Some observations on the vitamin B_{12} content of rat's milk

By D. L. WILLIAMS and G. H. SPRAY

Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford

(*Received* 26 *June* 1970—*Accepted* 17 *September* 1970)

1. Vitamin B_{12} activity in the milk of rats on various diets was measured by microbiological assay at intervals throughout lactation. The concentration of sodium and calcium in the milk was also determined.

2. At all stages of lactation the milk of rats fed on commercial rat cake contained much more vitamin B_{12} activity than that of rats receiving a semi-synthetic diet containing at least as much vitamin B_{12} as the rat cake. The milk of the latter group of animals contained more vitamin B_{12} than that from rats fed on the same diet without added cyanocobalamin. The levels of sodium and calcium were much less variable than those of vitamin B_{12} and the changes did not appear to be related to differences in diet, stage of lactation or litter size.

Weanling rats fed on a vitamin B_{12}-deficient diet were found to grow at normal rates unless they were bred from mothers that had received the deficient diet since mating. Also, the bodies of the offspring of such mothers contained less vitamin B_{12} than those of mothers receiving either a vitamin B_{12}-supplemented diet or rat cake (Williams, Spray, Newman & O'Brien, 1969). It had been shown previously that during the first few days of life, rats absorb about 90% of an oral dose of cyanocobalamin mixed with rat's milk, compared with only about 50% later (Williams & Spray, 1968). In view of these observations it seemed to be of interest to study the vitamin B_{12} content of the milk of rats receiving various diets.

EXPERIMENTAL

Animals and their management. Female albino rats of the Wistar strain received *ad lib.* a basal diet deficient in vitamin B_{12}. The diet contained, by weight, 60% soya flour (Soyolk; Soya Foods Ltd, London, EC3), 20·5% sucrose, 11·4% lactose, 0·5% choline dihydrogen citrate and mineral salts and vitamins (Williams *et al.* 1969). For some animals the diet was supplemented with 15 μg cyanocobalamin/kg diet. The rats were housed and managed as described previously (Williams *et al.* 1969). Rats receiving rat cake (modified diet 41 B; Herbert C. Styles (Bewdley) Ltd) were kept in ordinary rat cages. The animals were mated and those that became pregnant were transferred to individual breeding boxes, where they remained until the young were weaned. Where necessary, surplus young were removed or young of the same age from other mothers on the same diet were added, to maintain the litters at the desired size (Table 1).

Collection of milk. After being separated from their young for 18 h, the rats were lightly anaesthetized with ether and were given 0·1 ml synthetic oxytocin (Pitocin; Parke Davis & Co., Ltd; 10 i.u. oxytocin/ml) by intraperitoneal injection. After 5 min, milk was expressed manually into small Pyrex test-tubes and was stored at $-20°$ until required for assay.

Determination of vitamin B_{12} activity. Suitable quantities (5–25 μl) of milk were mixed with 0·2 ml 0·1 % (w/v) NaCN solution and 0·5 ml 0·4 M-acetate buffer, pH 4·5. The mixtures were diluted to 10 ml with water and were then treated as described for human serum by Spray (1955), the vitamin B_{12} content of the extracts being determined by microbiological assay with *Lactobacillus leichmannii* (Spray, 1955).

Determination of sodium and calcium in milk. In order to obtain information on variations in the concentration of some other constituents of milk, for comparison with possible changes in the levels of vitamin B_{12} activity, sodium and calcium were measured by flame spectrophotometry. Milk (25 μl) was diluted to 20 ml with conductivity water obtained by passage through an Elgastat deionizer (Elga Products Ltd, Lane End, Bucks). The solutions were analysed in a Unicam SP 900 recording flame spectrophotometer which had been calibrated by means of standard solutions prepared from NaCl and $CaCO_3$ (Specpure grade; Johnson Matthey & Co., Ltd, Hatton Garden, London, EC 1).

RESULTS

Recovery of added cyanocobalamin from rat's milk and the effect of storage on vitamin B_{12} activity. When 0·25 or 0·5 ng cyanocobalamin was added to portions (25 μl) of three separate samples of milk, the mean recovery was 91 % (range 73–120 %). The vitamin B_{12} content of three separate samples of milk was measured in triplicate soon after collection and after storage at $-20°$ for 1, 2 and 3 weeks. There was no marked change in vitamin B_{12} content during this time.

Changes in the vitamin B_{12} content of rat's milk during lactation and the influence of diet and litter size. Throughout lactation there was much more vitamin B_{12} activity in the milk of rats receiving the vitamin B_{12}-supplemented basal diet than in that from animals fed on the deficient diet (Table 1). Females whose diet was changed from rat cake to the deficient diet at the time of mating produced milk with a vitamin B_{12} content higher than that of milk from deficient rats but lower than that secreted by animals given a vitamin B_{12} supplement. Rather surprisingly, the levels in the milk of rats receiving rat cake were considerably higher than those found in the vitamin B_{12}-treated animals.

