Retention of Injected Hydroxocobalamin Versus Cyanocobalamin Versus Liver Extract-Bound Cobalamin

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PRIOR studies have indicated that intramuscularly injected hydroxocobalamin (500 or 1,000 μg.) is retained longer at the site of injection,[1,2] produces a more sustained rise in serum cobalamin level[1-5] and results in less short-term loss of cobalamin in the urine[1,3-5] than do injections of an identical quantity of cyanocobalamin. The present studies were undertaken in order to determine whether similar, relatively more sustained, rises in serum cobalamin level and similar lower cobalamin output in urine follow 100 μg. injections of these agents in normal subjects and in treated subjects with vitamin B_{12} deficiency due to inadequate intrinsic factor secretion. These parameters were also measured after the intramuscular injection of a solution of purified liver extract of activity equivalent to 100 μg. of cyanocobalamin.

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MATERIALS

Cyanocobalamin was supplied by the Merck Institute for Therapeutic Research as Redisol (0778V) containing 1,000 μg. of cyanocobalomin per ml. of solution. This stock solution was diluted 1:10 in sterile saline solution to provide 100 μg. in each ml. for injection. Assay of this material with Euglena gracilis showed its potency for this organism to be that of 103.6 μg. of cobalamin per ml. of solution.

Hydroxocobalamin was supplied by the Merck Institute for Therapeutic Research as Solution K-370 (C-1778) containing 1,000 μg. of hydroxocobalamin per ml. This stock solution was diluted 1:10 in sterile saline solution to provide for injection a solution containing 100 μg. per ml. Microbiologic assay of this material with E. gracilis showed 79.6 μg. of cobalamin activity per ml. of solution.

Liver extract cobalamin was provided by Eli Lilly and Co. as Solution Liver Extract, Purified, containing vitamin B_{12} activity equivalent to 20 μg. of cyanocobalamin per ml. This material contained 21 μg. of cobalamin activity per ml. for E. gracilis by microbiologic assay and consequently required 4.76 ml. for an injection equivalent to 100 μg. of cyanocobalamin. For convenience, 5 ml. was injected.

Subjects were eight healthy adult male physicians, one patient admitted for evaluation of a possible carcinoma of the esophagus (no pathologic condition was found) and five subjects with inadequate intrinsic factor secretion who, until one month before participating in the present study, had been receiving 30 μg. of cyanocobalamin intramuscularly monthly. These last five subjects included three with pernicious anemia in remission, one who had had a subtotal gastrectomy and one who had had a total gastrectomy.

TABLE

Effect of a Single 100 μg. Intramuscular Injection of Cyanocobalamin,

Subject	Date (1960)	Serum Cobalamin Level (μμg./ml.)			
		Baseline	1 day	2 days	3 days
					Cyanoco
V. H.*	3/3	815			
		(717)			
R. Z.*	3/17	915	600		
		(621)	(1,036)		
G. S.*	3/17	307	1,144		
			(1,304)		
D. A.*	5/18	609	1,328	1,424	1,856
		(504)	(1,049)	(794)	(867)
L. B.*	5/18	580	1,200	888	628
		(379)	(866)	(644)	(520)
M. K.*	5/18	466	1,464	1,094	956
		(428)	(724)	(647)	(671)
A. L.†	2/24	153	783		
		(181)	(582)		
					Hydroxo
V. H.*	3/17	947	2,336		
		(1,310)	(1,917)		
R. Z.*	3/3	468	1,520		
		(415)	(1,440)		
J. K.*	5/18	531	2,452	1,528	1,128
		(467)	(2,067)	(1,178)	(904)
C. M.*	5/18	818	2,432	1,668	1,395
		(770)	(2,353)	(1,671)	(1,422)
W. G.*	5/24	616	1,792	1,440	1,394
		(875)	(1,974)	(1,520)	(1,317)
J. D.‡	3/17	1,328	2,320		
			(2,390)		
P. M.§	4/5	400	2,485		
		(398)	(1,390)		
S. L.†	2/24	235	1,867		
		(250)	(2,200)		
W. M.†	3/21	367	1,573		
			(1,248)		
					Liver Extract
M. K.*	10/4	443	1,387	1,083	683
		(473)	(1,005)	(586)	(705)
J. K.*	10/4	424	693	501	469
		(388)	(598)	(512)	(573)
L. B.*	10/4	445	1,344	800	821
		(390)	(898)	(778)	(622)

* Normal subjects.
† Pernicious anemia in remission.
‡ Subtotal gastrectomy.
§ Gastrectomy.

