EXCRETION AND INTAKE OF B VITAMINS IN NEWBORN INFANTS

BY

R. F. A. DEAN and W. I. M. HOLMAN

From the Medical Research Council Department of Experimental Medicine, University of Cambridge

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The present work was undertaken in an attempt to throw more light on the metabolism of vitamin B in the newborn infant, and, in particular, by means of balance experiments, to seek evidence of the biosynthesis of B vitamins in the intestinal tract during the first week after birth. The investigations were confined to aneurin, riboflavin, and nicotinic acid and its metabolites.

Very little work has been done on this subject in the past and balance studies have not been attempted. Neuweiler (1943) estimated the urinary excretion of aneurin by infants one to ten days old and observed that the values were low, but higher during the first four or five days than later. Coulson and Stewart (1946) studied the ability of infants to methylate nicotinamide and, in the course of this work, estimated the normal urinary output of N-methylnicotinamide on the first day after birth. They reported an average urinary output of 3.2 mg. of N-methylnicotinamide per 24 hours. Hamil, Coryell, Roderuck, Kaucher, Moyer, Harris, and Williams (1947) examined the concentrations of aneurin, riboflavin, nicotinic acid, N-methylnicotinamide, pantothenic acid, and biotin in the urine of infants during the first week after birth. In the main their results are confirmed by the present findings in so far as the urinary concentrations of aneurin, riboflavin, and nicotinic acid and its metabolites are concerned, but they did not study the faecal concentration and output or attempt to relate the total output to the intake. Their values for the maximum concentration of aneurin in the urine were lower than those found by Neuweiler (1943) and in the present investigation; they also found somewhat lower concentrations of N-methylnicotinamide than are now reported. Their work came to our attention after our experiments had been completed.

Material and Methods

Urine and faeces were collected from male children, born in the 77th British General Military Hospital,

Wuppertal, and in the Landesfrauenklinik, Wuppertal. The mothers and the children themselves were, as far as could be ascertained, normal. The children were all full-term, delivery was without special event, and the birth weights were all between 2,750 and 3,500 g.

Urine passed during delivery or immediately after was collected whenever possible in a test-tube. Thereafter the urine was collected by means of a glass apparatus which enclosed the penis and scrotum and was held in place by loops of elastic fastened to an elastic belt round the child's waist. The distal end of the apparatus was narrow and had a rubber tube attached to it. This led normally into a dark glass collecting-bottle containing acetic acid and toluene that stood outside the cot. When the child went to the breast, however, the tube was clipped off near its end, and urine passed during feeding time collected in the apparatus and tube and was drained off into the bottle when the child returned to its cot. The children were not bathed during the experimental periods, but were washed without being immersed.

Meconium and faeces were caught in mackintosh squares inside the usual napkins, and this method was found to be satisfactory for all except the most liquid faeces. Rather surprisingly, the use of the mackintosh for several days on end did not cause any skin trouble, possibly because urine did not come into contact with the skin. Meconium or faeces adhering to the skin was removed with a spatula. The faeces were collected every four hours and were stirred up as soon as possible with a few drops of hydrochloric acid and toluene. They were kept in porcelain or dark glass containers.

At both the hospitals where this work was done it was the practice to give no fluid by mouth in the first 36 hours after birth, unless the child was obviously thirsty; he was then given 15 to 20 ml. of water sweetened with sugar. After about 36 hours the child was put to the breast. Of the children used for the investigation, all except one were wholly breast fed during the period covered by the collections.

Samples of milk were obtained by expression. They were not acidified, but were placed immediately in a refrigerator and analysed as soon as possible. Urines were protected throughout against daylight, which would have destroyed riboflavin, and were also analysed without delay. The faecal specimens were kept in a refrigerator until they could be analysed.

Aneurin was estimated by a modification of the thiochrome method similar to that described by Greenberg and Rinehart (1945). Each sample of food and faeces was extracted with a 0.65 % solution of HPO_3 , and the extract was incubated with takadiastase to convert cocarboxylase to the free vitamin. Urine was not treated with takadiastase. The extract, or the diluted sample of urine, was passed through a column containing 2 g. 'decalso,' and the adsorbed aneurin was eluted with a 25% solution of KCl containing sufficient HCl to bring the normality to 0.1. The aneurin in the eluate was oxidized to thiochrome by means of alkaline ferricyanide under rigidly controlled conditions. For the blank the ferricyanide was omitted. An internal standard was included in each determination by adding a known amount of aneurin to a portion of the extract or diluted urine before adsorption, and carrying out a second analysis on this solution. Under these conditions a linear relationship was obtained between the galvanometer reading and the amount of aneurin present in the sample.

