by lack of effect of LSD, since in dosage used, LSD has been found to inhibit the effect of injected(11) and of infused(12) serotonin on arterial pressure of anesthetized rats. Further confirmation is found in the fact that in rats pre-treated with iproniazid but not given pentolinium, reserpine was consistently pressor while serotonin was depressor. Clinical corroboration is found in the response to phentolamine of hypertensive patients under treatment with rauwolfia drugs whose response is commonly depressor(13); it seems likely that this reflects a state of underlying adrenergic excitation which in a degree mimics the status of patients with functioning pheochromocytoma.

Summary. In anesthetized rats pretreated with iproniazid, injected reserpine elicits a marked pressor response which from observations on effects of phenoxybenzamine, chlorpromazine and LSD seems primarily attributable to release of adrenergic amines, presumably norepinephrine. Experiments in conscious rats indicate that their response to reserpine is adrenergic. The data support conclusions reached by others in other species but do not support the view that serotonin plays a large role.

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Biliary and Fecal Vit. B_{12} Excretion in Man. An Isotope Study.* (23879)

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In spite of considerable progress in Vit. B_{12} research, it is still not known what amount normal humans have to absorb in order to maintain health; likewise, the optimum maintenance dose for pernicious anemia patients is unknown. One way to elucidate these questions is to study quantitatively the excretion of Vit. B_{12}. As judged from the minimum maintenance dose for pernicious anemia patients, normal humans seem to absorb at least one microgram *pro die* under physiological circumstances(1). When pure Vit. B_{12} is given *per os,* the upper limit of absorption lies well above this figure(2,3). On the other hand,

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normal humans excrete less than one microgram in the urine per day(4). This indicates that the vitamin is either broken down in the body or at least is excreted in urine as a microbiologically inactive compound, or, alternatively, is excreted mainly by routes other than the urine. Previous studies on animals(5,6) demonstrate the importance of the biliary and fecal routes in the excretion of "endogenous" (absorbed) B_{12}. Indications of the significance of these routes in man were obtained with the finding of high microbiological B_{12} activity in human bile(5,6). Also, as judged from a few observations reported in the literature(7,8,9), it seems that in man parenterally administered radiovitamin B_{12} appears in the bile and feces. However, in no case has this excretion been subject to a systematic study. Because of the relatively large doses administered, the short observation times, and the change in excretion pattern of radiovitamin during the first days after injection (Fig. 1), no definite conclusion can be drawn as regards the physiological excretion routes of Vit. B_{12}.

In the present study it was attempted to study systematically the biliary and fecal B_{12} excretion by administering parenterally minimal doses of radiovitamin B_{12} and by subsequently following for long periods its elimination with the bile and feces. It was felt that such an investigation could not be restricted to the fecal route alone, since the extensive synthesis and possible breakdown(10) of B_{12} taking place in the intestinal contents make it impossible to draw any conclusions as to whether the fecal radioactivity is derived from excreted Vit. B_{12}.

Material and methods. A single 0.5 μg dose of Co^{56} labelled Vit. B_{12} with a specific activity of approximately 2 mc/mg (the authors are greatly indebted to Dr. E. Lester Smith, Glaxo Ltd., England, for the generous gift of $Co^{56}B_{12}$) was injected intramuscularly to the following subjects: a) 5 healthy young adult females, whose feces were subsequently collected in one day portions for 26-29 days; 24-hour urine samples were collected the first 2 days after injection and thereafter at irregular intervals; b) 5 patients with a postoperative T-tube drain in the common bile duct and without clinical signs of disordered B_{12} metabolism and whose drain bile was collected in one day portions until the drainage was discontinued; c) 2 healthy young adult males, who were subjected to duodenal lavage 3 times during the 1st to 40th day after the injection. The duodenal lavage was performed in the usual manner by passing down the tube during X-ray fluoroscopy and by injecting magnesium sulfate through the tube. Before counting, the feces samples were liquefied by nitric acid digestion, and the bile and urine samples were concentrated by evaporation. In counting, scintillation technic was used. Since it is not possible, even by drainage, to collect the daily bile secretion quantitatively it was attempted to calculate the daily biliary Co^{56}-B_{12} excretion in the following manner: the bilirubin content of the bile samples was determined, and the radioactivity found was corrected assuming that the daily bilirubin excretion was 270 mg.†

Results. The excretion pattern changing markedly after the first few days following injection, the data have been grouped into an *early* (1st-4th day) and a *late* (4th-30th day) group (Fig. 1).

Urinary excretion. Even with the very small amounts of B_{12} administered in this study, considerable amounts of radioactivity are eliminated by this route during the first 2 days after injection. During the early period, more Vit. B_{12} is excreted by this route than by any other (Table I, Fig. 1). On the other hand, during the late period the urine contained less radioactivity than the feces. In contrast to the feces samples, which all contained demonstrable radioactivity, in some late urines no activity could be detected.

