Vitamin B_{12} absorption in the neonatal piglet

1. Studies in vivo on the influence of the vitamin B_{12}-binding protein from sows' milk on the absorption of vitamin B_{12} and related compounds

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1. The vitamin B_{12} in sows' milk is strongly attached to a specific 'binder' protein, which is present in excess. The influence of this 'binder' on the uptake and retention of cyanocobalamin and two natural analogues (cobinamide and Co-α-[2-methyladenyl]cobamide) was investigated with neonatal piglets.

2. Retention of a single oral dose of cyano[^{58}Co]cobalamin given before 7 d of age was consistently higher with suckled than with early-weaned piglets, as determined by measurement of whole-body radioactivity.

3. Efficiency of retention declined with age, more rapidly in early-weaned than in suckled animals; when the dose was given at 14 d approximately 30% was retained by both groups.

4. Distribution of the retained cyano[^{58}Co]cobalamin within the body of the piglets was the same in both groups; about half was present in the liver.

5. Foraging piglets may ingest adventitious vitamin B_{12} and its analogues, which are present in the sow's faeces and in contaminated litter. The influence of the vitamin B_{12}-binder in sows' milk on the uptake and retention of two non-cobalamin analogues, and the effects of the analogues on the uptake and retention of vitamin B_{12} from 2 to 14 d after parturition, were investigated with early-weaned piglets.

6. The analogues were detected in the liver but not in the body organs. They were also present in blood plasma, urine and bile, in high concentration relative to that of vitamin B_{12}. The content of analogues in the liver was very small in relation to the amounts ingested, and much less than that of vitamin B_{12}. There was no indication that the vitamin B_{12}-binder in sows' milk influenced uptake and retention of the analogues, or that ingestion of analogues affected the content of vitamin B_{12} in the body organs and fluids examined.

The milk of all the mammalian species that have so far been examined contains vitamin B_{12} in a protein-bound form and generally accompanied by a large excess of unsaturated binding protein. This binding protein is of the class of R-type binders, or cobalophilins, which are also present in saliva, tears, blood plasma and gastric juice (Allen, 1975; Stenman, 1976) and whose physiological role is unknown.

Ford *et al.* (1975) suggested that the vitamin B_{12}-binder in sows' milk promotes the absorption of vitamin B_{12} during the early post-natal period, and showed that sucking piglets absorbed and retained the vitamin efficiently in the first 2 weeks of life, despite the virtual absence of intrinsic factor in their gut. Thus, the unsaturated vitamin B_{12}-binder in sows' milk may be of direct nutritional advantage to the sucking piglet. It may also indirectly facilitate absorption of the vitamin by preventing its uptake by the intestinal microflora (Gullberg, 1973; Ford, 1974).

When reared under confined conditions in a farrowing pen, piglets consume substantial amounts of faeces and contaminated straw litter. Sansom & Gleed (1981) reported that piglets reared with the sow in a farrowing pen consumed on average 5–85 g sows' faeces per d during their first 21 d of life. The sows' faeces are rich in vitamin B_{12} (cobalamins) and even richer in the non-cobalamin analogues of vitamin B_{12}, mainly cobinamide (factor B),

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Co-α-[adenyl]cobamide (pseudo-vitamin B_{12}) and Co-α-[methyladenyl]cobamide (factor A) (Ford *et al.* 1953; Ford & Porter, 1953), which have no vitamin activity for higher animals. These non-cobalamin analogues of vitamin B_{12} are abundant in the large intestine, faeces, soil and other fermented or microbially-contaminated natural materials (Ford & Hutner, 1955; Kon & Pawelkiewicz, 1960). Bacterial synthesis of the analogues in the small intestine (Bhat *et al.* 1972; Brandt *et al.* 1977; Albert *et al.* 1980) may also contribute significant amounts of these compounds.

Reports that certain of the naturally-occurring non-cobalamin analogues are antagonistic towards vitamin B_{12} in higher animals (Coates *et al.* 1956, 1960, 1964; Siddons *et al.* 1975; Kondo *et al.* 1982), and that body tissues contain predominantly vitamin B_{12} (Ford *et al.* 1953; Quadros *et al.* 1976; Kolhouse & Allen, 1977; Kolhouse *et al.* 1978; Rickard & Elliot, 1982; Kanazawa & Herbert, 1983) suggest that there are mechanisms which prevent the absorption or retention, or both, of the analogues by the body tissues. Intrinsic factor is highly selective for vitamin B_{12} and binds corrinoids other than cobalamins weakly, and so it may operate selectively to protect the body against absorption of the non-cobalamin analogues (Mathan *et al.* 1974; Kolhouse & Allen, 1977). An additional mechanism involving the vitamin B_{12}-binders in blood, transcobalamin II and cobalophilins, may prevent dissemination to the body tissues of any analogues that have gained access to the blood (Kolhouse & Allen, 1977). Vitamin B_{12} bound to transcobalamin II is distributed to the body tissues, but the non-cobalamin analogues are supposedly sequestered by cobalophilins, delivered to the liver and excreted in the bile. In piglets, intrinsic factor does not begin to appear until the second week of life (Ford *et al.* 1975) and piglets may therefore have a different mechanism to ensure the preferential uptake and retention of vitamin B_{12} until their endogenous system has fully developed. It is possible that the vitamin B_{12}-binder in sows' milk may be involved and may selectivity promote the uptake and retention of vitamin B_{12}, since the affinity of this binder for cobalamins is more than twice that for the non-cobalamin analogues (Trugo, 1984).

