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The vitamin B_{12} content of meals and items of diet

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1. The vitamin B_{12} content of items of diet and of meals was measured after they had been prepared for consumption.

2. There was poor agreement between the vitamin B_{12} contents of meals estimated by measurements on meal homogenates and the contents estimated by calculations involving the weights and vitamin B_{12} contents of items.

3. The average amount of vitamin B_{12} absorbed per d from a hospital diet was found to be 1·7 μg.

Estimation of the dietary intake of vitamin B_{12} by weighing items of diet and referring to information on the vitamin B_{12} content of foodstuffs presented by McCance & Widdowson (1969), Robinson (1966), Chanarin (1969) and Love (1970) is hampered by lack of information about some items, paucity of information about the range of values likely to be encountered and by the fact that most of the information available relates to uncooked foodstuffs and therefore does not allow for weight changes in cooking (McCance & Widdowson, 1969) or for losses or destruction of the vitamin in cooking (Banerjee & Chatterjea, 1963; Heyssel, Bozian, Darby & Bell, 1966). Given the appropriate information it might be assumed that the vitamin B_{12} content of a meal could be calculated with accuracy if the weights of the items were known but this assumption has not been tested.

We therefore examined the vitamin B_{12} contents of prepared items of diet and of meals, with the objects of obtaining information not currently available, and of comparing the values for meals obtained by calculations involving the weight and vitamin B_{12} content of items with those obtained by measurements made on meal homogenates.

EXPERIMENTAL

The vitamin B_{12} content of items of diet and of meals was measured by microbiological assay using the method of Hutner, Bach & Ross (1956) and *Euglena gracilis* Z strain as the test organism.

Items of diet. Most of the items were prepared in the hospital kitchen and a few, mostly canned, were obtained commercially or from domestic sources. Most were weighed, before and after removal of inedible material if necessary, without treatment but portions of canned fish and curried beef were washed briefly under running water to remove oils, sauces and gravies. The portions, which finally weighed between 20 and 100 g, were then homogenized with suitable volumes of distilled water in a Waring Blendor for 10 min and samples stored at −20°. After thawing, and before assay, each sample was homogenized with a Silverson microhomogenizer for 2 min.

Table 1. *Mean values, ranges and standard deviations for vitamin B_{12} content ($\mu g/kg$ or $\mu g/l$) for various items of food prepared for consumption*

