The absorption of [^{58}Co]cyanocobalamin by unweaned rats

By D. L. WILLIAMS and G. H. SPRAY
Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford

(*Received* 17 *October* 1967—*Accepted* 1 *December* 1967)

1. [^{58}Co]cyanocobalamin was given by stomach tube to Wistar albino rats aged between 6 h and 24 days. The fraction of the dose absorbed was assessed by measuring the amount retained by the animals after 7 days, by total body counting.

2. Rats up to 4 days old absorbed about 90% of the test dose, compared with a mean of 83% at the age of 8 days, 66% at 16 days and 47% at 24 days.

3. The proportion of the test dose absorbed did not appear to be affected by starvation for up to 24 h, by increasing the dose from 0·05 ng to 0·25 ng/g body-weight, or by giving the [^{58}Co]cyanocobalamin mixed with rat's milk.

4. The results support earlier suggestions that newborn rats absorb vitamin B_{12} by a different mechanism from that in adults.

During attempts in this Department to produce deficiency of vitamin B_{12} in rats by dietary depletion, it became necessary to breed from rats already receiving a deficient diet. It therefore seemed to be of interest to attempt to determine what proportion of the available vitamin B_{12} was absorbed by young rats. Our results show that rats up to 4 days old absorb about 90% of a test dose of [^{58}Co]cyanocobalamin, but the absorption decreases to adult levels by 24 days. Boass & Wilson (1963) had previously shown that intestinal segments from foetal and newborn rats took up much more [^{57}Co]vitamin B_{12} than similar segments from adult rats, but the uptake declined towards adult levels by about 14 days. The stomach wall of foetal and newborn rats contained much less intrinsic factor than that of adult rats, though the levels increased rapidly after the 1st week of life.

EXPERIMENTAL

Animals. Young rats were bred in the Department from albino parents of the Wistar strain, which were fed *ad lib.* on a diet of standard rat cake (modified diet 41 B; Herbert C. Styles (Bewdley) Ltd).

Administration of [^{58}*Co*]*cyanocobalamin and management of animals.* The young rats were left with their mothers until just before dosing, when they were marked and weighed. An aqueous solution of [^{58}Co]cyanocobalamin (Radiochemical Centre, Amersham, Bucks; specific activity 40 μc/μg) was given through a fine polythene stomach tube, the quantity being adjusted so that each rat received 0·05 ng cyanocobalamin/g body-weight. This dose gave satisfactory counts of radioactivity even in very small rats. It was chosen because Taylor, Mallett, Witts & Taylor (1958) showed that in adult rats weighing 200–250 g there was a linear relationship between the amount of cyanocobalamin absorbed and the dose, in the range 1–10 ng. In a few experiments (Table 4) five times as much cyanocobalamin was given. In experiments to study the effect of milk on absorption (Table 5), [^{58}Co]cyanocobalamin of specific activity 150 $\mu c/\mu g$ was mixed with rat's milk, the vitamin B_{12} content of which had been measured by microbiological assay with *Lactobacillus leichmannii*. The total vitamin B_{12} content of the mixture was calculated from the assay results and the amount of [^{58}Co]cyanocobalamin used, and the rats received 0·05 ng total vitamin B_{12} activity/g body-weight.

After dosing and counting, the young rats were returned to their mothers and weighed daily. It was found that animals in which the oesophagus was damaged during dosing did not grow satisfactorily. Any which failed to gain weight daily were therefore discarded. Whenever possible, different members of each litter were dosed at different ages to allow for possible variation between litters in ability to absorb vitamin B_{12}. There was no evidence of transfer of ^{58}Co from treated to untreated litter-mates.

Measurement of radioactivity. Radioactivity in the rats was counted by placing each animal in a small cardboard box resting on a polythene block in the plastic phosphor well scintillation counter described by Warner & Oliver (1962). A radioactive standard containing a known amount of the dosing solution, made up to 4 ml with water in a plastic tube, was also counted. The radioactivity was counted immediately after the rats had been dosed and 7 days later. The fraction of the dose retained after 7 days was taken as the amount absorbed. In some experiments the radioactivity was also counted at intervals before the 7th day.

