may be due to obstructed mesenteric lymphatics(1), and is often accompanied by disturbed B_{12} and iron absorption(2,3). For this reason, the role of the mesenteric lymphatics in transport of iron and Vit. B_{12} from the intestinal wall to the blood was studied in 2 dogs with cannulated thoracic ducts.

Materials and methods. Two beagles (weight 35 and 22.5 lb) were fasted for 12 hours. The left thoracic duct was isolated and cannulated at its entrance into the left jugular vein under ether anesthesia. The external jugular vein on the opposite side was identified, cannulated, and an external shunt established between the thoracic duct and this jugular vein. The shunt was in operation whenever lymph or blood samples were not drawn.

Both dogs were given 3.9 μc/4 μg of Co^{58}-B_{12} and 4 μc/1 μg of Fe^{59}-citrate by stomach tube. Blood and lymph samples were taken, and lymph flow rates measured at half hourly or hourly intervals during 8 hours. Radioactivity was measured in a Penco 100-channel pulse-height analyzer equipped with a 3 inch well-type scintillation detector. The 0.81 Mev Co^{58} and 1.10 Mev Fe^{59} peaks were employed. Counts were corrected for the contribution of Co^{58} summation radiation in the Fe^{59} peak and the Fe^{59} Compton radiation in the Co^{58} peak.

Total hourly radioactivities reaching the blood stream *via* lymph were approximated by assuming that the mean of 2 consecutive flow rates represented the true mean flow rate during that time. Total plasma volume was assumed to be 4.2% of total body weight (4).

The state (bound or free) of the radioactivity in the lymph samples was assayed by concentration dialysis against a 25% (w/v) solution of polyvinyl pyrrolidone. The dialysis method for determining free B_{12} binding capacity has been described(5).

Animals were sacrificed 10 hours and 7 days, respectively, after ingestion of isotopes. Excreta, intestinal contents, and intestines were collected, liquefied with HNO_3, concentrated by evaporation, and radioactivity was measured.

Results. At both times of sacrifice, most of the B_{12} was recovered in the intestine and excreta, whereas most of the iron had been absorbed (Table I).

In plasma, the time pattern was practically identical for both tracers, and there was little variation between the 2 animals. Serum peaks occurred 4-5 hours after ingestion for both B_{12} and iron (Fig. 1-2, Table II).

In lymph, both radio-iron and radio-B_{12} concentration-peaks came 2 to 3 hours after ingestion, *i.e.*, before the corresponding serum peaks. This was true for both animals (Table II, Fig. 3, 4).

Since more of the radio-iron was absorbed, Fe^{59} concentrations were higher in lymph and plasma than radio-B_{12} concentrations (Table II). However, while radio-iron concentration in lymph did not differ significantly from that in serum, radio-B_{12} concentration was 10 to 20 times higher in serum than in the lymph for both dogs. The total cumulative fraction of iron and B_{12} reaching the blood *via* the lymph was only about 1.3% of total iron dose given and 0.05% of total B_{12} dose given (Fig. 2, 4). Thus, most of the iron and B_{12} absorbed does not go *via* the lymph.

In those lymph samples where radio-iron concentration was low (0.06 mμg Fe^{59}/ml), no iron was dialyzable, but where it was high (0.2-0.3 mμg Fe^{59}/ml), 70% of the iron was dialyzable. Since the average unsaturated iron binding capacity in normal human serum