Food and method of preparation	No. of samples	Vitamin B_{12} Mean	Vitamin B_{12} Range	Vitamin B_{12} SD	Mean recovery (%)
	Meat and poultry				
Beef: brisket, boiled	6	11·9	10·6–13·6	1·0	108
rump, braised	6	8·0	3·7–13·1	3·4	131
sirloin, roast	6	10·2	6·7–12·9	2·7	118
shoulder, curried	7	13·1	5·0–22·2	6·4	96
stewed	5	13·9	9·6–20·6	6·6	102
minced, stewed, with gravy	6	8·7	5·2–17·2	4·5	135
corned, canned (Fray Bentos)	6	17·4	11·4–27·0	5·8	80
Beefburger, fried	6	16·3	11·1–28·9	6·7	103
Beef olive, braised	5	13·9	11·6–15·1	1·4	94
Chicken: red and white meat, casserole	5	3·3	2·0–4·8	1·2	113
curried	6	5·6	2·7–13·0	3·9	63
fricassee	3	2·5	1·9–2·9	—	66
roast	5	5·5	2·9–8·0	2·4	104
breast, canned (Ye Olde Oake)	6	2·1	1·0–2·5	0·8	86
Ham: baked	6	5·2	3·4–6·9	1·4	112
gammon, boiled	6	3·1	1·8–6·6	1·8	108
Ham and chicken roll, canned (Crosse and Blackwell)	6	8·9	7·5–10·6	1·2	56
Ham and pork, chopped, canned (Plumrose)	6	5·5	4·9–6·2	0·6	82
Kidney: sheep, braised	5	791	526–1104	252	81
ox, braised	3	58	48–71	—	59
Lamb: chop, braised	7	13·5	4·5–23·1	6·7	103
leg, roast	6	16·3	12·6–22·2	3·9	110
Liver, ox, braised	6	759	535–1115	193	65
Luncheon meat, canned (Ye Olde Oake)	7	7·9	5·9–12·3	2·0	75
Meat roll	3	15·6	9·2–25·3	—	100
Mutton: shoulder, boiled	5	12·6	4·2–16·7	5·0	111
leg, roast	2	10·6	8·3–22·9	—	95
Mutton pie	2	3·2	2·5–3·8	—	90
Pork: chop, braised	2	4·0	3·2–4·7	—	118
leg, roast	6	4·5	1·3–8·2	2·3	93
Sausages: beef, links, braised	6	6·7	3·2–15·4	4·2	76
slices, fried	6	9·2	6·4–12·8	2·3	79
beef and pork, canned (Royal Dane)	6	4·0	3·0–5·1	0·9	105
Sausage roll	2	1·1	1·0–1·2	—	98
Spam	6	5·9	2·9–9·3	2·2	91
Sweetbreads, poached, with sauce	4	9·4	5·7–13·0	3·1	95
Steak and kidney pie	3	37·4	4·8–71·1	—	108
Tongue: ox, boiled	6	33	22–49	10·3	95
lamb, canned (Gold Dish)	6	38·7	30·6–51·8	8·0	80
Tripe, poached, with sauce	4	3·0	1·7–7·9	3·8	67
Turkey, breast, roast	6	22·3	4·1–32·9	18·1	96
Veal fricassee	3	9·1	5·2–15·8	—	105
jellied	6	14·4	9·5–21·7	4·2	89
Veal and ham pie	2	2·3	2·3–2·4	—	96

Table 1 (*cont.*)

Food and method of preparation	No. of samples	Vitamin B_{12} Mean	Range	SD	Mean recovery (%)
		Fish			
Anchovy, fillets, canned (Bermeo)	6	218·4	167–288	42	84
Brisling, Norwegian, canned (John West)	6	110	97–139	14	88
Crab, Japanese, canned (Grand Prix)	6	5·3	3–8·5	1·8	102
Cod, fillet, fried	2	8·5	7·0–10·1	—	86
Cutlet, poached	3	8·6	6·6–10·1	—	109
Fishcake, grilled	6	6·9	3·0–11	2·8	109
Fishfingers, grilled (Birds Eye)	6	4·7	2·6–6·2	1·5	98
Haddock: fried	6	9·3	7·0–12·1	1·9	87
grilled	7	11·8	5·4–18·0	5·4	65
poached, boiled, steamed	11	7·0	5·2–12·8	2·2	93
Herring: fillets, canned (John West)	6	85	49–118	26	81
grilled	5	53	35–88	22	107
pickled	6	80	62–96	12	86
Kipper: fillets, canned (John West)	6	101	63–132	26	96
poached	4	65	36–102	28	88
Pilchards, canned (John West)	6	119	91–148	18	57
Prawns, canned (John West)	6	5·5	3·3–8·2	2	104
Salmon, canned (John West)	6	25·9	19·5–37·0	6·0	75
Sardines, canned (John West)	6	124	96–148	20	86
Shrimps, canned (John West)	6	7·9	6·5–9·9	1·5	97
Tuna, canned (John West)	6	46	32–58	11	72
Whiting, fried	3	10·0	2·8–19·3	—	83
		Dairy products			
Cheese: Brie	4	12·9	8·9–14·9	2·8	86
Cheddar	6	7·7	4·3–10·4	2·1	82
Cheshire	4	8·9	5·5–13·3	3·5	83
Danish Blue	6	11·8	8·8–16·2	2·9	90
processed	7	8·3	4·4–14·0	4·1	99
Ice-cream	7	4·1	3·0–6·1	1·1	75
Milk: whole, fresh	7	6·5	3·6–10·4	2·3	71
whole, pasteurized	10	3·9	2·7–5·9	0·9	95
evaporated, unsweetened (Carnation)	7	1·5	0·9–1·8	0·3	75
Yoghurt	6	1·8	0·9–2·9	0·7	73
		Miscellaneous			
Black pudding	4	2·9	2·4–3·5	0·4	68
Haggis	6	10·7	7·2–15·1	3·6	73
Rice Krispies	2	1·3	1·3–1·4	—	65
Scotch egg	6	7·5	5·4–12·8	2·8	79
Egg: boiled	6	10·3	3·5–18·7	6·0	81
fried	6	6·1	1·9–12·0	3·9	37
scrambled	6	3·0	2·0–4·1	1·1	69

