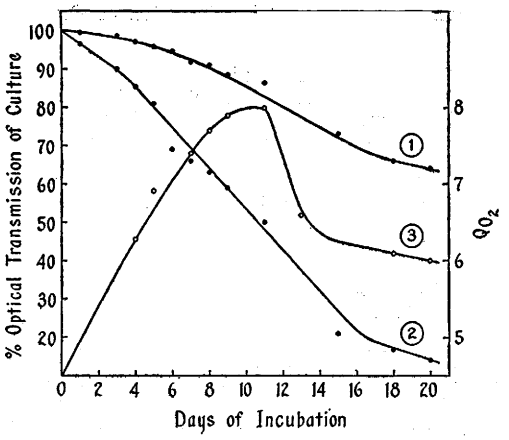

FIG. 3. Effect of age of culture on rate of lactate oxidation by washed cell suspensions of *M. tuberculosis* strain H37Ra. Curve 1 = % optical transmission of culture. Curve 2 = optical transmission of culture concentrated 5 times. Curve 3 = Q_{O_2}. 0.066 M phosphate buffer, pH 6.4; 0.022 M lactate; 6-9 mg dry wt bacterial cells/cup; Sauton's medium.

age of the bacterial cells. It is manifest from the data given that rate of lactate oxidation by washed cell suspensions increases with age of the cells, reaching a maximum between the 10th and the 12th day of incubation which coincides with the middle of the logarithmic phase of growth. Upon continued incubation, rate of lactate oxidation falls rapidly reaching a minimum during the stationary phase of the bacterial growth cycle. Thus, it is evident that the tubercle bacillus responds in this respect in a similar manner to other micro-organisms(6) and suggests that young cells derived from dispersed cultures might properly be more widely used in studies of the physiology of this organism. Although admittedly these data were obtained using only one strain of the tubercle bacillus and inspecting only one enzyme system, the results suggest further study along these lines. Furthermore, since it has been established that the lipid content of tubercle bacilli increases with age(7), which may well influence the permeability of the older intact cells, it would appear desirable to utilize the younger cells for investigative purposes.

Summary. A study of lactate oxidation by washed cells of *M. tuberculosis* strain H37Ra revealed a marked influence of the age of the cells. Cells obtained during the logarithmic phase of growth were the most active metabolically.

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Biliary Excretion of Co^{60} Labeled Vitamin B_{12} in Dogs.* (24254)

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Previous reports indicate that radioactivity is present in bile and feces after injection of labeled B_{12}, but observation periods were short and data not critically analyzed(1,2,3). The following study was instituted to evaluate the rate of Co^{60} excretion in bile and its role as a source of fecal radioactivity after intravenous

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TABLE I. Total Biliary Excretion in 5 Dogs following Intravenous Administration of Co^{60} Vit. B_{12}.

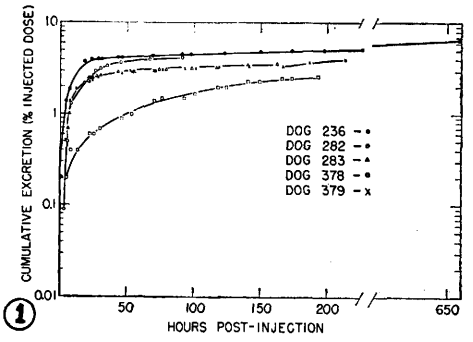
Dog No.	Sex	Wt, lb	Total dose inj., μc	Total vol bile collected, ml	Period of collection, hr	% of inj. dose in bile
282	♂	52	3.48	250	96	4.2
283	♂	38	3.48	661	162	3.6
236	♂	37	3.48	3245	658	6.6
378	♂	48	2.096	889	193	2.6
379	♂	48	2.096	1342	213	4.0

Lot V 5-6-6—20 cc vial contains 22.96 μc equivalent to 25.96 μg.
Lot V 5-7-7— 5 " " " 5.24 " " " 7.52 ".

administration of labeled Vit. B_{12} to dogs.