Fecal excretion. Although the values observed early after the injection are distinctly higher than later on, this effect is much less marked than in the urine (Table I, Fig. 1). During the late period, the mean 24-hour excretion of radioactivity varies relatively little

† This was calculated as follows: Normal total body hemoglobin is approx. 750 μg(20), corresponding to 29.0 g of heme. Calculating with a breakdown of 1/120/day, this corresponds to excretion of 230 mg of bilirubin. To this are added 40 mg from non-hemoglobin porphyrins(21,22).

TABLE I. Daily Excretion of Radioactivity, Calculated as B_{12}, after Intramuscular Injection of 0.5 μg Co^{56}-B_{12}.

			mμg/day	Range	% excreted of total B_{12}-Co^{56} in body	No. patients	No. of 24-hr collections analyzed
Early (1st-4th day)	Bile	Observed	4.8 ± 1.4†	.6–10.5		7	18
		Calculated	13.2 ± 3.9	2.6–29.1		7	18
	Feces		4.6 ± 2.0	1.5– 8.6		5	15
	Urine*		25.8 ± 2.4	12.9–32.9		5	10
Late (4th-30th day)	Bile	Observed	.7 ± .2	.4– 1.6		7	19
		Calculated	5.1 ± 1.3	1.4–11.5	1.1 ± .3	7	19
	Feces		1.4 ± .1	1.1– 1.7	.3 ± .02	5	123
	Urine		.1 ± .09	.0– .5	.02± .02	5	10

* Urine collected for 2 days only. † Mean ± S.E.

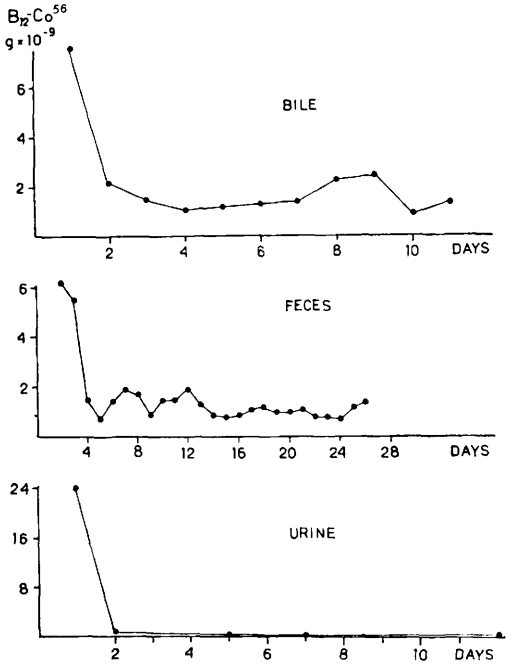

FIG. 1. Difference between *early* (1-4th day) and *late* (4th-30th day) period after parenteral administration of vit. B_{12}-Co^{56} as regards the pattern of radiovitamin excretion in 3 cases. Regarding excretion in bile and urine, those cases were chosen in which the largest number of samples were obtained. As regards fecal excretion, the case was chosen at random. There was considerable day-to-day variation, probably explained by differences in frequency of defecation and quantity of stools passed on different days. The curve represents successive mean values.

in different subjects. No feces samples were found to be nonradioactive. The average amount of radioactivity excreted by this route per day during the late period (calculated as Co^{56}-B_{12}) corresponds to 0.3% of the radioactivity present in the body (injected amount *minus* that excreted during the early period).

Biliary excretion. The early values are distinctly higher here also. The mean late excretion value is 5.1 mμg/day, corresponding to 1.1% of the amount of radiovitamin B_{12} present in the body after the early period (Table I).

The calculated corresponding excretions of "endogenous" vitamin are seen in Table II.

Discussion. The question naturally arises, whether the behavior of the injected radiovitamin is representative of the fate of the nonlabelled vitamin normally contained in the body.

The results of other workers(11-14) who have studied the distribution of radioactivity in different organs after oral or parenteral administration of radiovitamin B_{12}, indicate a

TABLE II. Daily Excretion of Endogenous Vitamin B_{12}. The calculations have been based on the assumption that the injected radiovitamin mixes with the body stores.

	Mean ± S.E.	Range
	μg	
Total amt of B_{12} in body*	3,900	790–11,100
Corresponding daily excretion of endogenous B_{12}		
Bile	43 ± 12	9 –122
Feces	12 ± 1	2 – 33
Urine	.8 ± .8	.2– 3

* Obtained by adding microbiological B_{12} contents of liver, kidneys, heart, spleen, brain, and muscles in 4-22 autopsies (varying for different organs)(15,16). The corresponding excretion of endogenous B_{12} is calculated using percentages given in Table I. Stand. errors are based only on isotope studies and do not include those of microbiological assays.

relatively good mixing of the introduced isotope with the body B_{12} pool. The distribution of radioactivity closely resembles the natural distribution of microbiological B_{12} activity (15,16).