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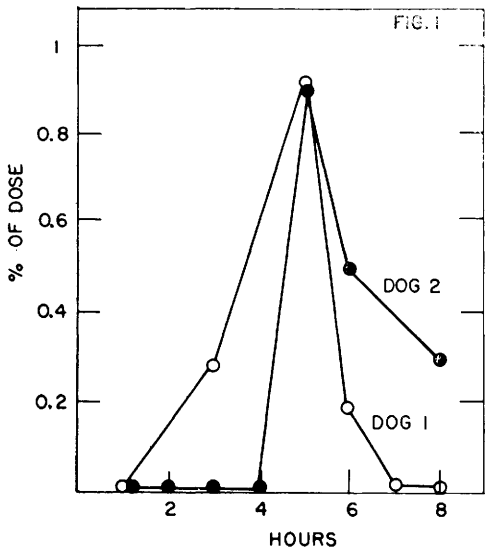
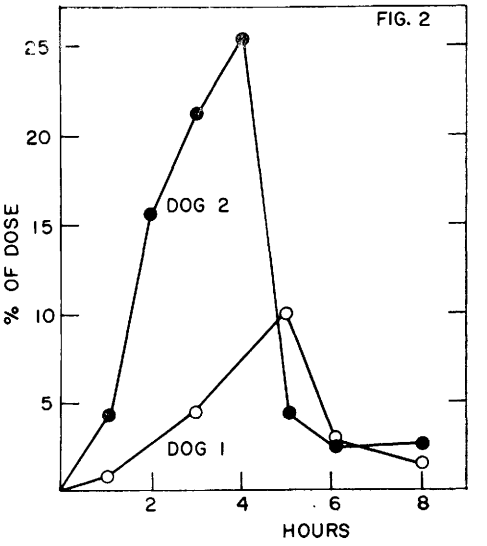
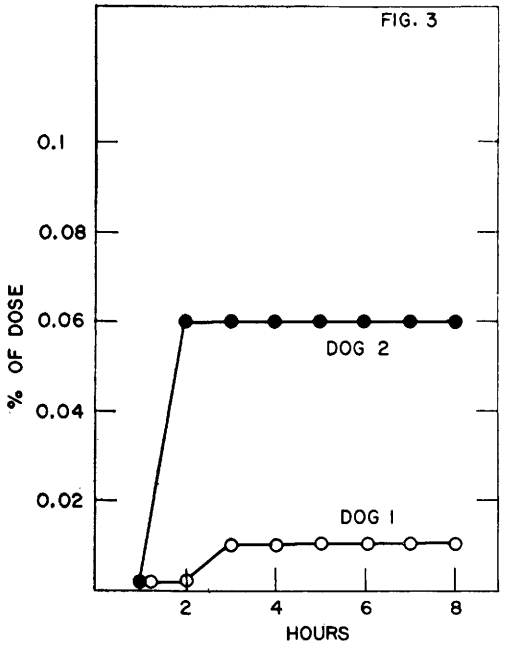
TABLE I. Radio-B_{12} and Radio-Iron Recovered in Excreta, Intestinal Contents and Intestines after Administration via Gastric Tube.

Dog	Times, admin./sacrifice	Amt B_{12} given, μg	Amt Fe given, μg	Amt B_{12} recovered μg	Amt B_{12} recovered % of dose	Amt Fe recovered μg	Amt Fe recovered % of dose
1	Nov. 17, 0800/Nov. 17, 1800	4	1	3.75	94	.33	33
2	Jan. 12, 0900/Jan. 19, 1200	4	1	1.76	44	.35	35







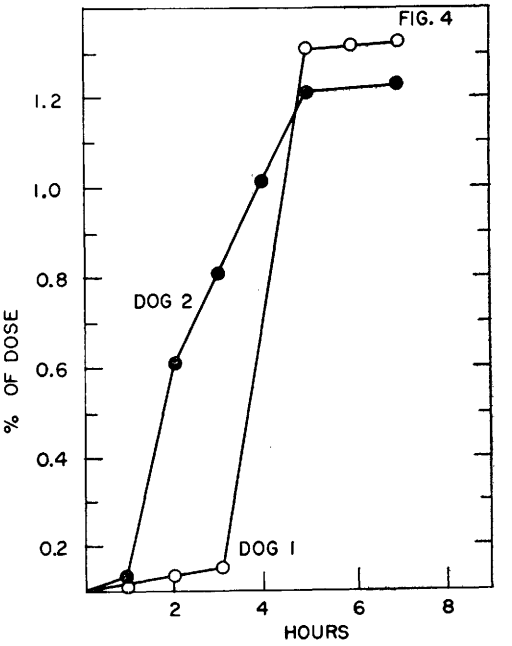


FIG. 1. B_{12} in total plasma—vol; % of dose.
FIG. 2. Fe in total plasma—vol; % of dose.
FIG. 3. Cumulative Co^{58}-B_{12} reaching blood via lymph; % of dose.
FIG. 4. Cumulative Fe^{59} reaching blood via lymph.

TABLE II. Concentrations of Co^{58}-B_{12} and Fe^{59} in Lymph and Plasma after Gastric Tube Administration.

		Peak concentration				Hr after ingestion	
		Lymph		Plasma			
	Dog	mμg/ml	% of given dose/ml	mμg/ml	% of given dose/ml	Lymph	Plasma
Fe^{59}	1	.34	34×10^{-3}	.46	46.4×10^{-3}	4	5
	2	.17	17×10^{-3}	.17	17.3×10^{-3}	2	4
Co^{58}-B_{12}	1	.003	$.07 \times 10^{-3}$	.07	1.7×10^{-3}	3	5
	2	.005	$.12 \times 10^{-3}$	.06	1.5×10^{-3}	2	5

is about 2 μg/ml(6) the iron binding capacity in dog lymph seems to be less than 1/1000 of what is found in human plasma.