Hydroxocobalamin or Liver Extract Cobalamin on Cobalamin Serum and Urine

E. gracilis (L. leichmannii)				Urine Cobalamin Excretion (μg. total) E. gracilis (L. leichmannii)		
7 days	14 days	21 days	28 days	1st Day	2nd Day	3rd Day
balamin						
943	947			59.4		
(789)	(1,310)			(50.5)		
1,080	928			105.3		
(544)	(597)			(63.5)		
704	530			71.8		
	(206)			(56.9)		
400	800	648	437	75.6	2.1	3.5
(743)	(654)	(607)	(585)		(0.15)	(0.07)
328	458	656	916	90.9	2.3	1.9
(510)	(445)	(403)	(419)	(67.0)	(0.04)	(0.01)
440	444	596	461	82.6	2.1	2.3
(557)	(425)	(486)	(451)		(0.72)	
275	271	285	461	36.2		
(352)	(250)	(258)	(363)	(41.9)		
cobalamin						
1,325	1,115			34.5		
(1,033)	(826)			(8.5)		
814	915			9.9		
(644)	(621)					
341	584	728	408	24.2	1.5	1.9
(658)	(547)	(612)	(458)		(2.2)	
731	840	836	544	77.2	4.5	5.6
(1,012)	(927)	(877)	(1,143)	(8.2)	(0.12)	(0.05)
944	692	597	932	7.8	12.3	2.3
(1,126)	(865)	(960)	(894)	(7.6)	(0.1)	(0.04)
1,311	1,025			18.6		
				(9.1)		
				11.7	5.0	5.3
(598)	(543)			(9.3)	(0.2)	(0.2)
483		387	611	15.5		
(578)		(362)	(339)	(10.5)		
				11.0		
				(7.0)		
Cobalamin						
512	424	483	688	22.1	0.25	0.21
(512)	(485)	(474)	(480)		(0.10)	
272	430	387		2.65	0.26	0.23
(498)	(466)	(3,150)	(478)	(1.59)	(0.03)	(0.03)
323	328	595	374	9.6	0.21	0.21
(538)	(424)	(402)	(372)		(0.01)	

Microbiologic assays for cobalamin activity were made at the Thorndike Memorial Laboratory using Euglena gracilis var. bacillarus and the methodology of Lear et al.[6] with various trivial modifications.

Microbiologic assays for cobalamin activity for Lactobacillus leichmanii were made at the Merck Institute for Therapeutic Research, using the methodology of Skeggs et al.[7]

Each subject was given either 100 μg. of cyanocobalamin or hydroxocobalamin in 1 ml. of solution by intramuscular injection into the deltoid muscle, or 100 μg. of liver extract cobalamin in 5 ml. of solution by intramuscular injection into the gluteus maximus. Samples of blood were obtained just prior to injection and one, two, three, seven, fourteen, twenty-one and twenty-eight days after injection. Subjects were not fasting at the time blood samples were obtained. Blood samples were allowed to clot for approximately three hours, after which they were centrifuged and the serum was aspirated into acid-washed glass tubes and kept frozen at minus 20°c. until assayed. Urine was collected for twenty-four-hour periods using toluene as a preservative. Aliquots were then kept frozen at minus 20°c. until assayed. Serum and urine samples were coded prior to assay and the code was not broken until after the results had been recorded. Five subjects (V. H., R. Z., L. B., J. K. and N. K.) each received injections of two different agents, as indicated in Table 1, which also indicates the time interval between injections. All other subjects received one injection of a single agent.

RESULTS

Table 1 presents the data obtained.