Partly because this study deals with a human material it is difficult to establish definitely the time required for the complete mixing of the isotope among the body B_{12}-pool. In the present study the fourth day was chosen as the limit, because by this time the excretion pattern had undergone a marked change from the dominantly urinary excretion pattern into one characterized by a steady fecal output of radioactivity. In addition, other investigators(13,14) have found that by this time the distribution of parenterally injected radiovit. B_{12} has assumed the "final" pattern with maximal activity over the liver area. Although the percentage excretion of radioactivity does not seem to be much affected by the fixing of the limit to a few days later than the 4th day after the injection, the question on the mixing of injected radiovit. B_{12} has to be subjected to further study. Until such studies are concluded, the data given on the excretion of endogenous Vit. B_{12} should be interpreted with caution.

Irrespective of whether the mixing within the period of time studied in the present paper is absolutely complete, the conclusion seems well founded that in man Vit. B_{12}, or its cobalt containing breakdown product, is excreted mainly by the fecal route. The source of the fecal material may well be the bile, which was found to have relatively high radioactivity and which is rich in microbiological B_{12} activity(5,6). Our data indicate that at least two-thirds of the biliary radioactivity is reabsorbed from the intestine, and thus that Vit. B_{12} is subject to an enterohepatic circulation as are other components of the bile. It is possible that this reabsorption is aided by the gastric intrinsic factor and/or the Vit. B_{12} binding substance found to occur in bile(5,6). An impaired intestinal reabsorption may be a factor in the pathogenesis of certain B_{12} deficiency states. Preliminary results with simultaneous collection of bile and feces in the same subjects seem to confirm the concept of an intestinal reabsorption of Vit. B_{12}(17).

That absorbed Vit. B_{12} is excreted by the bile-feces route probably explains the fact that in performing fecal excretion tests, some radioactivity can be detected in the stools for a long time after the administration of the oral dose. Biliary excretion with subsequent isotope dilution in the intestine may explain the observed inhibition of radiovitamin B_{12} absorption by parenteral administration of nonlabeled B_{12}(18).

Provided the introduced radiovitamin is uniformly mixed with the nonlabeled vitamin contained in the body, it should be possible to calculate from the present data the corresponding excretion of endogenous B_{12} if the size of the B_{12} pool be known. Thus far tentative calculations have been performed, in which the size of the pool has been estimated on the basis of microbiological assays performed by other authors(15,16) on autopsy material. The results are listed in Table II. Even allowing for considerable errors, the data seem to indicate that the daily loss of Vit. B_{12} may amount to several micrograms, and thus the requirement of B_{12} may be larger than hitherto assumed(1). The order of the magnitude of the daily loss of Vit. B_{12} receives some support in recent investigations on the hepatic storage of radiovit. B_{12}(11,19), indicating a loss of several micrograms of radiovitamin per day.

Summary. It was attempted to study Vit. B_{12} excretion in man by investigating the elimination of small doses of parenterally administered radiovitamin Co^{56}-B_{12}. Relatively large amounts of radioactivity were excreted in the bile, feces and urine shortly after injection. After this period, the excretion pattern changed, and radioactivity of relatively constant value was excreted with the bile and feces, indicating that these are the main excretion routes in man, whereas the urine samples exhibited low radioactivity. The fecal radioactivity was only about one-third of the calculated biliary excretion, which indicates intestinal reabsorption of biliary B_{12}. If it were assumed, that the radiovitamin mixes uniformly with the body stores of Vit. B_{12}, it could be estimated that normal subjects may lose considerable amounts of B_{12} per day, and consequently, the requirement of

B_{12} could be larger than hitherto suspected. The findings may have therapeutical implications.

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Effects of Δ^4-Cholestenone in Animals and in Man. (23880)

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Feeding of large amounts of Δ^4-cholestenone* will lower the levels of serum cholesterol in rats(1,2), dogs and chickens(3). This effect on serum cholesterol may be related to suppression of endogenous cholesterol biosynthesis by cholestenone, shown first by Tomkins, Sheppard and Chaikoff in acute experiments(4) and confirmed in this laboratory in chronic feeding experiments(1). However, high dose levels (1 g/kg/day) of cholestenone are highly toxic to rats, causing sharp arrest of normal growth rate and accumulation in tissues of dihydrocholesterol, a major product of cholestenone metabolism(5-7). The other outstanding effect of chronic feeding of cholestenone at high dose levels is a remarkable hypertrophy of the adrenal gland to 7 times normal weight(1). This hypertrophy is associated with sharply reduced rate of secretion of corticosteroids—both corticosterone and aldosterone(8). The present studies were carried out to evaluate general toxicity of cholestenone in different species; to study absorption and metabolic fate of the compound, particularly degree of conversion to dihydrocholesterol; and to further explore adrenal changes induced by large doses.

Methods. Male Osborne-Mendel rats were maintained on basic diet of following percentage composition: casein, 34.5; sucrose, 31.0; corn starch, 21.5; cottonseed oil, 6.5; salt mixture(9), 5; cod liver oil, 1; choline, 0.1 and supplements of vitamins. Cholestenone,† dihydrocholesterol and cholesterol were

* Referred to subsequently simply as cholestenone.

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