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The absorption and excretion of [^{58}Co]cyanocobalamin by rabbits

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Rosenthal (1959) studied the absorption of orally administered [^{60}Co]cyanocobalamin in rabbits using doses of between 0·05 and 0·15 μg/kg body-weight. He concluded that the amount of cyanocobalamin absorbed is limited and that the mechanism of absorption is similar to that in man. Our earlier studies (Simnett & Spray, 1961, 1965) suggested that normal rabbits ingest daily up to 100 μg cyanocobalamin through coprophagy. It therefore seemed of interest to study the absorption and excretion of cyanocobalamin with larger doses than those used by Rosenthal (1959). Our results indicate that rabbits can absorb much larger amounts of cyanocobalamin than other species so far studied.

EXPERIMENTAL

Animals and diet. Nineteen rabbits, ten males and nine females, aged between 9 and 12 months, were used for the experiments. Some animals were used more than once, after all the isotope from previous doses had been excreted. The rabbits were fed without restriction on a diet of oats and hay.

Administration of [^{58}*Co*]*cyanocobalamin and collection of samples.* For the experiments the animals were kept in separate galvanized iron cages with metal grid floors and devices for separating urine from faeces. In most experiments coprophagy was prevented by collars similar to those used in our earlier work (Simnett & Spray, 1961, 1965). The collars were put in place about 18 h before the [^{58}Co]cyanocobalamin was given, and the animals were starved overnight before administering the dose and for 6 h afterwards.

The quantities of cyanocobalamin given ranged from 0·055 to 150 μg. For doses of 0·055 and 0·1 μg, [^{58}Co]cyanocobalamin of specific activity 18 μc/μg (Radiochemical Centre, Amersham, Bucks) was used. For the larger doses, 1 μg (approximately 1 μc) of [^{58}Co]cyanocobalamin was mixed with the appropriate amount of non-radioactive cyanocobalamin (Cytamen; Glaxo Laboratories Ltd). The cyanocobalamin was administered by stomach tube in about 4 ml water and was washed into the stomach with a further 10–15 ml water. Faeces were collected for each period of 24 h until 2 successive days' faecal collections each contained less than 1% of the radioactivity in the dose; urine was collected similarly until 2 successive days' collections each contained less than 0·01% of the dose.

In a few experiments radioactivity was measured in blood, which was collected

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from an ear vein into bottles containing heparin 2, 4, 8, 11, 20, 24 and 48 h after dosing.

Measurement of radioactivity. Urine and faeces for each period of 24 h were transferred to conical waxed-cardboard containers, and water was added, if necessary, to bring the total volume in each container to about 350 ml. The radioactivity was measured in the plastic phosphor-well-counter described by Warner & Oliver (1962). The counts were compared with those from a standard containing 10% of the radioactivity in the dose. The standard was contained in the same volume as that of the samples and was kept in a similar waxed-cardboard container.

For the measurement of radioactivity in blood, 4 ml samples of blood were pipetted into flat-bottomed specimen tubes ($2 \times \frac{5}{8}$ in), which were placed in a small well-type scintillation counter in which the phosphor was a thallium-activated sodium iodide crystal. The counts from the samples of blood were compared with those from a standard containing 1% of the radioactivity of the dose in a total volume of 4 ml.

RESULTS

Excretion of isotope in faeces after oral doses of [^{58}Co]cyanocobalamin. When the amount of isotope excreted in the faeces in 24 h had fallen to less than 1 % of that in the dose, further amounts were disregarded in calculating the total quantities excreted. The effect of coprophagy on the pattern of excretion in four rabbits is shown in Table 1. When coprophagy was prevented, most of the isotope appeared in the first 2 or 3 days and the amount excreted had fallen to below 1% of the dose after 4 days in one animal and after 8 days in the other. When coprophagy was allowed, maximum excretion was not reached until the 3rd day, and the amount excreted daily did not fall below 1% of the dose until the 13th day.

In the experiments in which coprophagy was prevented (Table 2), there was a significant relationship between the amount of cyanocobalamin given and the amount absorbed. The linear regression of amount absorbed on dose was significant at the 0·1% level ($b = 0{\cdot}133$, $t = 18{\cdot}4$, $P \ll 0{\cdot}001$). Over the whole dose range the proportion of the dose absorbed varied between 7·7% and 39% (mean 16·2%), with no apparent trend attributable to changes in the size of the dose.

Urinary excretion of radioactivity after oral doses of [^{58}Co]cyanocobalamin. Amounts of isotope excreted in the urine in 24 h that were less than 0·01% of that in the dose were disregarded when the total amounts excreted were calculated. When coprophagy was allowed, the animals excreted within 24 h more than 0·01% of the dose for longer periods than when coprophagy was prevented (Table 1). In the experiments in which coprophagy was prevented, the fraction of the dose excreted in the urine varied from 0·2% to 2·8% without showing any trend due to variations in the amount of cyanocobalamin given (Table 2). There was a significant relationship between the amount excreted in the urine and the dose ($b = 0{\cdot}0195$, $t = 8{\cdot}58$, $P < 0{\cdot}001$).