The present paper describes experiments in vivo on the effects of the vitamin B_{12}-binder in sows' milk on uptake of cyano[58Co]cobalamin. It extends and complements the report of Ford *et al.* (1975) by comparing the uptake and retention of vitamin B_{12} by piglets reared either with or without vitamin B_{12}-binder in their diet. Further experiments were carried out using early-weaned piglets to determine whether the uptake and retention of two vitamin B_{12} analogues (factor A and factor B) were influenced by the presence of the binder in the diet, and whether the uptake and retention of vitamin B_{12} were influenced by the presence of these analogues.

<div align="center">EXPERIMENTAL</div>

<div align="center">*Expt 1. Uptake and retention of cyano[58Co]cobalamin*</div>

A Large White sow (of the herd maintained at the Agricultural Research Council Institute for Research on Animal Diseases, Compton) and her litter of eight piglets (litter 1) were taken immediately after farrowing to a pen in an isolation unit. Two of the newborn (< 1 d) piglets were each given, by stomach tube, 5 ml sows' milk (unsaturated vitamin B_{12}-binding capacity (B_{12}-UBC) 109 ng/ml) to which had been added 0·45 μg cyano[58Co]cobalamin of specific activity 1·08 μCi/μg (Amersham International plc, Amersham, Bucks). At 1 h after dosing, the whole-body radioactivity of the piglets was determined as described by Ford *et al.* (1975). The determinations were repeated at intervals of 2 d for a period of 14 d, during which time the animals were suckled by the sow. The remaining six sucking piglets were similarly dosed with the mixture of cyano[58Co]cobalamin and sows' milk, two at 3 d, two at 7 d and two at 14 d post partum and subsequently whole-body radioactivity was determined as decribed for their littermates.

Another litter of eight piglets (litter 2) was used as a control. They were dosed with $0.45 \mu g$ cyano[^{58}Co]cobalamin mixed with 5 ml sterilized evaporated cows' milk (Carnation Foods Co., London) diluted with 2 vol. water (B_{12}-UBC 0.04 ng/ml), according to the schedule described for litter 1. These control piglets were taken from the sow at 2 d of age and given the sterilized cows' milk *ad lib.* throughout the experiment. The whole-body radioactivity was determined as described for litter 1.

Measurements of the whole-body radioactivity of each individual piglet were expressed as a percentage of that observed 1 h after dosing with cyano[^{58}Co]cobalamin. After whole-body counting 14 d after dosing, the piglets were killed by electrical stunning and exsanguinated. The thoracic and abdominal organs were removed, and the distribution of radioactivity in the empty carcass, liver, spleen, heart, lungs, pancreas and kidneys was determined.

Expt 2. Uptake and retention of vitamin B_{12} and non-cobalamin analogues

Nine piglets were removed 2 d post partum from each of two Large White sows of the herd maintained at the National Institute for Research in Dairying (NIRD). Two (one from each litter) were killed and the concentrations of vitamin B_{12} and its analogues in their bodies were determined. The remaining piglets were allocated to treatments according to a 'split-plot' design. Each litter of eight piglets was divided into two groups of four, one group to be killed at 8-d-old and the other at 14 d. The piglets within each group were allocated in a random manner to four test diets. These comprised (1) 300 ml/d of a basal diet (B_{12}-UBC < 0.1 ng/ml) to which had been added a concentrate of corrinoids (for the composition of this preparation see p. 248), (2) 200 ml/d basal diet and 100 ml sows' milk (B_{12}-UBC 140 ng/ml) to which had been added the concentrate of corrinoids, (3) 300 ml/d basal diet, and (4) 200 ml/d basal diet and 100 ml sows' milk. Cyanocobalamin was added to diets 1, 3 and 4 to raise their content of vitamin B_{12} to equal that in diet 2. Thus the diets supplied, per day: $2 \mu g$ vitamin B_{12} (diets 1, 2, 3 and 4); $8.8 \mu g$ non-cobalamin analogues of vitamin B_{12} (diets 1 and 2); $14 \mu g$ B_{12}-UBC (diets 2 and 4); $0.03 \mu g$ B_{12}-UBC (diets 1 and 3).

