Vitamin B_{12} content of some articles of Indian diets and effect of cooking on it

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Vitamin B_{12} deficiency due to dietary inadequacy is not uncommon in India (Banerjee, Hosain & Chatterjea, 1959; Banerjee & Chatterjea, 1960). To know how to prevent this deficiency, it is necessary to have precise information on the distribution of vitamin B_{12} in Indian foods and the availability of the vitamin after cooking. Our study was undertaken to provide this information.

EXPERIMENTAL

The substances analysed were muscles from fourteen varieties of fish and prawn, muscle and liver of the goat, duck egg (yolk), cow's milk and twenty items of vegetable origin, including rice and pulses. Materials were analysed in (*a*) the natural raw (uncooked) state, (*b*) after boiling, (*c*) after cooking (frying and boiling).

Raw material. The fresh material (5 g) was homogenized with distilled water and transferred into a flask. To this homogenate, 25 mg of powdered papain (Biddle Sawyer & Co. Ind. Pvt. Ltd) and 0·1 ml of 1% (w/v) sodium cyanide solution were added, and the mixture was incubated under toluene at 37° for 72 h. For vegetables, 10 mg papain were used. After incubation, 40 ml of acetate buffer (pH 4·5) were added to the homogenate, which was then autoclaved at 10 lb pressure for 15 min and cooled, and the volume was made up to 200 ml. Next the extract was filtered and from the clear filtrate a measured portion was analysed for its vitamin B_{12} content.

Boiled material. The fresh material (5 g) was boiled with distilled water for 15 min and homogenized. The homogenate, together with the boiling water, was transferred to a flask and was treated similarly to that from the raw material.

Cooked material. The fresh material (5 g) was fried with mustard oil in an open aluminium pan for 10 min at a temperature varying between 110° and 120°. The fried material was then boiled with distilled water for 15 min and homogenized. The homogenate, together with the cooking water, was transferred to a flask and treated in the same way as the raw materials.

Control. The requisite amount of powdered papain and 0·1 ml of 1% (w/v) sodium cyanide solution were added to 30 ml distilled water and incubated under toluene at 37° for 72 h. After incubation, the mixture was treated in the same way as the raw material and its vitamin B_{12} activity was measured.

Vitamin B_{12} assay. Vitamin B_{12} was assayed by the method of Ross (1952) with *Euglena gracilis* var. *bacillaris* as test organism.

RESULTS

Vitamin B_{12} in the raw materials. Articles of vegetable origin did not show any vitamin B_{12} activity. Results obtained with those of animal origin in the raw state, after boiling or after cooking, are recorded in Table 1. Results shown in the table are the means of three observations for each sample. Some of the materials were analysed on two different occasions and are represented by two mean values.

Goat liver was found to be the richest source of vitamin B_{12} examined. A fair amount was present in nine out of fourteen varieties of fish, of which Tapsey (*Polynemus paradiseus*) and Tangra (*Mystus vittatus*) seemed to be the richest. Goat muscle was found not to be a good source of the vitamin. Egg yolk was a better source than goat muscle or some fish.

Table 1. *Vitamin B_{12} activity* (Euglena gracilis *assay*) *of articles of Indian diets, and effect of boiling and cooking*

Item	Local name	Vitamin B_{12} activity (μg/100 g fresh weight) Raw	Boiled	Cooked	Loss after cooking as a percentage of activity of boiled material
Fish (muscle):					
Catla buchanani	Catla	0·7	1·6	0·48	70·0
		2·08	4·5	0·61	86·4
Lates calcarifer	Vetki	3·4	1·0	0·5	50·0
Labeo rohita	Rui	3·6	—	—	—
		6·7	1·3	0·08	93·8
Mystus vittatus	Tangra	1·18	3·08	1·0	67·5
		7·5	4·85	3·7	23·7
Heteropneustis fossilis	Singee	4·04	1·36	0·8	41·1
Clarias batrachus	Magur	3·7	2·9	0·3	89·6
Anabas testudineus	Kai	1·9	1·1	0·65	40·9
Mugil parsia	Parsey	3·6	1·7	1·1	35·2
		5·6	2·7	2·0	25·9
Glossogobius giuris	Bele	2·2	—	2·2	—
Ophiocephalus striatus	Shole	1·0	—	0·3	—
Apocryptes lanceolatus	Guley	2·6	—	0·24	—
Wallago attu	Boal	4·6	1·36	1·32	—
Hilsa ilisha	Ilish	4·0	4·5	0·16	96·4
Polynemus paradiseus	Tapsey	8·0	1·0	0·47	53·0
Prawn	Bagda, chingree	1·01	—	0·45	—
Meat:					
goat muscle	Mangsha	1·0	0·26	0·08	69·6
		1·26	—	—	—
goat liver	Mete	40·0	104	26·0	75·0
		100	—	—	—
egg, duck	Dim (patihans)	—	6·0	—	—
Milk, cow's	—	—	4·8*	—	—

* μg/l.