RESULTS

Retention of isotope after oral doses of [^{58}Co]cyanocobalamin. Preliminary experiments (Table 1) showed that in rats dosed at or before 8 days of age there was an initial loss of isotope before the 5th day, with little or no further loss between the 5th and

Table 1. *Percentage of test doses of [^{58}Co]cyanocobalamin retained by rats at different times after dosing*

		% of test dose retained on:			
		5th day		7th day	
Age when dosed (days)	No. of observations	Mean	Standard deviation	Mean	Standard deviation
0·25	20	90·7	4·3	90·8	4·3
1	3	94·0	5·2	87·0	3·0
8	15	88·7	5·4	86·0	4·8
24	9	63·1	8·8	56·0	5·8

7th days. In 24-day-old rats the early loss was larger, with some excretion continuing between the 5th and 7th days, although this was much smaller than before the 5th day and was not significant on the basis of these nine observations ($t = 2·02$, $0·1 > P > 0·05$). These results suggested that by using the fraction of the dose retained after 7 days as a measure of the amount absorbed, we obtained an accurate assessment of absorption in rats up to 8 days old, but possibly a slight over-estimate in older animals. This error, however, would decrease rather than magnify the differences shown by our results.

Absorption of [^{58}Co]cyanocobalamin at different ages. The 323 observations on rats aged from 6 h to 24 days show that during the first 4 days of life the animals absorbed on average about 90% of a test dose of [^{58}Co]cyanocobalamin (Table 2). The mean absorption fell to 83% in animals aged 8 days, to 66% in those aged 16 days and to only 47% in those aged 24 days. The results for the older animals showed a wider scatter than those for the younger ones.

Table 2. *Percentage of test doses of [^{58}Co]cyanocobalamin (0·05 ng/g body-weight) absorbed by rats of various ages*

Age when dosed (days)	No of observations	% of test dose absorbed		
		Mean	Standard deviation	Range
0·25	42	89·9	6·0	78–100
0·5	17	89·5	7·6	75–100
1	61	91·1	6·4	75–100
2	41	86·8	5·5	67–100
4	45	88·2	7·4	61–100
8	40	82·7	8·5	56–98
16	32	66·0	15·4	29–86
24	45	46·8	17·8	11–96

Table 3. *Percentage absorption of test doses of [^{58}Co]cyanocobalamin by rats of different ages, after starvation, compared with that by their unstarved litter-mates*

Age when dosed (days)	Period of starvation (h)	No of. litters studied	% of test dose absorbed by: Starved rats			Unstarved rats		
			No. of animals	Mean	Standard deviation	No. of animals	Mean	Standard deviation
1	6	4	16	90·2	8·6	14	93·0	4·3
	12	3	12	90·5	6·6	10	89·8	7·0
4	6	1	3	92·0	3·5	3	92·3	3·2
	12	3	12	90·0	2·9	11	86·6	5·7
8	12	2	8	88·1	2·9	8	84·2	5·6
	24	1	3	81·0	2·6	3	79·3	1·2
16	18	2	7	66·4	14·9	5	49·2	11·3
	24	1	2	72·0	—	2	56·0	—

Effect of starvation. All the results summarized in Table 2 were obtained with animals that had been with their mothers until just before the [^{58}Co]cyanocobalamin was given. It was thought that to starve such young animals before dosing would be difficult and might be fatal. To see whether the presence of food in the stomach affected absorption, some rats were removed from their mothers for periods up to 24 h before the cyanocobalamin was given. Their litter-mates were left with the mothers and were dosed at the same time. The results (Table 3) show that fasting had no apparent effect on absorption in rats dosed when less than 8 days old. In 16-day-old rats the results were highly variable, as was found in the main study with rats of this age. However, there was no significant difference between the results for 16-day-old rats starved for 18 h and those for the corresponding unstarved animals ($t = 2.17$, $0.1 > P > 0.05$).

Absorption of [58Co]cyanocobalamin from doses of 0·25 ng/g body-weight. The effect of giving five times more [58Co]cyanocobalamin than was used in the main study was investigated in a few animals aged between 6 h and 16 days. The results (Table 4) were all similar to those found in animals of corresponding ages receiving 0·05 ng cyanocobalamin/g body-weight (Table 2).

Effect on absorption of giving [58Co]cyanocobalamin mixed with rat's milk. The results (Table 5) show that the proportions of the test dose absorbed when the vitamin was mixed with rat's milk were similar to those absorbed by animals of similar ages when the cyanocobalamin was in aqueous solution.