The piglets were placed in metabolism cages of the type described by Braude *et al.* (1969). The diet given daily to each piglet was divided into three 100 ml portions, placed in stainless-steel dishes and fed at 09.00, 13.00 and 18.00 hours. The basal diet, containing dried skimmed milk (146 g/l) and soya-bean oil (54 g/l), was homogenized and pasteurized and supplemented with fat-soluble vitamins, as described by Newport (1980). Sows' milk was obtained from several Large White sows of the NIRD herd, between 2 and 4 weeks of lactation, bulked, and stored at $-20°$ until required.

Collection and preparation of samples. The urine produced by each piglet during successive periods of 24 h was collected in bottles containing 50 ml 70% ethanol and the volumes recorded. Samples (20 ml) of each 24 h urine collection were concentrated in a rotary evaporator to remove ethanol and then reconstituted to 20 ml by addition of water. The floors of the metabolism cages were scrubbed and disinfected daily. Care was taken to prevent spillage of diets into the collection trays and contamination of the urine with diet or faeces.

Two piglets from each dietary treatment were killed after 6 d on the experimental diets (8-d-old piglets), and the remaining two after 12 d (14-d-old piglets), by intracardiac injection with sodium pentobarbitone, 16–18 h after the last feeding. Blood samples were taken from the heart by syringe into heparinized bottles, centrifuged at 1500 *g* for 15 min and the plasma collected. Bile was collected from the gall-bladder, by syringe. Liver, spleen, kidneys, heart and pancreas were removed, weighed and minced, and samples (approxi-

mately 10 g) were homogenized in 50 ml ice-cold distilled water. The reconstituted urines, blood plasma and tissue homogenates were stored at $-20°$ until required for analysis.

Preparation of a concentrate of corrinoids from sows' faeces. Freshly voided faeces from several sows were collected and to each 1 kg was added 1 litre aqueous solution containing 0·2 g sodium cyanide and 30 ml 2·5 M-sodium acetate buffer, pH 4·6. The mixture was homogenized, autoclaved at 110° for 60 min, cooled to room temperature and extracted with aqueous acetone and sodium cyanide as described by Ford & Porter (1953). The acetone was removed by evaporation under reduced pressure, and the residue centrifuged. The supernatant liquor was extracted with benzyl alcohol and the extract diluted with water-saturated diethyl ether. A dark-brown aqueous layer formed, and was separated and concentrated by rotary evaporation. The concentrates obtained from each 1 kg faeces were combined (10 ml) and applied to a column (600 × 25 mm) of Sephadex G-25 (Pharmacia Fine Chemicals, Hounslow) and eluted with 0·02 M-sodium chloride. The effluent was collected in 10-ml fractions. The presence of corrinoids in several fractions was indicated by their reddish-brown colour and confirmed by assay with *Escherichia coli*; these fractions were combined. Sow's faeces weighing 6 kg yielded a final concentrate of 131 ml, containing 11·2% of the original vitamin B_{12}-activity. On differential microbiological assay the concentrate was found to contain 7·2, 3·7 and 0·9 μg vitamin B_{12}-activity/ml as measured with *E. coli*, *Lactobacillus leichmannii* and *Ochromonas malhamensis* respectively. Thus, cobalamins constituted only a small proportion of the total corrinoid content. Bioautographic analysis (see below) of the concentrate confirmed that the corrinoid present in greatest amount was factor B, followed by factor A.

Analysis of the vitamin B_{12}-activity in the test samples. The 'vitamin B_{12}' compositions of plasma, urine, bile, tissue homogenates and of the concentrate of corrinoids, were assessed by differential microbiological assay, after extraction of the test samples in the presence of cyanide as described by Gregory (1954). The basal diet and the sows' milk were analysed in a similar manner except that the extraction procedure involved digestion of the test samples with cyanide-activated papain (Gregory, 1954). Assays with *O. malhamensis* (CCAP 933/1A, Cambridge Collection of Algae and Protozoa, Cambridge) were conducted as described by Ford (1953). This organism is highly specific and responds only to cobalamins, which are active also for higher animals. *Lactobacillus leichmannii* (NCDO 301, National Collection of Dairy Organisms, NIRD, Reading) responds to cobalamins and also to pseudo-vitamin B_{12} and factor A, and was used according to the method of Skeggs *et al.* (1950) modified as described by Gregory (1954). *Escherichia coli* mutant C-181 (NCDO 744) responds to cobalamins, pseudo-vitamin B_{12}, factor A, and also to factor B. This organism was employed in a tube assay technique described by Gregory & Holdsworth (1953) and based on the assay procedure of Burkholder (1951).