Samples were assayed in duplicate on at least two occasions. If the second assay result differed from the first assay result by more than 20% the sample was assayed for a third time. At least one recovery experiment was performed with each sample. For this purpose cyanocobalamin was added to the assay tubes so that the concentration should have been increased by 10 pg/ml above that due to the sample. The difference between the observed and expected increase was expressed as a percentage.

Meals. The meals examined were replicates of those prepared in the hospital kitchen and served to patients; each item was weighed and reweighed after removal of inedible material and the meal homogenized as described above. In addition to the standard assay and recovery procedures the effect of added cyanide was examined in ten samples. Preliminary studies on the effect of adding sodium cyanide to assay tubes containing cyanocobalamin in concentrations of 5–50 pg/ml showed that the growth of *Euglena* was not inhibited until 40 μl of 100 mM-sodium cyanide had been added. In this study 40 μl of 10 μM-sodium cyanide were added, this being well below the toxic level for *Euglena* yet adequate for conversion of other forms of vitamin B_{12} into cyanocobalamin.

RESULTS

Preliminary investigations showed a marked fall in microbiological activity of many samples when assayed for the second time. This was considered to be related to refreezing and was abolished by homogenizing the thawed sample. This practice was therefore adopted routinely and only results using this procedure are reported.

With items of food, the second assay result was within $\pm$ 20 % of the first assay result in 329 out of 491 samples (67 %) and, with meals, in 23 out of 45 samples (51 %).

Addition of cyanide to meal homogenates before assay had no effect on the microbiological activity in any of the samples examined.

Items of diet which had values of less than 1 μg per kg solid item or per l liquid item were regarded as being devoid of vitamin B_{12} and were obtained from at least one sample of apples, apricots, bananas, French beans, baked beans, white bread, brown bread, broth, cabbage, carrots, custard, corn flakes, jams, macaroni, oranges, peas, peaches, pears, porridge, potatoes, pineapple, rice, sago, soups, spaghetti, sprouts, tea and turnip. Items which had values greater than 1 μg per kg solid item or per l liquid item are listed in Table 1, that is meat and poultry, fish, dairy products and miscellaneous items (including eggs), together with relevant information and results.

All forty-five meals examined had vitamin B_{12} contents greater than 0·1 μg; the results are shown in Table 2, in which are also given the vitamin B_{12}-containing items in each meal, a range of calculated vitamin B_{12} values for each meal based on those shown in Tables 1, and the protein and energy contents of the meals calculated by reference to McCance & Widdowson (1969). The recoveries in assays of meal homogenates were similar to those found for items and ranged from 55 to 130 % with a mean of 83 %.

When the mean values for items were used, the calculated meal value was greater than the meal homogenate value in twenty instances and less in twenty-five. The magnitude of the difference between the calculated and homogenate values, expressed as a percentage of the homogenate value, varied considerably and details are given in Table 3. When the range of values for each item was used, the calculated ranges for the meals were about equal to the homogenate values in twenty-six out of forty-five instances, the homogenate value being greater in thirteen and less in six.