As Najjar and Ketron (1944) have pointed out, the thiochrome method gives erroneous results when N-methylnicotinamide is present, for example in the estimation of aneurin in urine. However, since the error varies directly with the amount of N-methylnicotinamide present, and since N-methylnicotinamide was estimated in every sample which contained this compound, it was possible to apply a correction. The correction used, which was determined by experiment, was as follows:

$$\text{Corrected aneurin concentration } (\mu\text{g./g.}) = \text{uncorrected aneurin concentration } (\mu\text{g./g.}) - 0.009 \times \text{N-methylnicotinamide concentration } (\mu\text{g./g.})$$

Riboflavin was estimated by the method of Slater and Morell (1946), using an artificial source of ultra-violet light instead of sunlight for the destruction of riboflavin in the pyridine-butanol extracts. The tubes containing the extracts were placed in a circular rack which could

be made to rotate slowly, by means of an electric motor, around an ultra-violet lamp. In this way all extracts received the same amount of irradiation. Riboflavin was extracted from all samples of foods and faeces by autoclaving in 0.25 N H_2SO_4 solution for 35 minutes at 120° C. An internal standard was included in each determination in the manner described by Slater and Morell. The relationship between the galvanometer deflection and the amount of riboflavin present was linear.

Nicotinic acid was estimated in foods and faeces by the method of Dann and Handler (1941). Nicotinic acid and its acid-hydrolysable derivatives in urine were estimated by the method of Perlzweig, Levy, and Sarett (1940); in this method concentrated hydrochloric acid is used for the hydrolysis. N-methylnicotinamide was estimated by the method of Huff and Perlzweig (1947). Since this method was worked out only for urine, an attempt was made to confirm the results obtained for faeces by another method. The colorimetric method of Sarett (1943) for total derivatives of N-methylnicotinic acid was tried, but accurate results could not be obtained, since the colouring matter in the extracts was not entirely removed by the preliminary absorption treatment. A red colour was, however, produced after the addition of benzidine, thus indicating that N-methylnicotinamide was present, and confirming, to some extent, the results obtained by the method of Huff and Perlzweig.

Results

Urinary Concentrations and Output. Before the balance experiments were started a preliminary study was made of the levels of urinary excretion of the vitamins on each successive day during the first week after birth. Some of the results are given in Table 1. Total N was also estimated in the urine of one infant. The length of the first period was in every case less

TABLE 1
URINARY EXCRETION OF B VITAMINS AND TOTAL N BY INFANTS IN THE FIRST WEEK AFTER BIRTH

Day	Infant No. 1					Infant No. 2		Infant No. 3	
	Aneurin		N-methylnicotinamide		Output Total N (mg.)	Riboflavin		Riboflavin	
	Concentration ($\mu\text{g./ml.}$)	Output ($\mu\text{g.}$)	Concentration ($\mu\text{g./ml.}$)	Output ($\mu\text{g.}$)		Concentration ($\mu\text{g./ml.}$)	Output ($\mu\text{g.}$)	Concentration ($\mu\text{g./ml.}$)	Output ($\mu\text{g.}$)
1	0.17	3(a)	45	769(a)	44(a)	0.23	4(b)	—	—
2	0.70	5	82	621	31	0.31	6	1.50	15(c)
3	0.83	29	89	3,104	247	0.49	9	0.38	4
4	0.57	3	72	430	24	0.09	7	0.03	0.2
5	0.20	6	23	652	52	0.04	5	0.29	3
6	0.19	19	17	1,623	205	0.04	4	0.14	16
7	0.14	4	12	355	77	—	—	—	—
8	—	—	11	698	95	—	—	—	—

(a) 0-14 hours after birth.

(b) 0-20 hours after birth.

(c) Collection began 17 hours after birth.

than a full day but the later periods were always 24 hours long.

Perhaps the most striking feature of the results is the relatively large amount of N-methylnicotinamide which is apparently excreted by the newborn child. This phenomenon was not especially commented on by Coulson and Stewart (1946) but was discussed by Hamil *et al.* (1947). As a confirmatory measure, the N-methylnicotinamide in the urine produced by an infant on the first day after birth was estimated by the method of Sarett (1943) as well as by that of Huff and Perlzweig. The two results were almost identical; one indicated an excretion of 3,136 $\mu\text{g.}$ per day and the other 3,065 $\mu\text{g.}$ per day.