Results obtained with *O. malhamensis* were taken as a measure of cobalamins ('true' vitamin B_{12}), and the higher values sometimes obtained with *E. coli* and *L. leichmannii* were taken to indicate the presence of non-cobalamin analogues in the test extracts. The analogues differ widely in their growth-promoting activity and are less potent than cobalamins under the test conditions employed in the tube assays. The difference between the results obtained with these latter two organisms and *O. malhamensis* will therefore consistently underestimate the amount of non-cobalamin analogues present. The growth responses of the test organisms are expressed as vitamin B_{12}-activity, and represent the growth response to cobalamins for *O. malhamensis* and to cobalamins plus non-cobalamin analogues for *E. coli* and *L. leichmannii*.

Bioautographic analysis. Test extracts from livers prepared for microbiological assay, and the concentrate of corrinoids from sows' faeces, were examined by the bioautographic technique. Extracts and reference standards containing about 0·1 μg vitamin B_{12}-activity/ml

(*E. coli* assay) were applied as 1–10 μl spots to 200 × 200 mm sheets of Whatman no. 1 paper, and chromatographed for 16 h at 25° by the ascending technique, with water-saturated 2-butanol containing 7 μg sodium cyanide/ml. The vitamin B_{12}-active compounds were identified on the chromatograms by the bioautographic technique with *E. coli*, as described by Ford & Holdsworth (1953).

Measurement of unsaturated vitamin B_{12}-binding capacity

The capacity of samples of the basal diet, sterilized cows' milk (Expt 1) and sows' milk to bind added cyano[G-^{3}H]cobalamin (specific activity 3·25 mCi/ml; Amersham International plc) was measured by the charcoal absorption method of Gottlieb *et al.* (1965), modified in that the charcoal was coated with polyvinylpyrrolidone (PVP-360, Sigma Chemical Co., Poole, Dorset) instead of albumin.

Statistical analysis of results

The results obtained with the differential microbiological assay methods were assessed separately by the analysis of variance procedure for a split-plot design (Cochran & Cox, 1950). The treatments were in a 2 × 2 factorial arrangement, the factors being sows' milk and non-cobalamin analogues. Differences between dietary treatments were assessed statistically using the 'within-group' (sub-unit) error mean square (6 df).

RESULTS
Expt 1. Uptake and retention of cyano[^{58}Co]cobalamin

Fig. 1 shows the percentage retention of ^{58}Co at different times after dosing in early-weaned and suckled piglets given an oral dose of cyano[^{58}Co]cobalamin. It was assumed that retention of radioactivity might be equated with the retention of cyanocobalamin. The whole-body radioactivity of each piglet measured at 1 h after dosing was taken as 100%, and the radioactivity retained in the body on the following days was expressed as a percentage of this initial measurement. There was a consistent difference, which increased with time after dosing, between the suckled and the early-weaned piglets dosed up to 7 d of age, the retention values being consistently higher for the suckled group. In piglets dosed at birth the mean percentage retention after 14 d was 84 in suckled and 70 in early-weaned piglets. When the animals were dosed at 3 d post partum the corresponding mean retention values were 83 and 63%. After dosing at 7 d the mean retention value remained high (73%) in the suckled group and had fallen to only 7% in the early-weaned group. However, the test dose given at 14 d post partum was no more efficiently retained by the suckled than by the early-weaned piglets, the retention value being about 30% after 14 d in both groups.

Table 1 shows the distribution of the retained cyano[^{58}Co]cobalamin within the body of the piglets. The radioactivity of the whole body at slaughter was taken as 100% and the values for the carcass and organs expressed as percentages. The difference between the whole-body radioactivity (100%) and the sum of that in the carcass and organs varied from 0·7 to 12·6 (SE 5·8 to 4·9)% in the suckled piglets and 0 to 7·6 (SE 3·5 to 3·3)% in the weaned piglets, and was probably due to loss of activity in the gut and in the blood. It was evident that most (about 90%) of the radioactivity measured in the whole body corresponded to absorbed radioactivity. Most of this was in the liver, which contained on average 46% (range 35–53%), as against 3·8, 1·6, 0·8, 1·2 and 1·7% in kidneys, lungs, heart, spleen and pancreas respectively. The remainder of the radioactivity (approximately 41%) was in the carcass. No differences were found in the distribution of radioactivity between early-weaned and suckled piglets.
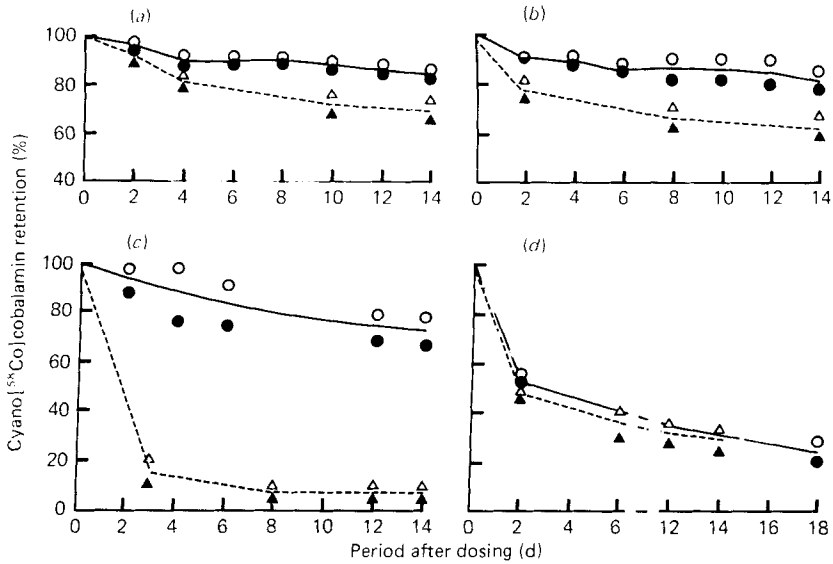
Fig. 1. Percentage retention over a period of 14 d of a test dose of cyano[⁵⁸Co]cobalamin given orally to suckled and weaned piglets at (a) birth and at (b) 3, (c) 7, and (d) 14 d post partum. Suckled piglets: (○, ●), individual values; (——), mean. Weaned piglets: (△, ▲), individual values; (---), mean. The two piglets dosed at birth in the weaned group were kept with the sow for 2 d after dosing. At day 2 post partum they were then weaned with the remaining six piglets from the same litter.

