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Metabolic Studies of Vitamin B_{12} (Depinar) (24886)

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When a large dose of Vit. B_{12} is given parenterally, a major portion is excreted in urine and therefore is not available for metabolic functions. Hence, it is desirable to explore any preparation containing cyanocobalamin which reduces such rapid excretion. This report presents evidence that both in experimental animal and man, Vit. B_{12} administered by either intraperitoneal or intramuscular route as its tannate derivative (Depinar) is excreted much more slowly in urine and maintained at significantly higher levels in blood than when B_{12} is given in aqueous form. Data are presented to demonstrate that Vit. B_{12} Co^{60}-tannate administered to the rat results in increased organ content of the radioactive component and presumably due to Vit. B_{12} molecule itself(1).

Methods. Animal experiments. Ten male adult rats of McCollum strain were divided into 2 groups of approximately equal average weight. One group was administered 0.84 μg of Co^{60} B_{12}-tannate (Co^{60} "Depinar"). The second received 0.84 μg of Co^{60} B_{12} (both of 180 μc/mg specific activity) intraperitoneally. Animals were housed in individual metabolism cages. Urine was collected daily under toluene. Urine samples and washings were evaporated to 50 ml on steam bath in graduated 100 ml brown bottle. After 20 days the 10 rats were sacrificed. Liver, kidney and intestines were preserved in frozen state and subsequently were individually homogenized in stainless steel cup on Waring Blender. Radioactivity of urine samples and of total organ homogenates was measured by well scintillation counter. *Human Vit. B_{12} serum levels.* Male, healthy adults were administered either 500 μg of crystalline Vit. B_{12} or 500 μg of Vit. B_{12} as its tannate derivative (Depinar). Blood was obtained by venipuncture prior to administration as well as 8, 24, 72, 144, 216, 504 and 672 hours (4 weeks) after administration of Vit. B_{12} or "Depinar." Serum obtained by centrifugation was stored in frozen state until assayed for Vit. B_{12} by microbiologic assay with *Lactobacillus leichmannii* ATCC 4797 according to method of Skeggs and co-workers(2). All urine was collected for duration of study and aliquots were analyzed at 6, 24, 72, 96, 124, 144 and 168 hours by above technic. *Materials.* "Depinar" is a Vit. B_{12} tannic acid derivative prepared and kindly supplied by Thompson & Hecht of Armour & Co. Radioactive Vit. B_{12}, Co^{60} Vit. B_{12} (Co^{60} B_{12}) preparations employed for these tests were kindly supplied by Dr. Chas. Rosenblum of Merck & Co. Specific activity of 180 μc/mg. "Resting" cells of *Lactobacillus leichmannii* ATCC 4797 was described previously(3).

Results. In Fig. 1, urinary excretion of

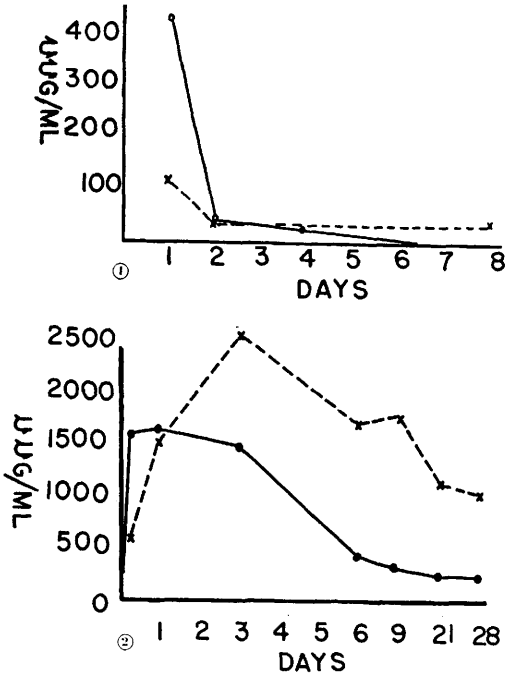

FIG. 1. Urinary excretion of radioactive vit. B_{12} and "Depinar" administered to rats intraper. Each point represents avg value of 5 rats receiving 0.84 μg vit. B_{12} or "Depinar"/100 g body wt. ○—○ Vit. B_{12} Co^{60}. ×—× "Depinar" Co^{60}.

FIG. 2. Serum vit. B_{12} levels of human subjects receiving cyanocobalamin or its tannate derivative "Depinar." ●—● Vit. B_{12}. ×—× "Depinar."

