were, nevertheless, significantly less than controls. No greater milk yield resulted from injection of more than 1 mg lactogen. These data indicate that lactogen is necessary for maintenance of milk secretion in intact rats and strongly imply that since lactogen alone was incapable of restoring milk secretion 100%, other pituitary hormones are necessary for full milk secretion and perhaps might be susceptible to discharge by nursing stimuli.

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Excretion, Enterohepatic Circulation, and Retention of Radiovitamin B_{12} in Pernicious Anemia and in Controls.* (25067)

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The routes and patterns of Vit. B_{12} excretion have been studied in animals(1,2) and in man(3,4,5). In human studies, some indications were found of an intestinal reabsorption of Vit. B_{12} excreted in bile. The purpose of the present paper is to confirm this finding and to extend the studies to patients with deficient B_{12}-absorption. Retention of B_{12} in serum, and B_{12} excretion in controls and in patients with B_{12}-deficiency were also studied.

Material and methods. Controls were 11 healthy, non-hospitalized males between 20 and 34 years old (normal controls), and 4 female and 2 male hospital patients between 16 and 69 years old (control patients). Their diagnoses were asthenia and achlorhydria, allergic bronchial asthma, diabetes mellitus, and acute or subacute nephritis (3 cases). Random serum samples showed normal B_{12} concentrations. All control patients had normal Vit. B_{12}-absorption tests (21-55%) by the technic described earlier(5). Four additional patients with a post-operative drain in the common bile duct were employed to obtain several bile samples within a short time. The 13 cases with disturbed B_{12} metabolism (Table I) had either an acute Vit. B_{12} deficiency as evidenced by low Vit. B_{12} levels in serum and by megaloblastic anemia reacting to B_{12} treatment, or disturbed Vit. B_{12}-absorption, mostly both. Case No. 4 is insufficiently studied and results based on material involving this case are in brackets (Table IV). Between 0.3 and 0.6 μg Vit. B_{12} labeled with Co^{56} or Co^{58} and with approximate specific activity of 2 mc/mg were injected intramuscularly. Two persons received 1 μg because of the decay of radioactivity. Twenty-nine serum samples were obtained from 17 persons between 3-17 days after injection of labeled B_{12}. All urine was collected from 24 persons during 3 days after injection. From 29 persons 136 24-hour feces collections were obtained, starting on the average 6.8 (4-10) days after radiovitamin injection. Feces were liquefied by nitric acid digestion and concen-

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TABLE I. Thirteen Cases with Disturbed B_{12} Metabolism.*

Sex	Age, yr	B_{12} absorption test Urinary excretion, % of given amt	Serum vit. B_{12}, $\mu\mu$g/ml	Megaloblastic bone marrow	Histamine fast gastric achlorhydria	Total body hemoglobin, g	Hb (g/100 cc)	RBC (mill.)	WBC (× 1000)
♂	49	2	165		+	560	12.6	4.0	5.6
♂	56	2	16	+	+	450	9.8	2.9	4.0
♀	69		40	+	+	433	12.8	3.7	5.3
♂	52				+	750	13.7	4.3	6.0
♂	26	11	115			405	9.1	3.8	7.0
♂	55	7	10	+	+	274	8.8	1.9	5.0
♀	45	9	165	+		365	8.2	2.3	3.8
♀	73	4	32		+	385	8.8	2.3	3.2
♂	67	6	14	+		350	8.6	2.4	6.1
♀	65		20	+	+	425	7.5	1.7	4.0
♂	62	5	170	+	+	448	4.7	2.5	3.5
♂	55		36	+	+	500	9.5	2.5	2.6
♂	59	1		+	+	230	5.6	1.3	4.5

* Space left empty = test not performed.