Table 1. *Distribution of the radioactivity retained in the bodies of piglets that had been given a test dose of cyano[⁵⁸Co]cobalamin*

(The radioactivity in each organ examined was calculated as a percentage of the whole-body radioactivity at the time of slaughter. Mean values for two piglets from each age group)

| Age at dosing (d)	Age at slaughter (d)	Percentage radioactivity (whole body 100%)						
		Carcass	Liver	Kidneys	Lungs	Heart	Spleen	Pancreas
		Suckled piglets						
< 1	14	40	46	2·3	0·1	0·1	0·1	0·1
		37	49	2·9	1·8	0·7	1·5	1·7
3	17	42	43	3·8	0·8	1·2	3·0	1·9
		33	52	5·3	4·8	0·5	1·8	7·2
7	21	45	35	0·3	0·9	0	0·1	0·5
		44	40	4·6	1·6	0	1·3	1·4
14	32	51	45	1·6	0	0	0	0
		48	47	1·4	0	0	0	0
		Weaned piglets						
< 1	14	35	53	5·7	1·7	1·5	2·0	1·4
		37	49	5·3	2·5	1·3	1·0	2·5
3	17	39	47	4·6	2·6	2·2	1·1	1·1
		45	42	6·7	1·9	1·1	2·1	2·0
7	21	34	44	2·2	1·4	1·6	1·9	2·0
		56	36	1·1	1·4	0·8	1·1	1·3
14	28	35	45	6·3	1·5	1·1	1·1	1·4
		34	50	5·7	1·6	0·5	1·1	1·9
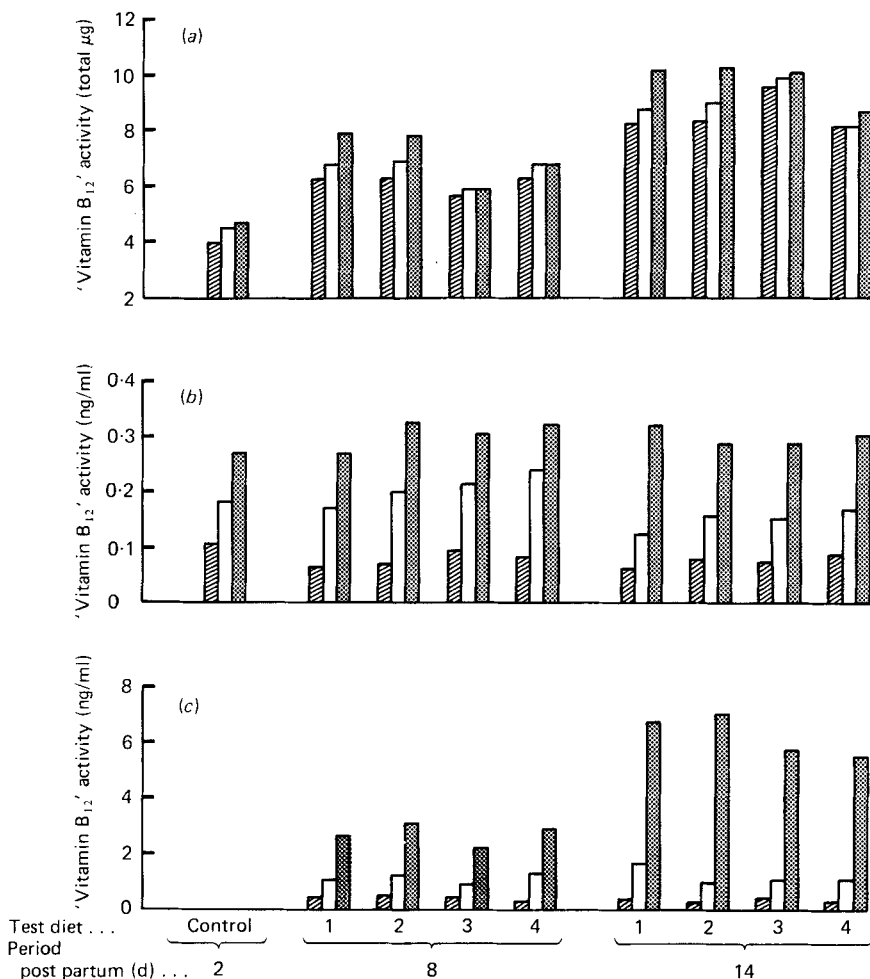
Fig. 2. 'Vitamin B$_{12}$'-activity in (*a*) liver, (*b*) plasma and (*c*) bile from control piglets killed at 2 d post partum (*n* 2) and from piglets weaned at 2 d of age on to four different test diets and killed at 8 d (*n* 2, for each diet) and at 14 d (*n* 2, for each diet) post partum. Test diets (for details see p. 247): (1) basal diet + non-cobalamin analogues; (2) basal diet + non-cobalamin analogues + sows' milk; (3) basal diet; (4) basal diet + sows' milk. 'Vitamin B$_{12}$'-activity was measured by microbiological assay with: (▨), *Ochromonas malhamensis*; (□), *Lactobacillus leichmannii*; (▦), *Escherichia coli*. Results are means of values obtained for each two piglets. The difference between the results obtained with the three assay organisms was significant for the liver (*P* < 0·01, controls and test diets 1 and 2) and for plasma and bile (*P* < 0·001).