In all the groups the mean concentration of vitamin B_{12} in the milk collected on the 24th day was higher than at the earlier stages of lactation. The results for the rats fed on rat cake suggest that early in lactation mothers with large litters secrete milk containing less vitamin B_{12} than those with smaller litters; this trend appears to be reversed later, although the mean values for rats with four young are based on limited numbers of observations.

Sodium and calcium in rat's milk. In the milk of rats fed on rat cake, the mean values for the concentration of sodium were between 0·8 and 2·0 mg/ml and for calcium, between 2·5 and 3·4 mg/ml. The results did not show any trends which could be attributed to differences in litter size or stage of lactation. The values for rats fed on the vitamin B_{12}-deficient or vitamin B_{12}-supplemented diets were within the same limits at all stages of lactation. The results have been described in greater detail elsewhere (Williams, 1967).

Table 1. *Vitamin B_{12} content of the milk of rats receiving various diets on the 4th, 8th, 16th and 24th day of lactation*

(Mean values with their standard errors; no. of observations in parentheses)

Diet	No. of young	Vitamin B_{12} activity in milk (ng/ml)							
		4th day		8th day		16th day		24th day	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Basal vitamin B_{12}-deficient	6	—	—	6·0	0·7 (8)	3·8	0·4 (10)	7·6	0·8 (9)
Basal with vitamin B_{12} supplement	6	—	—	15·4	1·1 (10)	14·2	2·1 (11)	41·3	7·8 (11)
Modified 41 B* till mating,	4–6	—	—	11·3	1·8 (8)	7·1	1·0 (7)	11·9	4·0 (7)
then vitamin B_{12}-deficient	10	—	—	7·2	1·7 (5)	9·1	2·6 (5)	11·2	0·6 (3)
Modified 41 B	4	62·7	7·9 (9)	52·0	6·9 (9)	48·8	6·1 (5)	65·2	7·3 (5)
	8	47·0	3·4 (9)	40·4	8·2 (12)	43·2	4·8 (13)	82·7	8·6 (13)

* Rat cake.

DISCUSSION

Our results show that the level of vitamin B_{12} activity in rat's milk is influenced by the vitamin B_{12} content of the animals' diet and, apparently, by other factors. The amount of cyanocobalamin added to the vitamin B_{12}-supplemented diet was 15 μg/kg, and the modified diet 41 B was found by microbiological assay to contain 10–15 μg vitamin B_{12} activity/kg. Despite this similarity, rats receiving rat cake secreted milk containing considerably more vitamin B_{12} than that of vitamin B_{12}-treated females.

The vitamin B_{12} content of milk appears to change fairly quickly when the diet is altered; within 4 weeks of receiving the deficient diet instead of rat cake, the level of vitamin B_{12} in milk was not much higher than in the milk of rats that had been fed on the deficient diet since weaning.

Sodium and calcium were determined in the hope of obtaining some index of the quality of rat's milk at various stages of lactation. Measurement of fat, lactose or protein might have been more useful for this purpose, but was not attempted because it was often impossible to obtain sufficient milk. The consistency of the values for sodium and calcium suggests that neither the diet nor the progress of lactation had any marked effect on quality; therefore variations in vitamin B_{12} concentration are unlikely to have been related to differences in the quality of the milk from rats on different diets.

The values for the concentration of vitamin B_{12} in rat's milk were up to 100 times higher than those found previously in this laboratory for rat plasma or serum (Booth & Spray, 1960; Williams *et al.* 1969). The rat's mammary gland must therefore have an efficient mechanism for concentrating the vitamin from the blood. This mechanism appears to be effective at all stages of lactation, in contrast to results reported for iron; in early lactation the iron content of milk is several times greater than that of plasma but the concentrations become similar later (Ezekiel, 1965). The mechanism also seems to operate in the face of quite severe depletion of vitamin B_{12}, whereas in vitamin B_{12}-deficient human subjects the concentration of vitamin B_{12} in milk is no higher than that in serum (Baker, Jacob, Rajan & Swaminathan, 1962).

The results in this paper are presented to provide additional background information about the effects of the vitamin B_{12}-deficient diet described previously (Williams *et al.* 1969); feeding female rats on this diet from the time of mating ensures a reduced intake of vitamin B_{12} by the young during the period before weaning without noticeably affecting the breeding performance of the mothers.

REFERENCES

Baker, S. J., Jacob, E., Rajan, K. T. & Swaminathan, S. P. (1962). *Br. med. J.* i, 1658.
Booth, M. A. & Spray G. H. (1960). *Br. J. Haemat.* **3**, 288.
Ezekiel, E. (1965). *Biochim. biophys. Acta* **107**, 511.
Spray, G. H. (1955). *Clin. Sci.* **14**, 661.
Williams, D. L. (1967). The distribution of vitamin B_{12} in animals and the effects of dietary deficiency. DPhil Thesis, University of Oxford.
Williams, D. L. & Spray, G. H. (1968). *Br. J. Nutr.* **22**, 297.
Williams, D. L., Spray, G. H., Newman, G. E. & O'Brien, J. R. P. (1969). *Br. J. Nutr.* **23**, 343.

Printed in Great Britain