Radioactivity in blood after oral doses of [^{58}Co]cyanocobalamin. This was investigated in only four rabbits (nos. 1–4), which had been used previously for the experiment recorded in Table 1. Coprophagy was prevented, and the animals received 25 μg

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Table 1. *Excretion of isotope (as % of dose) in the faeces and urine of normal rabbits after oral doses of 25 μg (1 μc) [58Co]cyanocobalamin*

Days after dose	Coprophagy allowed				Coprophagy prevented			
	Rabbit no. 1		Rabbit no. 2		Rabbit no. 3		Rabbit no 4	
	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
1	8·8	0·03	7·6	0·05	49·2	0·02	27·7	0·23
2	10·7	0·01	12·3	0·01	18·9	0·01	16·3	0·20
3	18·8	0·04	20·8	0·04	3·5	0·04	11·5	0·28
4	15·0	0·06	4·2	0·06	6·8	0·03	5·2	0·18
5	7·7	0·08	6·3	0·06	0·4	0·02	7·3	0·26
6	5·3	0·02	4·2	0·03	0·5	0·02	3·5	0·26
7	2·9	0·06	2·1	0·03	—	0·01	2·8	0·22
8	2·0	0·04	2·1	0·02	—	0·003	1·4	0·18
9	1·1	0·02	1·8	0·02	—	—	0·2	0·10
10	1·6	0·02	1·5	0·02	—	—	0·3	0·005
11	1·9	0·01	1·4	0·02	—	—	—	—
12	1·0	0·01	1·1	0·01	—	—	—	—
13	0·4	0·004	0·3	0·004	—	—	—	—
14	0·4	—	0·2	—	—	—	—	—

Table 2. *Absorption and urinary excretion of [58Co]cyanocobalamin after oral administration to normal rabbits, with coprophagy prevented*

Rabbit no.	Dose of cyano-cobalamin (μg)	Amount absorbed		Urinary excretion	
		μg	% of dose	μg	% of dose
2	0·055	0·01	17·5	0·0014	2·5
3	0·1	0·02	21·9	0·0021	2·1
4		0·04	38·7	—	—
1	1·0	0·11	11·0	—	—
2		0·12	12·1	—	—
11		0·14	14·4	0·019	1·9
19		0·15	14·8	0·009	0·9
12	5·0	0·6	12·6	0·14	2·8
20		1·2	23·7	0·08	1·6
13		1·2	24·4	—	—
7	10	1·1	11·4	0·15	1·5
8		1·1	10·5	0·28	2·8
13		0·8	7·7	0·20	2·0
3	25	5·6	21·6	0·05	0·2
4		7·6	29·4	0·50	2·0
14		4·2	16·8	—	—
22		8·0	32·1	—	—
9	50	6·3	12·6	1·35	2·7
10		9·4	18·7	0·45	0·9
15		8·3	16·6	—	—
14		8·6	17·2	—	—
16	100	15·5	15·5	1·70	1·7
9		14·9	14·9	1·30	1·3
23		15·8	15·8	—	—
17	150	15·0	10·0	2·10	1·4
10		21·7	14·5	4·20	2·8
12		20·8	13·9	—	—

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(1 μc) [^{58}Co]cyanocobalamin. Isotope was detectable in the blood 2 h after dosing, and appreciable amounts were present after 4 h. The highest concentration was found after 11 h, after which the level declined slowly, with moderate levels still present after 48 h (Fig. 1).