Expt 2. Uptake and retention of vitamin B$_{12}$ and non-cobalamin analogues

Fig. 2 shows the vitamin B$_{12}$-activities, as determined by differential microbiological assay, in the liver, plasma and bile of piglets given the four test diets, and of piglets killed at 2 d post partum which had received only sows' milk. Piglets given diets containing the analogues (diets 1 and 2) had more of the analogues in their livers (Fig. 2(*a*)) (*P* < 0·05) than the corresponding controls (diets 3 and 4), but the amounts were small compared with the amounts ingested. The bioautographs of liver extracts revealed trace amounts of analogues in piglets that had not received them in the diet (diets 3 and 4), and in piglets killed at 2 d of age. The bioautographs also confirmed that in piglets reared on diets 1 and 2, the
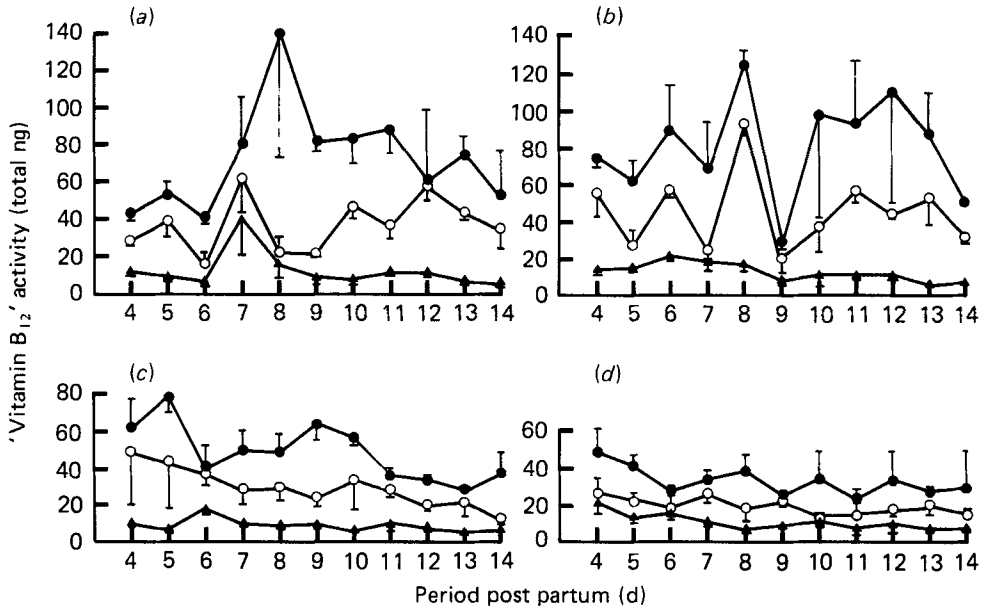
Fig. 3. 'Vitamin B_{12}'-activity in 24 h collections of urine (days 4–14 post partum) from piglets reared on four different test diets: (a) diet (1) basal diet+non-cobalamin analogues, (b) diet (2) basal diet+non-cobalamin analogues+sows' milk, (c) diet (3) basal diet, (d) diet (4) basal diet+sows' milk. 'Vitamin B_{12}'-activity was measured by microbiological assay with: (▲) *Ochromonas malhamensis*, (○) *Lactobacillus leichmannii*, (●) *Escherichia coli*. Points represent the mean values for four piglets from days 4 to 8 and for two piglets from days 9 to 14, with their standard errors represented by vertical bars.

