Serum Levels of Vitamin B_{12} Following Intramuscular Injection of a Depot Preparation

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In an earlier study[1] we presented clinical evidence showing that cobalamin administered intramuscularly in oil with aluminum monostearate was excreted into the urine over a prolonged period. This was in contrast with cobalamin administered in an aqueous medium. In the latter instance essentially all of the administered vitamin appeared to be recovered in the urine within twenty-four hours.

In view of the interesting effect of the oil-aluminum monostearate combination in prolonging the availability of the vitamin to the organism following injection, it appeared worthwhile to determine the extent to which the blood level of the vitamin might reflect the influence of the depot vehicle. Data on this point from studies with dogs are presented here.

METHODS

Control blood samples were taken from two groups of four dogs each apportioned as equally as possible on a weight basis. Following this, the vitamin in sterile water (Ducobee® "1000," 1,000 μg. vitamin B_{12} per ml.) or in sesame oil with 2 per cent aluminum monostearate (Ducobee Depot, 1,000 μg. vitamin B_{12} per ml.) was administered to each of the dogs intramuscularly (rectus femoris) in a dose of 15 μg. per kg. On a weight basis this amount approximated that given in the earlier clinical trial. Blood samples were then taken at one, two, three, four, seven, nine, eleven and fifteen days.

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Blood samples, 8 to 10 ml. from the jugular vein, were taken from the dogs the morning before feeding so that they represent fasting samples. They were chilled for one hour to promote shrinking of the formed clot. They were then centrifuged for a half hour and the clear serum was taken for assay.

For estimation of the cobalamin content of serum, 1 to 2 ml. aliquots were diluted 1:5 with pH 4.6 acetate buffer following the procedure of Gaffney et al.,[2] steamed for a half hour, cooled to room temperature and centrifuged. An aliquot was diluted 1:2 with 0.33M Na_2HPO_4 to bring it to neutrality and then further diluted as needed for microbiologic assay by the U.S.P. procedure using L. leichmannii as the test organism. The extent of growth of the organism was based upon titration with 0.05N alkali of the lactic acid produced over a seventy-two–hour incubation period at 37°C. A comparison of the lactic acid production in the tubes containing the serum samples with the acid in the tubes containing the reference vitamin B_{12} permitted calculation of the vitamin B_{12} content of the serum samples.

To determine whether the depot preparation would exercise any untoward effect upon continued injection, it was administered twice weekly to two dogs (beagle) and two monkeys (rhesus) for a total of forty-six injections to each over a period of 170 days. Each of the four extremities were utilized in rotation. Hematologic studies were carried out on the animals before medication was started and at six-week intervals thereafter. At the end of the injection period, all of the animals were

TABLE I

Serum Vitamin B_{12} Levels in Two Groups of Four Dogs Each Following the Intramuscular Administration of the Vitamin in Water or in a Depot Oil and Aluminum Monostearate Preparation

Determination	Dog No.	Weight (kg.)	Serum Vitamin B_{12} Following Its Intramuscular Injection (mμg. per ml.)							
			Initial	Day						
				1	2	3	4	7	9	11	15
Aqueous Preparation											
	1	7.8	0.33	1.94	0.735	0.72	0.62	0.56	0.41	0.53	0.475
	2	7.0	0.33	1.69	0.675	0.51	0.64	0.42	0.39	0.39	0.32
	3	13.0	0.22	1.88	0.92	0.66	0.52	0.38	0.36	0.32	0.21
	4	12.6	0.33	1.79	0.72	0.64	0.54	0.36	0.33	0.34	0.23
Average			0.30	1.82	0.76	0.63	0.58	0.43	0.37	0.40	0.31
Standard error			±0.03	±0.06	±0.17	±0.04	±0.03	±0.04	±0.02	±0.05	±0.06
vs. initial			...	...	...	...	p = <0.001	p = 0.04	p = 0.07	p = 0.11	Not significant
Depot Preparation											
	5	5.8	0.36	2.50	1.97	1.75	1.62	0.765	0.71	0.785	0.54
	6	9.8	0.30	3.60	1.57	1.47	1.32	0.85	0.795	0.775	0.40
	7	11.6	0.33	2.50	1.62	1.10	0.95	0.46	0.34	0.35	0.22
	8	13.8	0.24	3.80	2.82	2.00	1.57	1.20	0.71	0.55	0.34
Average			0.31	3.10	2.00	1.58	1.22	0.82	0.64	0.62	0.38
Standard error			±0.02	±0.35	±0.29	±0.19	±0.14	±0.15	±0.10	±0.10	±0.07
vs. initial			...	...	...	...	...	p = 0.012	p = 0.014	p = 0.02	p = 0.4
vs. aqueous			...	...	...	p = 0.0014	p = 0.0025	p = 0.04	p = 0.03	p = 0.09	Not significant

examined grossly, and one of the monkeys was sacrificed for tissue section studies. The injection sites were imbedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