Clinical diagnoses were, in case 1 chronic gastro-duodenal-jejuno-ileitis with malabsorption syndrome, in cases 2-3, 6, 8-13, pernicious anemia, in case 4 chronic pancreatitis with malabsorption syndrome, in case 5 mesenteric and pulmonary tuberculosis with malabsorption syndrome, and in case 7 malabsorption syndrome.

trated by evaporation. Bile, unless otherwise stated, signifies strongly bile coloured mixture of duodenal juices obtained by duodenal lavage (kindly performed by Dr. G. Perman) (6). Fifty-nine bile samples and 11 samples of bilirubin-free duodenal juice were taken from 30 individuals 12.8 (7-19) days after isotope administration, and after collection of feces. In some cases a satisfactory flow was easily obtained, in others bile secretion had to be stimulated by cholecystokinin or magnesium sulfate. Bilirubin determinations were performed within a few hours after duodenal lavage. Bile samples were kept in the dark at +4°C and concentrated by evaporation. Methods of determining B_{12}-binding (Table III), bioassays, and scintillation measurements will be described elsewhere(7). Radioactivity measurement times were adjusted to keep statistical error of counts between 1 and 10%. Daily bilirubin production was estimated individually in each case, and employed to calculate daily B_{12} excretion in the bile. The percentage saturation of hemoglobin with CO (COHb) was calculated from determinations of CO pressure in alveolar air (9,10). Simultaneously, total amount of Hb (THb) was estimated by determining the increase in COHb after rebreathing of a known amount of CO in a closed system(10). From THb and COHb the Hb destruction rate (d.r.) was calculated(10). Daily bilirubin production could be roughly calculated to be = (THb x 1/120 x d.r. x 4 x 619 x 584/619) x 1.05/66.700 mg, where 1/120 is the fraction of Hb normally destroyed/day. The factor 1.05 is due to the part of bile pigment derived mainly from heme-enzymes(10). In some instances the d.r. could not be calculated since the persons had inhaled CO (for instance by smoking) before the experiment. In these cases, the d.r. was assumed to be 1 for controls and 2 for cases with B_{12}-deficiency. The figure 2 is the lowest value recorded in cases of untreated megaloblastic anemia which had not been exposed to CO inhalation, both in

TABLE II. Excretion of Vit. B_{12} in Controls and B_{12}-Deficient Patients.

Excretion	No. of cases	Controls	No. of cases	B_{12}-deficient
Urinary, total excretion 1st-4th day	14	12.8 ± 1.87%*	6	8.7 ± 1.93%*
Fecal, daily excretion, later than 4th day	16	.5 ± .17%†	9	.3 ± .06%†
Biliary, daily excretion, later than 4th day	14	1.5 ± .31%†	8	.4 ± .11%†

* % of inj. dose. † % of inj. dose *minus* loss 1st-4th day. Means ± S. E. of mean.

TABLE III. Vitamin B_{12}-Binding Capacity* in Serum.

Controls	B_{12}-deficient patients
2.9 ± 1.16 mμg/ml (4 cases)	3.2 ± .67 mμg/ml (8 cases)

Means ± stand. errors of mean.

* Nondialysable after adding 200 mμg radio B_{12} per ml serum.

present and in previous materials(10). Daily radiovit. B_{12} excretion was estimated to be = C x (radiovit. B_{12} concentration) x (calculated daily bilirubin production) / (bilirubin concentration in sample). Since only a part of daily radiovit. B_{12} excretion in bile is measured, the factors by which this part is multiplied to obtain total daily excretion must not be too large. An attempt was made to correct for the fraction of bile pigments not giving the diazo reaction, and for the probable maximum amount of duodenal radioactivity not originating in pure bile. This correction constant (C) was calculated to be 0.76(7).

Results. Retention of Vit. B_{12}. Mean daily excretions of radioactivity in both urine, bile and feces were numerically higher in controls than in cases with subnormal Vit. B_{12} levels (Table II) indicating that more of the injected B_{12} is retained by B_{12}-deficient patients. Similarly, these patients show higher serum concentrations of radioactivity than the controls (Fig. 1). No significant difference could be observed, however, in B_{12} binding capacity between the 2 groups of sera (Table III). Distribution of all excretion values was markedly skew, and the difference is statistically significant only as regards biliary excretion ($0.005 > p$), and serum concentration (Fig. 1).