Co^{60} Vit. B_{12} and Co^{60} "Depinar" is plotted as function of time. Noteworthy is the initially large difference between Co^{60} Vit. B_{12} and Co^{60} "Depinar" treated groups. Each value represents average Vit. B_{12} level expressed in mμg/daily urine sample of 5 rats administered 0.84 μg of vitamin/100 g body weight intraperitoneally. The Co^{60} Vit. B_{12} group excreted an average of over 400 mμg during first day's collection period, whereas Co^{60} "Depinar" group slightly over 100 mμg. The second day urinary output of radioactive Vit. B_{12}, as measured as Co^{60} activity, was essentially equal for both groups. However, the Co^{60} "Depinar" treated group leveled off at approximately 35 mμg and remained at that level until at least the 8th day. The Co^{60} Vit. B_{12} treated group excretion declined sharply so that very little radioactivity appeared in urine after 4th day.

In Table I, radioactivity in selected rat organs, namely: liver, kidney and intestine, is tabulated. Comparison is made of these organs removed 20 days after intraperitoneal administration of 0.84 μg of Co^{60} Vit. B_{12} or Co^{60} "Depinar"/100 g body weight. These organs were removed from the same 10 animals employed for the urinary excretion study. Each value represents average of 5 animals.

In Group A (Vit. B_{12} treated animals) average body weight 215 g; radioactivity of the 3 organs analyzed was consistently lower than Co^{60} "Depinar" group (Group B). The greatest differences observed were in kidney and intestines; these levels being 106.2 $\pm$ 19 and 245.6 $\pm$ 4.2 mμg/total kidney and 29.4 $\pm$ 5 and 69.6 $\pm$ 7 mμg/total intestines, respectively for Co^{60} B_{12} and Co^{60} "Depinar" groups. Liver and kidney were chosen as target organs since Harte, *et al.*(4) previously demonstrated persistence of significant storage of Vit. B_{12} at least to several months in these 2 organs and easily detectable amounts after 20 days.

In Fig. 2, Vit. B_{12} serum levels of human subjects receiving cyanocobalamin (Vit. B_{12}) or its tannate derivative ("Depinar"), are plotted against hours after administration. With intramuscular administration of Vit. B_{12}, serum level, as measured by modification (5) of method of Skeggs, *et al.*(2) employing *L. leichmannii* ATCC 4797, reached maximum level of approximately 1700 $\mu\mu$g/ml at end of first day. After 3 days, the level was slightly

TABLE I. Radioactivity in Organs of Rats Administered Co^{60} Vit. B_{12} and "Depinar."

Group	Treatment	Body wt (g)	mμg vitamin activity/total organ		
			Liver	Kidney	Bowels
A	Vit. B_{12}	215	85.8 $\pm$ 28.8*	106.2 $\pm$ 19	29.4 $\pm$ 5
B	"Depinar"	200	100.6 $\pm$ 6.4	245.6 $\pm$ 42	69.6 $\pm$ 7

Organs removed 20 days after intraper. administration of 0.84 μg/100 g body wt of Co^{60} tagged vit. B_{12} or "Depinar."

Each value avg of 5 animals (rats/serum). * Stand. dev.

TABLE II. Excretion of Vit. B_{12} Activity in Urine of Subjects Receiving Intramuscular Cyanocobalamin or Its Tannate Derivative.

Group	Treatment	Hr after administration* 6	24	72	96	120	144	168
A	500 μg cyanocobalamin	300	72	—	—	—	—	—
B	500 μg vit. B_{12}-tannate	—	—	.79	.472	.32	.363	.104

— = No appreciable vit. B_{12} activity. 5 subjects were used in each study.
* Expressed as total γ/B_{12}.

less than 1500 $\mu\mu$g/ml, then it fell rapidly to basal level (380 $\mu\mu$g/ml). At 8 hours, a near maximum serum level was obtained with Vit. B_{12} (1600 $\mu\mu$g/ml) which was in marked contrast to serum level of 550 $\mu\mu$g at 8 hours of subjects given "Depinar." This was approximately equal to basal level. With intramuscular administration of "Depinar" there was no large increase in serum level of Vit. B_{12} activity until the end of first day (1600 $\mu\mu$g/ml). Vit. B_{12} activity in serum increased until end of third day (over 2500 $\mu\mu$g/ml) and started to decline so that by end of sixth day, when the B_{12} group had already returned to basal level, the "Depinar" treated group remained at high serum level (1600 $\mu\mu$g/ml). This level did not change significantly during next 3 days. At the end of 21 days, serum Vit. B_{12} activity was still elevated (approximately 1100 $\mu\mu$g/ml) and even after 28 days approximately 850 $\mu\mu$g/ml at which time experiment was terminated. Vit. B_{12} activity of first day serum samples of both groups was approximately equal (1700 $\mu\mu$g/ml). At this time, mean serum value of soluble Vit. B_{12} group began to decrease, whereas "Depinar" groups continued to increase markedly for the next 48 hours. At end of 3 days and until ninth day, there was a constant and sustained difference of approximately 1100 $\mu\mu$g/ml between the 2 groups.