Table 2. *Protein, energy and vitamin B_{12} contents of forty-five meals*

(The vitamin B_{12} contents were obtained by direct assay of meal homogenates and also by calculations using the values in Table 1. Only the vitamin B_{12}-containing items in each meal are listed and small amounts of milk, as used with tea, have been disregarded)

			Vitamin B_{12} (µg)		
				Calculated	
Vitamin B_{12}-containing items	Protein* (g)	Energy* (MJ)	Direct assay	Mean	Range
Breakfast:					
Fried egg; braised (sheep) kidney	21	1·57	52·0	27·12	18·0–38·0
Fried bacon; fried egg; milk	21	2·21	2·08	0·59	0·35–1·04
Fish, poached cutlet	17	1·23	1·90	0·69	0·53–0·81
Scrambled egg; fried sausage	22	1·74	1·64	0·97	0·65–1·34
Fried bacon; scambled egg	23	1·92	0·32	0·26	0·18–0·36
Rice Krispies; fried bacon; milk	15	1·60	0·42	0·51	0·33–0·69
Rice Krispies; poached egg; milk	15	1·73	1·38	0·86	0·52–1·17
Rice Krispies; fried sausage; milk	19	2·16	1·14	0·92	0·93–1·62
Fried sausages; milk	14	4·13	0·32	0·59	0·39–1·04
Lunch:					
Fried haddock	23	2·20	0·33	0·66	0·49–0·86
Minced beef	62	3·70	0·60	1·31	0·78–2·58
Roast beef	26	2·17	7·15	0·62	0·41–0·79
Boiled beef	30	2·37	1·75	0·81	0·72–0·93
Fried haddock	22	1·93	1·46	0·64	0·48–0·84
Poached haddock; ice-cream	24	1·64	0·96	1·02	0·76–1·35
Roast beef	31	2·72	1·34	0·45	0·29–0·57
Braised (ox) liver; fried sausages	41	3·52	77·1	59·4	41·9–87·4
Roast lamb; ice-cream	21	2·15	0·94	1·09	0·83–1·49
Sweetbreads	27	2·50	0·35	0·48	0·29–0·66
Minced beef; ice-cream	25	2·64	1·73	1·37	0·84–2·62
Meat roll	19	2·10	0·78	0·67	0·40–1·09
Poached haddock	25	1·98	1·04	0·48	0·38–0·82
Curried beef	18	2·27	1·70	1·06	0·41–1·79
Steak and kidney pie	23	2·03	0·56	4·26	0·55–8·11
Meat roll	18	2·58	0·62	0·89	0·53–1·44
Veal fricassee	37	1·63	0·58	1·02	0·58–1·77
Lamb chop	17	2·12	0·22	0·31	0·10–0·53
Roast lamb	21	1·61	1·10	1·00	0·77–1·36
Fish, poached cutlet	32	2·60	1·16	0·47	0·39–0·86
Mince-pie	30	2·05	0·91	0·80	0·48–1·58
Roast beef	40	3·85	0·45	0·62	0·41–0·79
Steak pie	36	3·59	0·68	0·73	0·54–1·13
Minced beef; ice-cream	37	2·67	0·74	1·59	0·97–3·07
Pickled herring	16	1·10	11·0	7·9	6·1–9·4
Sweetbreads	33	2·69	0·89	1·02	0·61–1·4
Mince pie; ice-cream	23	2·31	1·51	0·62	0·46–1·41
Boiled egg; milk	21	2·03	0·91	1·26	0·75–2·04
Supper:					
Boiled chicken; milk	22	2·36	0·14	0·44	0·22–0·44
Chicken croquette	11	0·50	0·11	0·10	0·06–0·15
Fishcake	9	1·02	0·51	0·44	0·18–0·69
Mutton pie	15	1·66	0·23	0·30	0·21–0·36
Minced beef	27	1·33	0·65	0·73	0·44–1·44
Boiled egg; cheese; milk	17	2·06	0·76	0·43	0·30–1·12
Chicken fricassee	25	1·14	0·19	0·23	0·17–0·26
Spam	5	0·71	0·20	0·21	0·10–0·32

* Calculated by reference to McCance & Widdowson (1969), but energy expressed in MJ instead of kcal.