Twenty-four hours after injection, the rise in serum cobalamin level was greater after hydroxocobalamin than after cyanocobalamin or liver extract cobalamin. This relatively greater increment in serum cobalamin level after the injection of hydroxocobalamin was sustained for several days, but then disappeared gradually. The increment in serum cobalamin levels produced by all three agents disappeared gradually over several weeks so that by twenty-eight days after injection serum cobalamin levels had returned to the initial values (Table 1).

In the first twenty-four hours after injection, most of the cyanocobalamin, considerably less of the hydroxocobalamin, and still less of the liver extract cobalamin was excreted in the urine. During the second and third twenty-four-hour periods further small increments of cobalamin were excreted in the urine following injection of the cyanocobalamin and hydroxocobalamin forms, but little appeared in the urine of the subjects given liver extract cobalamin.

In general, after injection of hydroxocobalamin, serum cobalamin levels were relatively higher and urinary excretion of cobalamin relatively lower in subjects who lacked adequate intrinsic factor secretion than after injection of cyanocobalamin. This was also true for normal subjects.

It is possible that the relatively large excretion of cobalamin in the urine of one subject (V. H.) after the hydroxocobalamin injection was due to the fact that he received this injection only two weeks after receiving an injection of cyanocobalamin (Table 1). It should be noted that in another subject (R. Z.), who received the same two agents separated by the same interval of time but in the reverse order, almost all of the injected cyanocobalamin was excreted within twenty-four hours (Table 1).

COMMENTS

The present studies support the findings of others (using larger doses)[1–5] that there is a more sustained rise in the serum cobalamin level of normal subjects following injection of hydroxocobalamin than of cyanocobalamin. This also appears to be true in subjects with vitamin B_{12} deficiency due to inadequate intrinsic factor secretion who had been maintained in remission by monthly injections of cyanocobalamin. During the first three days after the injection of 100 μg. of material in such patients, as in normal subjects, the loss of cobalamin in the urine is much greater after cyanocobalamin than after hydroxocobalamin.

The lowest urinary loss of cobalamin followed injection of the equivalent of 100 μg. of cobalamin in 5 ml. of liver extract. Whether this is due to slower release from the site of injection due to the greater volume of the mass injected (5 ml. given as a single injection to one subject and as a 2.5 ml. injection in each buttock to the other two subjects) as compared to the volume injected (1 ml.) of the hydroxocobalamin or cyanocobalamin, or whether this retention is

related to the nature or binding of the liver extract cobalamin itself remains to be determined.

The lower urinary excretion rates of cobalamin and the higher serum cobalamin levels which exist for a number of days after injection of hydroxocobalamin but not after cyanocobalamin injection (although the significance of this findings in therapeutic terms is unknown) probably result from the greater retention of hydroxocobalamin at the site of injection, presumably as a result of its greater binding affinity.[8,9] Earlier workers[10] also have found relatively great retention of cobalamin supplied in liver extract, as manifested by low urinary excretion of cobalamin after injection of this agent, as compared to cyanocobalamin. There is reasonably good evidence[11] that liver extract contains predominantly hydroxocobalamin with small amounts of sulfitocobalamin, and that the cobalamin is not protein-bound.

It is interesting that, after injection of the cobalamin forms, the cobalamin levels measured by E. gracilis tend to be higher than by L. leichmannii. This is especially true of the urine samples. One possible explanation for this may be that a variable amount of the material injected may be excreted in the urine as a cobalamin more active for E. gracilis than for L. leichmannii. Another possible explanation is that the cobalamin standard for E. gracilis may have been low, or that for L. leichmannii may have been high. Both are repeatedly checked, however.

SUMMARY

It is demonstrated in normal subjects and in subjects with vitamin B_{12} deficiency maintained in remission by monthly injections of cyanocobalamin that the rise in serum cobalamin levels is more sustained after the intramuscular injection of 100 μg. of hydroxocobalamin, than after the injection of 100 μg of cyanocobalamin or liver extract cobalamin of equal microbiologic value. Urinary excretion of cobalamin is considerably greater for several days following injection of cyanocobalamin than following injection of hydroxocobalamin or liver extract cobalamin. However, because the serum levels at convenient intervals for therapeutic injections, such as two or four weeks, are not significantly greater after the injection of hydroxocobalamin, any therapeutic advantage of this agent remains to be shown.

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