The concentrations of aneurin, riboflavin, and N-methylnicotinamide in the urine tended to be much higher on the first three or four days after birth than subsequently, and the maximum concentrations occurred on the second or third day. These results confirm the findings of Neuweiler (1943) and of Hamil *et al.* The alterations in concentration followed a regular pattern and were probably due, to a large extent, to alterations in water balance. The total daily excretions were much more irregular and for this there may have been several reasons. The simplest would be that the collections were incomplete. The supervision of the infants was, however, so close that it is unlikely that enough urine was lost to affect the results appreciably. It was more probable that the amounts of urine which were collected had been formed over periods whose length could not be exactly determined, because the bladder was not always completely emptied when the infants passed urine spontaneously. The most likely reason is that there is an inherent irregularity in the metabolism of the newborn infant. The total output of N was also irregular, presumably for the same reasons: similar irregularities in the N excretion of infants have been

observed by other workers (Reusing, 1895; Birk, 1912; Thomson, 1944; Barlow and McCance, 1948), and the question has been discussed by Barlow and McCance.

Hamil *et al.* reported a sharp falling-off in the excretion of the B vitamins towards the end of the first week. These authors did not, however, actually measure the daily urinary volumes of the infants they studied. Instead they took average values for urinary volume found by other investigators and used them for the calculation of the vitamin excretions.

Balance Experiments. The results of the two balance experiments are summarized in Tables 2, 3, and 4. Each period was a full 24 hours, the first starting at the time of birth. Both infants were fed by their mothers, but one of them (No. 5) received supplementary feeds of cow's milk and oat flour. These feeds were analysed, but the milk of the mother was so scanty that none could be obtained for analysis. The mother of infant No. 4 had plenty of milk, and results obtained by analysing a sample taken on the sixth day were used in calculating the B vitamin intakes of both children. This may have given values for the earlier days which were somewhat too high, because the concentration of B vitamins in the milk is greater at the end of the first week of lactation than at its beginning (Roderuck, Williams, and Macy, 1946; Roderuck, Coryell, Williams, and Macy, 1946).

In general, the data for the urinary excretions confirmed the results of the preliminary studies.

Aneurin. Meconium passed at birth by child No. 5 was analysed, but no aneurin was found; apparently none had entered the gut up to the time of birth, or it had been destroyed. Excretion by both children began on the day after birth (Table 2) and tended to rise towards the end of the week. Nevertheless, the balances were probably positive by the fourth or fifth day. The aneurin excreted

TABLE 2
ANEURIN BALANCES IN THE FIRST WEEK AFTER BIRTH

Day after Birth	Infant No. 4					Infant No. 5				
	Intake ($\mu\text{g.}$)	Output ($\mu\text{g.}$)			Balance	Intake ($\mu\text{g.}$)	Output ($\mu\text{g.}$)			Balance
		Urine	Faeces	Total			Urine	Faeces	Total	
1	0	13	2	15	Negative	0	—	7	—	Negative
2	9	—	11	—	Negative	7	—	11	—	Negative
3	26	17	19	36	Negative	34	—	2	—	Doubtful
4	37	22	57	79	Negative	40	6	14	20	Positive
5	48	21	—	—	Doubtful	52	9	25	34	Positive
6	37	3	26	29	Positive	65	10	19	29	Positive
7	37	11	—	—	Doubtful	75	5	50	55	Positive

may have been derived partly from desquamated cells and partly from food residues; it is possible that some had been produced by bacterial synthesis.

Riboflavin. The urine passed by child No. 4 at birth, and the urine and meconium passed then by child No. 5 contained small amounts of riboflavin (Table 3). On the first day both children excreted fairly large quantities of the vitamin, mostly in the meconium and faeces: in the next days the excretion tended to fall, and by the end of the week the two balances were positive. The results suggest the rapid exhaustion of a store in the gut derived from amniotic fluid, cells, or secretions, and although it

is possible that by the end of the week some synthesis had begun, the more likely source of the riboflavin which was excreted was the food.

Nicotinic Acid and its Metabolites. The meconium passed at birth by child No. 5 contained considerable amounts of N-methylnicotinamide (Table 4), and in the next days large quantities of this metabolite were excreted, most of it in the urine. The balances were almost certainly negative all the week, but became less so towards the end.* No conclusions can be drawn from the values for the excretion of nicotinic acid by the children. The high figures for the urinary excretion of N-methylnicotinamide may be partly explained by the finding (Dean and Holman, 1949) that newborn infants excrete on an average only about one-third as much N-methyl-2-pyridone-5-carboxylamide as N-methylnicotinamide, whereas adults excrete almost twice as much. It was

* In the calculation of the intake of nicotinic acid, no allowance was made for amounts which might have been derived from tryptophane. Evidence has recently been produced that the acid can be formed in this way by children aged 3 to 24 months (Snyderman, Ketron, Carretero, and Holt, (1949) and by adults (Holman and de Lange, 1950). The ability of the newborn infant to effect the conversion obviously needs investigation.