Table 4. *Percentage of test doses of [58Co]cyanocobalamin (0·25 ng/g body-weight) absorbed by rats of various ages*

Age when dosed (days)	No. of observations	% of test dose absorbed: Mean	Range
0·25	6	92·2	84–97
0·5	3	93·3	93–94
1	2	93·0	89–97
8	3	79·3	78–80
16	2	56·0	—

Table 5. *Percentage of test doses of [58Co]cyanocobalamin, mixed with rat's milk, absorbed by rats of different ages (each animal received 0·05 ng total vitamin B_{12} activity/g body-weight)*

Age when dosed (days)	No. of observations	% of test dose absorbed: Mean	Range
4	5	93·5	91–96
8	6	87·9	83–93
16	6	72·4	66–80
24	6	55·0	48–60

DISCUSSION

In order to interpret our results we have made the usual assumption that the distribution of ^{58}Co represents the distribution of [58Co]cyanocobalamin. According to Boass & Wilson (1963), little or no intrinsic factor is produced by the rat stomach during the 1st week of life. Our results show that during this period rats absorb about 90% of a test dose of [58Co]cyanocobalamin, presumably by a mechanism independent of intrinsic factor. Although it is difficult to compare results for isolated segments of intestine with those for intact animals, this high absorption is in agreement with the high uptake by intestinal segments of newborn rats (Boass & Wilson, 1963). After the 1st week the absorption of cyanocobalamin decreases and probably becomes increasingly dependent on intrinsic factor. By the 24th day the mean absorption had fallen to levels found in adult rats by other authors, e.g. 33·5% (Watson & Florey, 1955), 42·9% (Clayton, Latner & Schofield, 1957), 52·3% (Taylor *et al.* 1958 and 57% (Smith & Ellis, 1965).

A possible criticism of our results with very young rats might be that owing to the technical difficulty of intubating the stomach of such small animals, the test dose might be introduced into the tissues rather than into the stomach. We feel that this is unlikely, because results were only included from those rats which grew normally after dosing. Also, when the wall of the oesophagus was accidentally pierced, the catheter could be seen under the skin and was usually stained with blood when removed. If the dose entered the lungs it was expectorated by the rat. Animals in which such abnormalities were noted were discarded.

The reason for the greater variation of the results in older rats as compared with those in younger animals is not clear. The results of Boass & Wilson (1963) suggest that considerable variations occur in the amount of intrinsic factor secreted by rats aged between 7 and 17 days. Thus the change in the mechanism of absorption of cyanocobalamin from being independent of intrinsic factor to dependence on intrinsic factor may occur at different ages in different individuals. This could account for the variability. Likewise, we can only offer speculative explanations for the absorption of less than 20% of the test dose by some 24-day-old rats.

Absorption does not appear to be affected by starvation (Table 3) or by giving the cyanocobalamin with rat's milk rather than in aqueous solution (Table 5). Rats up to 16 days old appear to absorb 0·25 ng cyanocobalamin/g body-weight as efficiently as they absorb 0·05 ng/g (Table 4). This is in agreement with the results of Smith & Ellis (1965), who found that adult rats weighing 250–300 g absorbed roughly the same proportion of doses of either 10 or 90 ng cyanocobalamin.

The milk of rats on stock diets contains up to 100 ng/ml of vitamin B_{12} activity for *Lactobacillus leichmannii* (D. L. Williams & G.H. Spray, unpublished observations). The present results suggest that most of this vitamin B_{12} is absorbed by baby rats until they are at least a week old, assuming that the absorption of [^{58}Co]cyanocobalamin reflects that of vitamin B_{12} from milk. The normal absorption of cyanocobalamin when given with rat's milk, supports this assumption. To try to obtain further information, nursing mothers were injected with [^{58}Co]cyanocobalamin and the radioactivity in their litters was counted afterwards. The young rats did not take up sufficient isotope for accurate counting until 3 days after the mothers were injected, so that the results could not be compared with those already described.

We are grateful to Mr J. Carter and Mr M. Clarke for technical assistance.

REFERENCES

Boass, A. & Wilson, T. H. (1963). *Am. J. Physiol.* **204**, 101.
Clayton, C. G., Latner, A. L. & Schofield, B. (1957). *Br. J. Nutr.* **11**, 339.
Smith, A. D. M. & Ellis, H. (1965). *Lancet* ii, 1277.
Taylor, K. B., Mallett, B. J., Witts, L. J. & Taylor, W. H. (1958). *Br. J. Haemat.* **4**, 63.
Warner, G. T. & Oliver, R. (1962). *Br. J. Radiol.* **35**, 349.
Watson, G. M. & Florey, H. W. (1955). *Br. J. exp. Path.* **36**, 479.

Printed in Great Britain