Excretion routes. It has been demonstrated (3,4) that, physiologically, most Vit. B_{12} in man is excreted by the fecal route, and that Vit. B_{12} reaches the gut mainly with the bile. The present study shows that radioactivity is present not only in bile, but also in other constituents of duodenal juice. Mean radiovitamin concentration in bile (*i.e.*, duodenal juice mixed with bile) was 19 times higher than that in bilirubin-free duodenal juice.

Enterohepatic circulation. Radioactivity calculated to be excreted daily in bile was larger in control cases than the amount/day in feces (Table IV). In spite of large individual variations, the difference between mean daily biliary excretion and mean fecal excretion is statistically significant ($0.01 > p > 0.005$). Likewise, the mean of the differences is significantly ($0.005 > p$) higher than zero. In patients with deficient Vit. B_{12} absorption, on the other hand, daily excretion in bile was smaller than or equal to fecal excretion in about half of the cases (Table IV). The difference between mean biliary excretion and mean fecal excretion is not statistically significant in this group.

Discussion. Distribution of radioactivity in the body resembles that of B_{12}(12). There is evidence that radioactivity in liver derives from microbiologically active Vit. B_{12}(11). Such facts have been accepted previously(11) as indications that radioactivity really represents B_{12}. For this reason radioactivity was calculated as Vit. B_{12}. The standard deviation of B_{12}/bilirubin ratios in 39 samples obtained from 8 patients was 26% of corresponding mean values. Time lapse between 2 samples was 1 hour, 1 month. In comparing

TABLE IV. Enterohepatic Circulation of Vitamin B_{12} in Controls and in Cases with Deficient Vitamin B_{12} Absorption.

Group of subjects	Biliary excretion		Fecal excretion		No. of cases with indications of enterohepatic circulation
	No. of cases	%/day*	No. of cases	%/day*	
Normal controls	9	1.5 ± .36	10	.5 ± .23	9
Control patients	5	1.4 ± .59	6	.5 ± .16	5
Total controls	14	1.5 ± .31	16	.5 ± .17	14
Cases with deficient B_{12} absorption	11 (12)	.9 ± .31 (.9 ± .30)	12 (13)	.3 ± .05 .4 ± .07	6 (6)

* % of inj. dose *minus* loss during 1st-4th day.

Means ± stand. errors of mean.

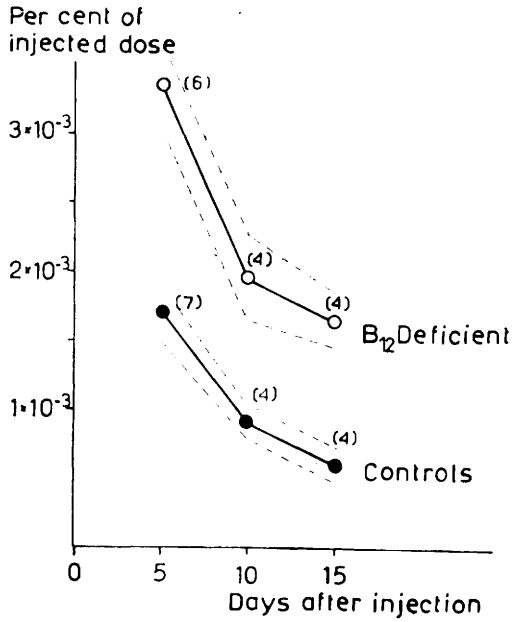

FIG. 1. Serum radioactivities after radiovitamin administration. Differences between controls and B_{12} deficient cases are statistically significant; $p < 0.005$ (15 days), $0.5 > p > 0.02$ (5 and 10 days). ——— means, – – – – – stand. error. Figures in parentheses indicate No. of samples.

controls with patients with B_{12}-deficiency, the difference in age and sex should be taken into account.

Vit. B_{12} retention. Previous studies on retention of Vit. B_{12} in patients with B_{12}-deficiency as compared to controls have yielded controversial results(11,13,14), possibly due to composition and size of control materials (14) or to therapeutic amounts of B_{12} given to pernicious anemia patients prior to experimental period(11). The present data suggest that, under present experimental conditions, more B_{12} may be retained by patients with Vit. B_{12}-deficiency. At any rate, more of the radio-B_{12} in the body is in the serum of these patients than in controls, indicating that in B_{12}-deficiency serum B_{12} might constitute a larger part of total body B_{12} than under normal conditions. Since the amount retained in serum is very small compared to B_{12}-binding capacity, even significant differences in the latter(13,15) are unlikely to explain differences in retention.