Material and methods. Co^{60} Vit. B_{12} was received in 2 lots with specific activities of 0.885 and 0.95 uc/ug, respectively. Prior to the experiment dogs were kept in isolation for at least one month, during which time they were wormed and immunized to canine hepatitis and distemper. All animals were young and healthy. Hematologic values were normal. Dogs were maintained under anesthesia by intermittent injections of sodium pentothal through catheter in femoral vein. The labeled vitamin was administered through this vessel. Under aseptic conditions the common bile duct was exposed and a polyethelene catheter inserted and tied at a point below the major bifurcation. The other end of catheter was connected to plastic bag for collection of bile under sterile conditions. Postoperatively, the dogs were fed a low fat diet including 0.3 g of Desicol. Two mg of Menadione were injected intramuscularly daily. At varying intervals the bag was emptied, volume of bile measured, and radioactivity/ml determined in a well-type scintillation counter. Counts/minute were converted to microcuries by reference to an aliquot of the radiovitamin injected.

Results. Table I shows dose of Co^{60} B_{12} injected, period of collection, volume of bile excreted, and total Co^{60} radioactivity measured. The experiment was terminated by irreversible plugging of the lumen or dislodgment of tubing from the common duct. Periods of collection ranged from 96 to 658 hours. Fig. 1 shows cumulative excretion of Co^{60} in bile of 5 dogs. Subjecting these excretion curves to graphic analysis a 2-component pattern was noted in dogs No. 379, 283 and 236. (illustrated in Fig. 2 for dog No. 379.) The half-times for the first (fast) components were 6.0, 6.5, and 7.5 hours, respectively. The slow components had half-times of 130, 90, and 295 hours, respectively. In dog No. 282 the collection period was not long enough to analyze the curve adequately, but 2 components were probable. The fifth



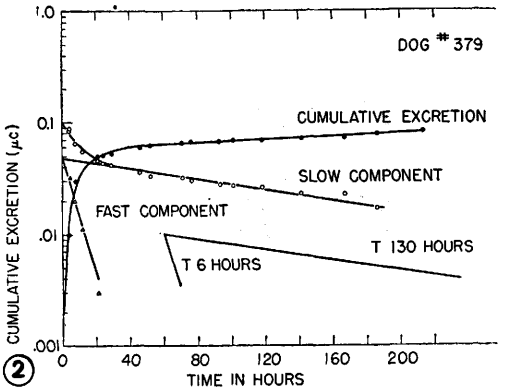


FIG. 1. Cumulative biliary excretion of Co^{60} in 5 dogs given intrav. cobalt labeled vit. B_{12}.

FIG. 2. Example of two-component biliary excretion of Co^{60} after intrav. inj. of cobalt labeled B_{12}.

TABLE II. Excretion of Co^{60} in Urine and Feces of Normal and Biliary Fistulae Dogs following Labeled B_{12} Injection.

	Dog No.	μc inj.	Days post-inj.	μc excreted in		Total μc	% excreted in		Total %
				Feces	Urine		Feces	Urine	
Normals	279	3.48	2		.05			1.4	
	281	3.48	5		.10			2.9	
	274	13.90	25	2.0	5.8	7.8	14.4	41.7	56.1
	237	4.85	54	1.9	.6	2.5	39.1	12.4	51.5
	275	8.12	235	2.9	1.4	4.3	35.7	17.2	52.9
	284	6.96	245	1.5	2.6	4.1	21.5	37.4	58.9
Biliary fistulae	282	3.48	5		.03			.9	
	283	3.48	7		.10			2.9	
	378	2.096	21 (12 days)*	.2	.05	.25	9.5	2.4	11.9
	379	2.096	21 (12 days)*	.2	.03	.23	9.5	1.5	11.0
	236	3.48	28	.55	.18	.73	15.8	5.2	21.0

* Fecal and urinary data available for 12 days only.

animal, No. 378, had a single component of excretion with a half-life of 145 hours (Fig. 2d).

Fecal excretion of radioactivity is shown in Table II for 5 dogs with biliary fistulae and 7 normal animals. It is clear that Co^{60} activity of bile does not explain total fecal excretion since dogs with biliary fistulae show radioactivity, though to a much lesser degree, in their feces.

Discussion. In general, presence of a 2-component excretion pattern indicates existence of 2 or more compartments in which the radioactive tracer is presumably diluted. Whether these compartments are in parallel, turning over the labeled vitamin independently and at different rates, or whether they are connected in series and are mutually dependent, cannot be ascertained in these experiments.