The free B_{12} binding capacity in lymph, after adding 25 mμg/ml of Co^{60}-B_{12}, was 0.7 and 1.7 mμg/ml, respectively, which is comparable to that found in the plasma from 1 dog (3 mμg/ml) and to that in normal human plasma (6 mμg/ml) (Fig. 5).

Discussion. It has been demonstrated previously that iron transport takes place mainly *via* portal blood(7-10). This is confirmed by the present study, which indicates that Vit. B_{12} also is transferred directly from the intestinal wall to the portal blood without going *via* the lymph.

The time relationship between concentration of absorbed radio-iron in plasma and lymph has not previously been studied. The fact that both radio-iron and radio-B_{12} concentrations in lymph reached their peaks earlier than those in blood indicates that a small fraction of these substances is actually absorbed *via* the lymph while intestinal concentration is high. Later, most of the iron found in the lymph probably comes from the plasma(7,10). Since the concentration gradient between plasma and lymph is higher for radio-B_{12} than for radio-iron, and since it has been shown that plasma-radio-B_{12} escapes rapidly into the extravascular space(11,12) it is probable that much of the radio-B_{12} found in the lymph is also derived from plasma, at least after plasma concentration has reached its maximum.

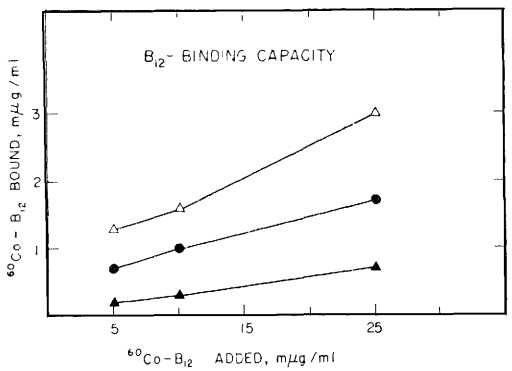


FIG. 5. Unsaturated B_{12}-binding capacity in dog lymph (filled symbols) and dog plasma (empty symbols).

There are indications that substances with low molecular weights such as fatty acids with less than 10 carbon atoms(13), amino acids (13-15), and hexoses(13), are mainly transferred directly from the intestine to the portal blood, whereas larger molecules, such as fatty acids with more than 10 carbon atoms(13,15) or native proteins(16,17), are transferred into the lymph. The possible malabsorption of B_{12} in steatorrhea due to blocked lymphatics may be secondary to the presence of fat in the intestinal lumen, in analogy with the findings in pancreatogenic steatorrhea (18).

There has been considerable discussion whether Vit. B_{12} is absorbed as a compound with a molecular weight only slightly higher than that of pure crystalline B_{12}(19,20) or whether it is accompanied by the entire intrinsic-factor-molecule (molecular weight about 70,000). Such a large complex would probably be transported *via* the lymphatic route. The present observations indicate, therefore, that most of the absorbed radio-B_{12} is absorbed as a considerably smaller molecule.

If this is true, then the degradation of intrinsic factor must take place in the intestinal wall, for it has been shown that the enzymes present in the intestinal lumen are not capa-

ble of splitting the intrinsic factor-B_{12}-complex(21). As a matter of fact, during preparation of the present paper, such an intracellular enzyme in the intestinal wall was described(22,23). Defects in this intracellular enzyme may explain the resistance to hog intrinsic factor in patients. It is also conceivable that the time lag before B_{12} appears in the blood (Fig. 1, dog 1)(24) is explained by this process in the intestinal wall.

Simultaneously with this work, experiments in rats by Taylor and French were published (25), which are confirmed by our results.

The emerging picture of B_{12}-absorption resembles that of iron absorption. During several hours after entering the intestinal wall, the major portion of an originally protein-bound molecule is retained in the intestinal wall, where it is separated from its original binder before being transferred into the portal blood. Meanwhile, a very small fraction, possibly still bound to the original binder is absorbed *via* the lymph.

Summary. Transport of radio-iron and radio-B_{12} from the intestinal wall to the blood was studied in 2 dogs with cannulated thoracic ducts. Most of both substances was directly transferred into the blood without going *via* the lymph. Since large molecules are generally absorbed *via* lymph, this indicates that the large B_{12}-intrinsic factor is split in the intestinal wall prior to transfer.

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