Excretion routes. Previous studies in rats with ligated common bile ducts(3) and in dogs with biliary fistulae(1) indicate that Vit. B_{12} can reach the gut by another route besides the biliary one. The results can be confirmed by present investigations in man under more physiological conditions. It appears probable that the major part of non-bilirubin-containing duodenal juice consists of pancreatic juice. This could be consistent with the observation of a high radioactivity in the pancreas of a pig receiving injections of radiovit. B_{12}(7).

Enterohepatic circulation. The fact that calculated biliary excretion of radioactivity is consistently and considerably higher than fecal excretion favours the concept of an enterohepatic circulation. If estimated daily bilirubin production, and thus daily B_{12} excretion, are too high this could simulate an enterohepatic circulation. However, the mean B_{12}/bilirubin ratio in controls was 35 $\mu\mu$g radio-B_{12}/mg bilirubin and mean daily fecal excretion in the same cases was 2000 $\mu\mu$g radio-B_{12}/day. If no enterohepatic circulation were present, this would correspond to a mean daily excretion of bilirubin of less than 60 mg/day, which is impossible. Finally, in one case of pernicious anemia in relapse (case No. 13) more radioactivity was actually collected by duodenal lavage within an hour than was excreted/day in the feces. Duodenal lavage was performed after completing feces collection. No attempts to express quantitatively mean daily excretion of B_{12} to the gut by other routes than the biliary are justified by present studies since it is difficult to estimate the daily volume of such excreta. However, existence of this extra-biliary excretion implies that the calculated amount of radioactivity reaching the upper part of the intestine is minimal.

Summary. 1) After parenteral administration of radiovit. B_{12}, radioactivity reaches the gut mainly with the bile, but other components of duodenal juice also contain radioactivity. 2) The calculated daily biliary excretion of radioactivity in controls is invariably larger than daily fecal excretion, suggesting enterohepatic circulation. In cases with defective B_{12}-absorption, there are indications of an enterohepatic circulation in only about half of the cases. 3) A higher percentage of radio-

vitamin administered is retained in serum, and a numerically lower percentage is excreted/day in urine, bile, and feces of patients with B_{12}-avitaminosis than in controls. These facts suggest that more B_{12} is retained in states of deficiency.

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Effect of Relaxin on Mammary Gland Growth in Female Mice.* (25068)

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In a previous paper it was reported that relaxin in synergism with estrogen stimulated lobule-alveolar growth of mammary gland of intact and castrate male mice pretreated with diethylstilbestrol to stimulate extensive duct growth(1). The extent of gland development induced experimentally was checked by whole mounts of the glands and by determination of total desoxyribosenucleic acid (DNA) content of mammary glands. The present report concerns the synergistic role of relaxin and estrogen in stimulating growth of the lobule-alveolar system of intact female, ovariectomized, and ovariectomized-hysterectomized mice. For comparison, the type of growth and DNA content of mammary gland of comparable mice injected with estrogen and progesterone are presented.

Procedure. Female virgin albino mice weighing approximately 28 g were fed a commercial mouse feed. The single or concomitant doses of relaxin, estradiol benzoate, and progesterone were injected subcutaneously on the back in 0.1 ml of olive oil daily for 10 days. On 11th day mammary glands were removed. Seven glands (4 posterior and 3 anterior) were analyzed for DNA by Webb and Levy method(2), and 3 anterior glands were prepared for whole mounts for morphological examination. Relaxin‡ was washed with anhydrous ether to remove possible progesterone contamination, dissolved in small amount of distilled water, lyophilized and suspended in olive oil by homogenization.

In one group the ovaries were removed whereas in a second group both ovaries and uteri were removed. Experiments were started 12 days after surgery.

Results. Intact female mice injected with estradiol benzoate 0.75 μg daily had mammary glands containing a mean of 2.541 mg

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