Table 3. *Magnitude of the differences in the vitamin B_{12} content of forty-five meals determined by calculations using the vitamin B_{12} content of items and by assay of meal homogenates. The differences are expressed as percentages of the homogenate value*

	Differences in vitamin B_{12} content											
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120
Calculated > homogenate	3	3	3	3	3	0	0	1	2	0	1	1
Calculated < homogenate	2	5	3	2	3	5	2	1	0	1	0	1
Total	5	8	6	5	6	5	2	2	2	1	1	2

DISCUSSION

Two technical aspects of the study merit comment. The marked fall in activity of samples which had been frozen on more than one occasion had not been encountered with human or animal tissues and was, therefore, unexpected. Although known in analytical chemistry, the effect does not appear to have been reported previously in relation to microbiological assays for vitamin B_{12}. Whatever the nature of the effect, all that is required from the practical aspect is foresight and the necessity for further homogenization. Addition of cyanide augments the yield in assays of vitamin B_{12} of human tissues with *Lactobacillus leichmannii* (see Chanarin, 1969), but has a statistically non-significant effect when *Euglena gracilis* is used (Anderson, 1965)—the lack of effect was not surprising.

Some values for items of diet differ from those presented by McCance & Widdowson (1969), the mean values reported here being greater for fresh milk, eggs, ox liver, salmon and sardines; and lower for beef, ham, pork, mutton and lamb, ox kidney, Cheddar cheese and herring. Whether the differences are related to the effects of cooking or to seasonal, geographical or other factors is not clear. The results obtained from fresh and pasteurized milks are in keeping with the reduction in vitamin B_{12} content during pasteurization reported by Chapman, Ford, Kon, Thompson, Rowland, Crossley & Rothwell (1957) and Karlin (1969), and there was no evidence of seasonal variation in the vitamin B_{12} content of pasteurized milk.

The average daily intake is used as an index of vitamin B_{12} nutrition. Values of 5–7 μg have been suggested by Jolliffe & Peterman (1956), Estren, Brody & Wasserman (1958), Gräsbeck (1960) and by a joint FAO/WHO Expert Group (WHO, 1970); and values of 2·7 μg for a grossly nutritionally inadequate diet, 16·0 μg for a low-cost diet and 31·6 μg for a high-cost diet were reported by Chung, Pearson, Darby, Miller & Goldsmith (1961). There are two reasons why the average daily intake is not a satisfactory index of nutrition with vitamin B_{12}. In the first place, it conceals the contribution by unusually high values; with the meal homogenate results, summation of the means of breakfasts (6·8 μg), of lunches (5·2 μg) and of suppers (0·35 μg) gives an average daily intake of 11·6 μg, but exclusion of two unusually high results reduces this to 3·1 μg. In the second place, the average daily intake does not account for the decrease in fraction, but increase in absolute amount, absorbed as the ingested mass is increased. An estimate of the average amount absorbed per d would be a better index; this can be obtained by assuming complete availability (Heyssel *et al.* 1966) and by taking the proportion absorbed from meals containing between 0·1 and 0·5 μg to be 75%, between 0·5 and 1·0 μg to be 60%, between 1 and 2 μg to be 50%, between 2 and 5 μg to be 33%, between 5 and 10 μg to be 33%, and above 10 μg to be 5%—these values being based on those reported by Chanarin (1969). Application of these proportions to the meal homogenate values gives an average amount absorbed from breakfasts of 0·79 μg, from lunches of 0·67 μg and from suppers of 0·22 μg. Assuming that the meals examined represented the hospital diet and that all items were acceptable, the figures sum to an average amount absorbed per d of 1·7 μg.

In assessments of nutritional status, calculations of intakes by dietary histories and reference to tables of values for items are held to be informative. It is important that the degree of accuracy of such procedures should be known; this was examined by comparing the intakes of vitamin B_{12}, calculated by weighing items, and by referring to the values in Table 1 with the results of meal homogenates. There was poor agreement both when the calculated values were obtained using a range of values for items and when the more convenient practice of using a mean value for an item was adopted, and from this it must be concluded that calculated estimates of dietary intake have a very limited place in the assessment of nutritional status with respect to vitamin B_{12}.

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