RESULTS

The results of the microbiologic analyses of the serum samples for vitamin B_{12} following the intramuscular injections of each of the preparations into the dogs are summarized in Table I. The average pretest serum vitamin B_{12} levels of 0.30 to 0.31 mμg. per ml. fall into the range of those noted for human beings by Chalmeras and Shinton[3] by assay with Euglena gracilis[4] and by Thompson and Hecht[5] by the procedure of Gaffney et al.[2]

From the average response to the administration of each of the vitamin B_{12} source preparations summarized in Table I and in Figure 1, it is seen that the dogs exhibited markedly elevated serum vitamin B_{12} levels. Those given the vitamin in an aqueous menstruum clearly had elevated levels through four days at which time their serum vitamin B_{12} values averaged 0.58 mμg. per ml. Since two or three of the four dogs of the group had serum vitamin B_{12} levels below 0.40 mμg. per ml. after four days, the significance of the average being above the pretest level may be questioned ($p = 0.04$ or higher).

It is interesting that the serum vitamin B_{12} levels of the four dogs given the vitamin as a depot preparation not only exhibited elevated levels over a longer period, between eleven and fifteen days, but also that the levels at-

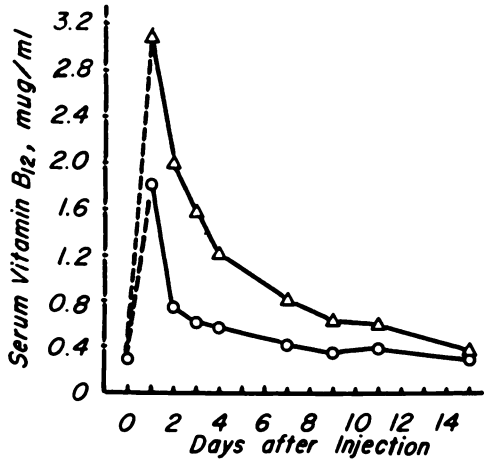

FIG. 1. Average serum vitamin B_{12} levels in dogs following the intramuscular injection of the vitamin in an aqueous (○—○) or depot (Δ—Δ) preparation.

tained averaged two to three times those of the dogs given the vitamin in an aqueous medium. This may reflect the fact noted earlier[1] that excess vitamin is quickly excreted following its administration in water. Release of the vitamin administered in oil containing aluminum monostearate took place more slowly as shown by prolonged urinary excretion. This is in accord with the above result which demonstrates elevated serum levels for a period of time about threefold longer than that following administration of the vitamin in the usual aqueous medium.

Of interest equal, perhaps, to the relative rates of excretion following the administration of the vitamin in an aqueous or depot preparation is the correlation between the data of this study and those of Buckwalter and Dickison.[6] Studying blood levels following the administration of procaine penicillin in oil and aluminum monostearate, these investigators noted that the vehicle supported elevated blood levels of the antibiotic for about twelve days. This is in striking agreement with the data given in Table I in which it is seen that the vitamin was clearly maintained at elevated levels for more than eleven (although less than fifteen) days.

With regard to the effects of the repeated injections in dogs and monkeys we may summarize briefly by noting that there was no apparent evidence of pain, redness or swelling at any time in any of the injection sites. No significant changes were observed in the total red and white blood cell counts, the differential counts, the hematocrit or in the hemoglobin concentration of the blood of the monkeys or the dogs. Microscopically, necrosis of muscle tissue was not seen although droplets of oil were observed in the connective tissue between the muscle bundles. Thus, it is seen that no adverse effects could be discerned and the preparation was completely tolerated as evidenced both by gross and microscopic examination of the sites of injection.

In conclusion, we may say that oil and aluminum monostearate serve as an effective depot vehicle for the intramuscular administration of vitamin B_{12} just as they do for procaine penicillin.

SUMMARY

Serum levels of vitamin B_{12} were determined in two groups of four dogs each following the intramuscular administration of vitamin B_{12} in water or in a depot oil and aluminum monostearate preparation.

Confirming earlier published observations based on the urinary excretion of the vitamin following its administration in each of these vehicles, the studies with the dogs showed that the vitamin administered in a depot form maintained elevated levels for a period about three times that following the administration of the vitamin in the usual aqueous medium.

It was noted, in addition, that the serum levels which followed the administration of the vitamin in the depot preparation were two to three times those which followed the administration of the vitamin in water.

Tolerance to injection of the preparation was established. No untoward blood or tissue effects were seen in dogs or monkeys following forty-six injections of the preparation over a 170-day period.

Accordingly, it is clear that administration of vitamin B_{12} in oil with aluminum monostearate results in prolonged and elevated serum levels of vitamin B_{12}.

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