corrinoid present in the greatest amount was vitamin B_{12}, followed by factor B. The liver was the only organ in which any significant content of non-cobalamin analogues was detected. The livers also contained much more vitamin B_{12} (4–10 μg) than any other organ (0·02–1·2 μg). The total amount of vitamin B_{12} stored in the liver during 12 d, calculated as the difference between the contents at 14 and 2 d of age, was in the range 4·2–5·9 μg. As about half the total vitamin B_{12} in the body of piglets is in the liver, it may be assumed that the total absorbed into the body was about 10 μg, or about 42% of the amount (24 μg) ingested during the 12 d. By comparing the differences between *E. coli* and *O. malhamensis* assay values for the livers with those for the diets, and assuming that the liver is also the main storage organ for the analogues, it may be similarly estimated that only 1% of the non-cobalamin analogues given in diets 1 and 2 was retained in the body.

A high concentration of non-cobalamin analogues, relative to that of vitamin B_{12}, was found in plasma (Fig. 2(b)) and in gall-bladder bile (Fig. 2(c)) from all the animals, whether or not they had received analogues in their diets. The concentration of vitamin B_{12} in plasma was significantly depressed ($P < 0·05$) when analogues were included in the test diets. There was a large increase with age in the excretion of the analogues in the bile of all the animals.

The high concentration of analogues in the plasma of the 2-d-old sucking piglets suggests that the analogues may have been ingested before the piglets were weaned. This supposition was confirmed when no analogues were detected in the livers and other organs, or in the bile or plasma from two piglets killed immediately after parturition.

The presence of sows' milk or non-cobalamin analogues in the test diets had no significant effect on the content of vitamin B_{12} in the liver. The concentration of analogues in the liver and bile was increased when analogues were present in the diet ($P < 0·05$), whereas sows' milk had no effect.

Fig. 3 shows the vitamin B$_{12}$-activity in urine from piglets given the four different test diets. The excretion of vitamin B$_{12}$ in urine, as measured with *O. malhamensis*, was fairly uniform throughout the test period and was not influenced by the presence in the diet of sows' milk or non-cobalamin analogues. Excretion of the analogues, however, as indicated by the assays with *L. leichmannii* and *E. coli*, was far more variable, espcially with test diets 1 and 2. As with vitamin B$_{12}$, there was no indication that the presence of sows' milk in the diet had influenced the excretion of analogues.

<div align="center">DISCUSSION</div>

Ford (1974) and Ford *et al.* (1975) suggested that, in the neonate, the vitamin B$_{12}$-binding protein present in milk constitutes an alternative mechanism for the absorption of vitamin B$_{12}$ until this function is taken over by the developing intrinsic-factor system. Expt 1 showed that from birth to 7 d of age, suckled piglets consistently absorbed and retained a higher proportion of a single oral dose of cyano[^{58}Co]cobalamin than did piglets receiving a diet containing no vitamin B$_{12}$-binder. The sucking animals retained their capacity to absorb the test dose for several days longer than did their early-weaned counterparts.

In the piglet, transport of intact macromolecules from the gut to blood ceases within 36 h of parturition, but uptake into the intestinal epithelium without subsequent transport to the blood ('internalization') may continue for up to 3 weeks. Closure of the gut, with the cessation of 'internalization', begins at the duodenum at about 36 h and proceeds caudally towards the ileum (Lecce & Morgan, 1962; Lecce, 1973). Ford *et al.* (1975) suggested that during the period of 'internalization', protein-bound vitamin B$_{12}$ might be efficiently taken up into the mucosal cells and there released for transport into the circulation. The decreasing uptake with increasing age at dosing, reported here for the weaned piglets, might then be explained by the absence of the vitamin B$_{12}$-binder from milk and the closure of the gut, which is greatly accelerated under the stress of early weaning.

A complication arose from the necessity to use piglets weaned 2 d post partum, in order to avoid the high incidence of severe scouring that occurs in piglets weaned at birth and so deprived of colostrum (Bowland, 1966; Barrow *et al.* 1977). Thus, vitamin B$_{12}$-binder received with the colostrum might have been taken up by the mucosal cells and continued to enhance absorption for several days, and the small degree of difference found between suckled and early-weaned piglets up to 3 d of age might have been attributable to the colostrum that the early-weaned piglets had received before weaning.