TABLE 3
RIBOFLAVIN BALANCES IN THE FIRST WEEK AFTER BIRTH

Day after Birth	INFANT No. 4					INFANT No. 5				
	Intake	Output (μg.)			Balance	Intake	Output (μg.)			Balance
		Urine	Faeces	Total			Urine	Faeces	Total	
At birth		1	—	—			1	8	9	
1	0	29	47	76	Negative	0	17	68	85	Negative
2	26	12	110	122	Negative	19	8	40	48	Negative
3	72	7	48	55	Positive	71	—	10	—	Positive
4	103	4	38	42	Positive	86	8	20	28	Positive
5	134	2	—	—	Positive	111	20	29	49	Positive
6	106	0.2	19	19	Positive	136	12	12	24	Positive
7	106	3	—	—	Positive	157	6	41	47	Positive

TABLE 4
NICOTINIC ACID BALANCES IN THE FIRST WEEK AFTER BIRTH

Day	INFANT No. 4					INFANT No. 5				
	Intake of Nicotinic Acid (μg.)	Output			Balance	Intake of Nicotinic Acid (μg.)	Output			Balance
		N-methyl-nicotinamide*		Nicotinic Acid			N-methyl-nicotinamide*		Nicotinic Acid	
		Urine (μg.)	Faeces (μg.)	Urine (μg.)			Urine (μg.)	Faeces (μg.)	Faeces (μg.)	
At birth		—	—	—			71	106	—	
1	0	2,759	113	77	Negative	0	1,587	1,006	211	Negative
2	121	939	230	30	Negative	88	241	289	230	Negative
3	330	1,756	196	42	Negative	462	310	25	—	Doubtful
4	473	2,920	153	47	Negative	544	818	162	142	Negative
5	616	1,927	—	45	Negative	707	1,393	42	162	Negative
6	484	182	43	19	Doubtful	870	1,271	17	—	Negative
7	484	1,111	—	68	Negative	1,006	339	121	236	Doubtful

* Expressed as nicotinic acid.

suggested that the difference could be accounted for by a lower level of enzyme activity in the infant. The method used for the estimation of the N-methylnicotinamide is probably not specific for this substance; other substances such as coenzymes are almost certainly estimated and they also may be excreted in relatively large amounts by the child.

Little is known about the nicotinic acid metabolism of the human foetus and of the newborn infant. However, it has been established (Dean and Holman, 1948) that the concentrations of nicotinic acid in the blood of the mother and child at birth are approximately equal, and it seems unlikely from this and other evidence (Lwoff, Morel, and Dignonnet, 1941) that the child at birth possesses any large store of the acid. In an attempt to throw further light on the question, amniotic fluid was analysed for N-methylnicotinamide, the method of Huff and Perlzweig (1947) being used. The results indicated the presence of amounts of the order of 0.6 to 1.4 $\mu\text{g./ml.}$, but owing to the unusually high 'blank' fluorescence, the concentrations cannot be stated with certainty. These amounts may originate entirely as the result of urinary excretion into the amniotic fluid by the foetus, and this fluid, swallowed by the infant before birth, may provide some of the vitamin found in the meconium and the first faeces. Small amounts may be derived from intestinal secretions or desquamated cells, but it is extremely unlikely that micro-organisms capable of synthesizing the vitamin are present in the gut before birth. The fall in the output after birth suggests an exhaustion of the original supply, and very little synthesis by intestinal organisms in the first few days of life. The possibility that a part of the faecal N-methylnicotinamide was derived from unaltered food residues was eliminated by analysing a sample of human milk for N-methylnicotinamide by the method of Huff and Perlzweig (1947). No detectable amount was present.

Discussion

Although our results may have contributed towards the definition of the status of the newborn child in regard to some of the B vitamins, they do not provide clear evidence of synthesis in the gut. For that purpose methods are needed which will serve to differentiate between amounts of the vitamins acquired during intra-uterine life and others derived from bacterial action or from food residues. It seems unlikely, on the basis of the present evidence, that synthesis of any of the three vitamins studied occurs in appreciable quantities before the end of the first week.

Summary

The excretion and intake of aneurin, riboflavin, nicotinic acid and its derivatives have been studied

in newborn infants. After preliminary studies of urinary excretion balance experiments were conducted on two infants.

The aneurin and riboflavin balances were probably negative for about four days, and then became positive.

The nicotinic acid balance remained negative until the end of the first week, largely because of the extremely high excretion of N-methylnicotinamide. This high excretion may be due to the fact that infants, unlike adults, appear to produce in their urine much less N-methyl-2-pyridone-5-carboxylamide than N-methylnicotinamide.

No clear evidence of bacterial synthesis of B vitamins was obtained.

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