At present it is not possible to explain the apparent 2 component biliary excretion of radioactive cobalt after intravenous injection of the labeled vitamin. These observations in dogs are consistent, however, with those of Grasbeck *et al.*(4) in patients.

The physiologic significance of this 2 component excretion will have to be determined by further investigation. Certain facts are known which will help design further experiments. Meyer *et al.*(5) have shown that plasma binds radioactive B_{12} promptly and very tightly. In our preliminary experiments (unpublished) with continuous flow paper electrophoresis, the radioactivity appears with the $\alpha 1$, globulin of plasma, suggesting that this protein fraction carries Vit. B_{12} from sites of absorption to deposition in tissue. That the parenchyma of liver is relatively permeable to this complex and permits its passage into the biliary passages is unlikely because plasma clearance(6) takes place in human beings too rapidly to allow this interpretation. Plasma clearance in the dog, however, has not been measured yet. The fast biliary excretion may represent loss of radioactive vitamin not bound to tissue components while the slower excretion may represent loss of cells or cellular debris containing radiovitamin or its cobalt degradation products, or loss of degradation products alone. We did not determine whether biliary radioactivity was associated with cells, large molecules, or was freely dialyzable. Even more pertinent is the question whether the radioactive cobalt still represents Vit. B_{12} or some of its degradation products. In fact, interpretation of all long term studies with radioactive Vit. B_{12} is dependent upon whether the radioactivity is still associated with B_{12} or its metabolic products. If radioactivity represents undegraded B_{12} it still may not be representative of B_{12} behavior. To be representative, the injected radiovitamin must be uniformly distributed in the total body pool of B_{12} and one must determine whether the ratios of labeled to cold B_{12} are constant

throughout the body. We are not aware of any such determinations.

Since stools of dogs with biliary fistulae contain Co^{60} radioactivity, it is evident that labeled B_{12} or one of its degradation products passes into the lumen of the gastrointestinal tract from sources other than bile. Fecal radioactivity is obviously enhanced when the flow of bile to the intestines is uninterrupted. Whether Co^{60} in bile is still part of the Vit. B molecule and can be absorbed for reutilization has not yet been determined. Okuda *et al.* (7) have also independently demonstrated the presence of radioactive B_{12} in bile and stools of rats after injection of labeled B_{12} in rats and believe that fecal B_{12} has a biliary and an intestinal source.

Conclusions. 1. Following intravenous administration of 2.09 to 3.48 μc Co^{60} Vit. B_{12} to dogs between 2.6 and 6.6% of radioactivity was recovered in the bile. 2. In 3 of 5 dogs the 2-phased excretion pattern showed fast and slow components of 6.0 to 7.5 hours and 90 to 295 hours, respectively. 3. Fecal excretion of radioactivity in dogs with biliary fistulae was considerably less than in intact normal animals.

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Oxygen Consumption of the Hypothermic Potassium Arrested Heart.* (24255)

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Cardiac arrest induced by intracoronary administration of potassium(1) or acetylcholine(2) has been found useful in cardiac surgery. However, revival of arrested hearts has not been completely satisfactory(3). Refractory ventricular fibrillation and inability of the previously asystolic heart to maintain an adequate cardiac output have been the principal difficulties. Previous studies(3) have indicated that the combination of cardiac hypothermia and potassium arrest resulted in the most rapid and complete post-arrest recoveries. The assumption was made that a reduction in myocardial oxygen requirement by cardiac hypothermia in the arrested heart was in large part responsible for these results and the present study was undertaken to test this asumption by quantitating the oxygen consumption of the normothermic and hypothermic arrested heart.

Methods. Fifteen technically satisfactory experiments were performed on open chest dogs anesthetized with sodium pentobarbital (30 mg/kg). The chest was opened bilaterally in the fourth intercostal space and the sternum transsected at this level. Following administration of heparin, the left coronary artery and the coronary sinus were cannulated. During control studies when the circulatory system was intact and the heart doing work, the source of blood for left coronary artery perfusion was either the dog's carotid artery or a pump oxygenator(4). When the pump oxygenator was used, blood was withdrawn from the right atrium at a rate

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