It is tempting to conclude from the findings in Expt 1 that vitamin B$_{12}$-binder in the sows' milk was directly responsible for the higher uptake and retention of cyano[^{58}Co]cobalamin by the suckled piglets. But there are other possibilities that might account for this difference between the weaned and suckled piglets. Earlier secretion of intrinsic factor in the weaned piglets might have been responsible for the decrease of vitamin B$_{12}$ uptake and retention, for Ford *et al.* (1975) reported that absorption of vitamin B$_{12}$ in sucking piglets decreased sharply after 21 d, when intrinsic factor was being actively secreted. It has also been reported that the absorption of vitamin B$_{12}$ by rats decreased with increase in age at dosing. The decrease coincided with the appearance of intrinsic factor, which was not produced during the early weeks of life (Boass & Wilson, 1963; Williams & Spray, 1968; Gallagher & Foley, 1972). These reports imply that intrinsic factor imposes a limit on uptake, and its precocious appearance in the early-weaned piglets might account for their lower uptake and retention of cyano[^{58}Co]cobalamin during the first 2 weeks of life. By day 14, the efficiency of uptake by the suckled animals had decreased to equal that in the early-weaned piglets, suggesting that by this stage intrinsic factor was regulating uptake in both groups. A further possiblity is that differences in the composition of the intestinal

microflora between suckled and weaned piglets might have influenced the efficiency of uptake of vitamin B_{12} from the intestine. In this connection, the milk binder might have facilitated uptake indirectly, by preventing uptake of vitamin B_{12} by intestinal bacteria.

The results from Expt 1 clearly demonstrated that the retention of radioactive vitamin B_{12} in the neonatal period was more efficient in the suckled than in the weaned piglets. It may be that vitamin B_{12} secreted with the bile was more efficiently reabsorbed in the presence of the milk binders. However, in Expt 2 no evidence was found that sows' milk influenced the uptake and retention of non-radioactive vitamin B_{12} and its non-cobalamin analogues in early-weaned piglets. The small numbers of animals used, the large variation between animals in their vitamin B_{12} endowment at birth, and the binder they received with colostrum before weaning, may together explain why no statistically significant effect of the binder could be detected. It is further possible that in these early-weaned piglets the precocious production of intrinsic factor masked any effect of the milk binder.

Expt 2 provided evidence that the ingestion of non-cobalamin analogues, predominantly factor B and factor A, did not affect the content of vitamin B_{12} in the body organs or in the blood plasma, bile and urine. The analogues did not accumulate in the liver at the expense of vitamin B_{12}, but they were delivered only to the liver and not to the other organs, in which no trace of the analogues was detected. Accumulation of the analogues in the liver has been reported to be associated with a decrease in the liver's vitamin B_{12} content in sheep (Ford et al. 1955; Rickard & Elliot, 1982), dogs (Brandt et al. 1975) and chicks (Coates et al. 1956). No such effect was observed in the present experiment, possibly because the analogues were given for only 6 or 12 d.

The presence of the non-cobalamin analogues in sucking piglets killed at 2 d of age, and in early-weaned piglets that had not received them in their diet and had been kept in cages without access to their faeces, suggests that they must have been derived from ingestion of sow's faeces before weaning at 2 d of age or from bacterial synthesis in the gut, or both. No analogues were present in piglets killed at birth.

In piglets, the liver is the main storage organ for vitamin B_{12}, and probably also for the analogues. The very small content of analogues in the liver, in relation to the large amounts ingested with test diets 1 and 2 in Expt 2, is conclusive evidence that they are absorbed inefficiently, or poorly retained, or both, Although the concentration of analogues in plasma, bile and urine was higher than that of vitamin B_{12}, the contribution of the analogues in plasma to the total content in the body was very small; their content in bile and urine was also small when compared with the amounts given in the diets. Vitamin B_{12} was retained much more efficiently than the analogues. Kolhouse & Allen (1977) concluded from experiments with rabbits that the specificity of intrinsic factor for vitamin B_{12} is a necessary but not sufficient condition for the preferential uptake of the vitamin. They postulated that there might be a separate mechanism in the ileum which recognizes the non-cobalamin analogues and prevents them from entering the portal blood.

The results of the present experiments cannot be interpreted unequivocally to demonstrate that the vitamin B_{12}-binder in sows' milk exerted a specific and direct effect on vitamin B_{12} absorption, because there were uncontrollable variables in the experimental design. Thus in Expt 1, a whole litter of piglets was allocated to each of the experimental classes ('suckled' and 'early-weaned') and differences between litters might have contributed to the treatment differences observed.

To avoid these difficulties and complement the in vivo findings, the influence of purified vitamin B_{12}-binder on the uptake of the vitamin has been investigated in vitro, using preparations of brush-border membrane vesicles isolated from the small intestine of piglets. These experiments are described in the third of this series of papers (Trugo et al. 1985).

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