Table II tabulates appearance of Vit. B_{12} activity in urine of human subjects receiving intramuscular cyanocobalamin or its tannate derivative "Depinar." Subjects receiving 500 μg cyanocobalamin, Group A, excreted significant amounts of Vit. B_{12} in urine the first 72 hours. Vit. B_{12} activity appeared in the 6 hour urine sample, persisted until 72nd hour, then no significant activity could be detected by microbiological technics. In Group B, the first urine specimens of subjects receiving "Depinar" equivalent to 500 μg cyanocobalamin contained no appreciable Vit. B_{12} activity. Such activity appeared in appreciable amounts in the 72-hour sample and persisted until at least 168th hour after administration, when collection of urine samples was discontinued.

Discussion. When Vit. B_{12} is administered at 500 μg or 1000 μg level intramuscularly to man almost all vitamin activity appears quickly in urine(6). In view of the use of other biological complexes to enhance body retention and increase blood levels of the substance in question (over the soluble form), "Depinar" was prepared. This is an insoluble complex of Vit. B_{12} and tannic acid which breaks down at slow rate to free Vit. B_{12} in the body. It was believed that if Vit. B_{12} could thus be provided in depot form so that it would be excreted at a slower rate and thus provide a continuous source of this vitamin for metabolic function, conservation and fuller utilization of Vit. B_{12} could be achieved.

Our data offer evidence that prolonged Vit. B_{12} activity was obtained. Table I, Fig. 1 and 2 indicate that urinary output of Vit. B_{12} activity is markedly decreased initially and sustained for considerably longer period of time when Vit. B_{12} is administered as "Depinar."

In view of prolonged retention of Vit. B_{12} when offered as its tannate derivative, it would be of interest to evaluate this Vit. B_{12}-tannate complex in management of hyperglycemia and glycosuria induced by prolonged injection or hypersecretion of cortisone. In diabetics with retinopathy, it has been reported(7) that such patients excrete significantly more of test intramuscular dose of Vit. B_{12} than nondiabetic subjects and secrete significantly more cortisone than diabetics without retinopathy. These patients likewise have elevated serum Vit. B_{12} levels. Increased urinary excretion and serum levels may well be the re-

sult of hypertrophy of the adrenals with resultant decrease in ability of tissue to retain Vit. B_{12}. Further investigation is necessary to ascertain whether "Depinar" would be retained to clinical advantage of these patients.

Conclusions. 1. When rats were administered "Depinar" and soluble Vit. B_{12} both tagged with Co^{60}, the group receiving the tannate derivative of Vit. B_{12} had elevated radioactivity in liver and markedly elevated radioactivity in both kidney and intestines, assumed to be due to B_{12} Co^{60}-tannate complex. 2. This material was likewise excreted much more slowly in urine of both rats and man than aqueous Vit. B_{12}. 3. Serum Vit. B_{12} activity of normal human subjects administered "Depinar" was significantly greater and sustained a much longer time than those receiving soluble Vit. B_{12}. 4. Whether Vit. B_{12} administered as tannate derivative is of any metabolic benefit over the soluble form to patients requiring supplements of this vitamin is yet to be investigated.

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Build-Up and Regression of Inhibitory Effects of Cholic Acid on *in vivo* Liver Cholesterol Synthesis.* (24887)

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That certain bile acids inhibit *in vivo* both synthesis and degradation of rat liver cholesterol has been demonstrated(1,2). Whenever dietary cholic acid inhibited liver cholesterol synthesis, there was a small but very significant increase in liver cholesterol level. It is well-known that increases in liver cholesterol levels are attended by decreases in liver cholesterol synthesis rates(3,4,5). Therefore, does cholic acid directly inhibit rate of liver cholesterol synthesis or does it initiate a change in level of liver cholesterol which in turn brings about the inhibition? The present studies were undertaken to clarify the mechanism of action of cholic acid by determining the order of changes in bile acid, cholesterol and phospholipid concentrations, in relation to changes in *in vivo* liver cholesterol synthesis rates. These studies have been made during early build-up and regression phases of effects of cholic acid.

Methods. Exp. A. Thirty-two Sprague-Dawley female albino rats weighing 250-280 g, were maintained *ad lib.* on basal diet consisting of 97% Rockland rat ration and 3% corn oil for 2-wk observation. The animals were then divided into 2 groups. Controls continued on basal diet *ad lib.*, while treated group received basal diet supplemented with 0.5% cholic acid. During experimental period, controls and treated animals consumed 17.8 $\pm$ 3.10 and 17.4 $\pm$ 1.86 g/day respectively. After dietary periods of 12 hours, 1, 2, and 3 days, 4 control and 4 treated rats received intraperitoneal injections of 50 μc of acetate-1-C^{14}. Six hours later the rats were sacrificed and samples of blood and liver removed for analysis. *Exp. B.* Thirty rats similar to those used in Exp. A were maintained on same basal diet. The animals were

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