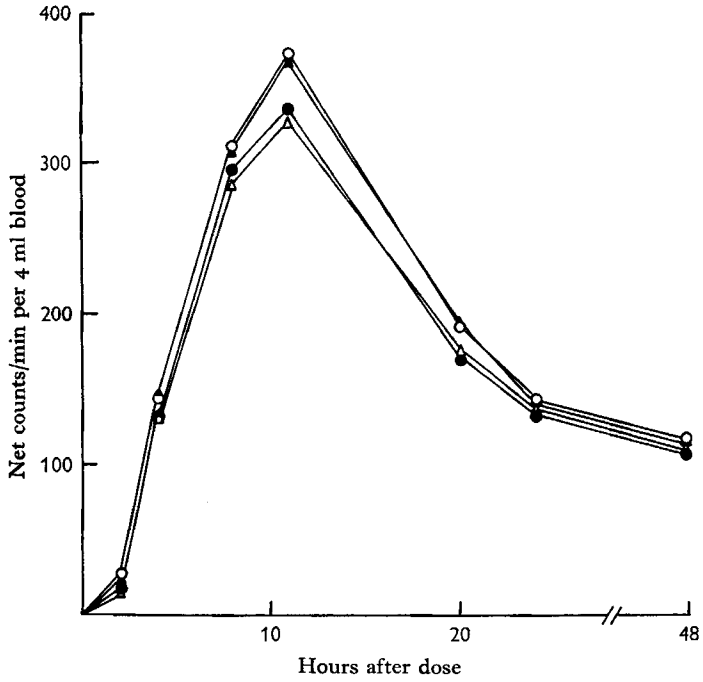

Fig. 1. Levels of isotope in the blood of four normal rabbits after a dose of 25 μg (1 μc) [^{58}Co]cyanocobalamin given by mouth, with coprophagy prevented. ○—○, rabbit no. 1; ●—●, rabbit no. 2; △—△, rabbit no. 3; ▲—▲, rabbit no. 4.

DISCUSSION

In order to interpret our results we have assumed that measurements of the distribution of ^{58}Co represent the distribution of [^{58}Co]cyanocobalamin. When rabbits were given [^{58}Co]cyanocobalamin in doses of up to 150 μg and coprophagy was prevented, they absorbed a relatively constant proportion of the dose over the whole dose range. After absorption, some of the isotope was excreted in the urine, and there was a significant correlation between the amount absorbed and the amount excreted in the urine by the seventeen rabbits for which results were obtained for both urine and faeces ($r = 0{\cdot}897$, $P < 0{\cdot}001$).

The larger doses used in these experiments were similar to the amounts rabbits normally ingest in 24 h by coprophagy. The smaller doses were similar to those used by Rosenthal (1959), who found that rabbits receiving 0·2–0·3 μg cyanocobalamin absorbed about 43% of the dose, compared with absorptions of 22% and 39% in our two rabbits given 0·1 μg.

The apparent ability of the rabbit to absorb such large amounts of cyanocobalamin when given large oral doses is in marked contrast to the behaviour of man, rat and

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sheep. According to Glass (1955), in man the fraction of the dose absorbed falls rapidly from 80–90% for doses of 0·5–1 μg to about 3% of a 50 μg dose. Rats absorb about 52% of the dose in the dose range 0·001–0·01 μg, but when the dose is increased to 0·5–2 μg the proportion absorbed falls to about 7% (Taylor, Mallett, Witts & Taylor, 1958). The sheep is reported to synthesize 600–1000 μg vitamin B_{12} daily in the rumen, but absorbs only about 3% of it (Marston, 1959). The high absorption by the rabbit probably explains the higher levels of vitamin B_{12} in the serum of rabbits than of other species.

Only tentative conclusions can be drawn from the four experiments on the blood levels of isotope after oral doses of 25 μg [^{58}Co]cyanocobalamin. It is noteworthy that the rate of appearance of the isotope in the blood after this dose closely resembled that found in the plasma by Rosenthal (1959). After giving less than 0·5 μg, he found the highest level 8 h after the dose; in our rabbits the levels were high after 8 h, but increased to maximum values at 11 h. This suggests that the rabbit absorbs doses of less than 0·5 μg and of 25 μg by similar mechanisms and confirms the ability of this species to handle by normal physiological processes much larger doses than other mammals. Doscherholmen & Hagen (1957) carried out similar experiments in man and distinguished three types of absorption curve, depending on the size of the dose. Our results with rabbits most closely resemble those with man after doses of 50 and 100 μg, which were interpreted to reflect a combination of two mechanisms of absorption, one mediated by intrinsic factor and the other independent of intrinsic factor. Further experiments with doses of different sizes would be necessary to determine whether rabbits absorb cyanocobalamin by only one or by both of these mechanisms.

SUMMARY

1. Rabbits were given doses of between 0·055 and 150 μg [^{58}Co]cyanocobalamin by mouth, and the excretion of the isotope in the faeces and urine was measured. The rate of appearance of the isotope in the blood was also measured in four rabbits, which received 25 μg [^{58}Co]cyanocobalamin by mouth.

2. Prevention of coprophagy resulted in shorter periods of elimination of the isotope in both faeces and urine than when coprophagy was allowed.

3. The rabbits absorbed from the gut between 7·7% and 39% of the dose and excreted in the urine between 0·2% and 2·8% of the dose. Neither the fraction of the dose absorbed nor the fraction excreted in the urine was influenced by the amount of cyanocobalamin given.

4. There were statistically significant relationships between the size of the dose and the amount absorbed, between the size of the dose and the amount excreted in the urine and between the amount absorbed and the amount excreted in the urine.

5. Some isotope was found in the blood of rabbits 2 h after 25 μg [^{58}Co]cyanocobalamin given by mouth. The levels increased to a maximum after 11 h, followed by a slow decline, with considerable amounts still present after 48 h.

6. The results suggest that the rabbit can absorb from the gut much larger amounts of cyanocobalamin than can man, rat or sheep.

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