Vitamin B_{12} in the boiled materials. The effect of boiling was studied in eleven varieties of fish; in nine, the values after boiling were lower than in the raw materials. In goat muscle also the value was lower after boiling. In three varieties of fish, and in goat liver on one occasion vitamin B_{12} activity was higher after boiling than in the raw

state. Higher values were noted on two occasions in a single variety of fish (*Catla buchanani*) and on one occasion in the other two types (*Mystus vittatus* and *Hilsa ilisha*). Egg yolk and cow's milk were found to contain significant amounts of the vitamin after boiling.

Vitamin B_{12} in the cooked materials. Except for one variety of fish (*Glossogobius giuris*), the vitamin B_{12} activity of the materials was lower after cooking than when they were either raw or boiled. This low value was noted even for the four articles that showed higher values after boiling. Vitamin B_{12} activity after cooking was less than that after boiling in twelve out of thirteen materials studied. The amount lost in cooking as a percentage of that left after boiling varied from 23·7 to 96·4.

DISCUSSION

The figures for the vitamin B_{12} contents of Indian fish agreed well with the results recorded by Teeri, Loughlin & Josselyn (1957) for some of the species of fish consumed in New England, USA. Our values also compare well with those recorded by Braekkan (1959) and Barrett & Widdowson (1960). But the values reported by Sreenivasamurthy, Swaminathan & Subrahmanyan (1955), Ueno (1956) and Love (1961) were somewhat higher. The vitamin B_{12} content of goat muscle was low and similar to those reported for mutton and lamb leg (Schweigert, Scheid & Marquette, 1951; Barrett & Widdowson, 1960). Goat liver was found to be the richest source of vitamin B_{12} examined, the value found being similar to that given by Shenoy & Ramasarma (1954) and also to that for beef liver reported by Schweigert *et al.* (1951). Our value for goat liver was much higher than that recorded by Sreenivasamurthy *et al.* (1955). A low value was also recorded for sheep liver (Barrett & Widdowson, 1960). Egg yolk contained a significant amount of vitamin B_{12}, similar to that reported by Sreenivasamurthy *et al.* (1955). A lower value for hen's egg was reported by Barrett & Widdowson (1960).

The vitamin B_{12} content of cow's milk was found to be close to that reported by other workers (Collins, Harper, Schreiber & Elvehjem, 1951; Antener, 1958–9; Sreenivasamurthy, Nambudripad & Iya, 1953; Macy, Kelly & Sloan, 1953). The vitamin B_{12} content of raw whole milk, determined by rat assay, ranged between 5·5 and 9·4 μg/l. (Hartman, Dryden & Riedel, 1956). Gregory, Ford & Kon (1958) reported wide variations in the vitamin B_{12} content of cow's milk. The vitamin B_{12} activity of goat liver, egg yolk and fish agreed well with the values for lamb liver, egg yolk and fish, respectively, as reported by Lichtenstein, Beloian & Murphy (1961).

A comparative analysis of the vitamin B_{12} contents of the raw and boiled materials would indicate that boiling as done in our experiments may lead to a considerable loss of the vitamin. This observation cannot be satisfactorily explained, since cyanocobalamin itself was not destroyed. The explanation may lie in the method of preparing the samples for assay. To begin with, the raw food was homogenized and incubated, without prior heat treatment, under toluene for 72 h at 37° in presence of papain. Toluene did not necessarily prevent all bacteria from growing, and there was the possibility that bacterial synthesis of vitamin B_{12} had occurred during this incubation

period. Further, the natural enzymes present in the raw food might have been active. In contrast, in the heated foods there was less likelihood of bacterial growth occurring, and the natural enzymes had been inactivated. These two factors could result in a higher vitamin B_{12} activity being measured in the raw foods, either because the natural enzymes were more effective in releasing the vitamin than papain or because bacterial synthesis of vitamin B_{12} had occurred.

Increase in vitamin B_{12} activity, as found in three varieties of fish and goat liver, could be due to better extraction of the vitamin by boiling.

Reports on the effect of cooking are scanty. We found that the vitamin B_{12} activity of almost all our materials was much lower in the cooked state than in the boiled state. Thus cooking entailed considerable loss in activity. In comparing the activities of the boiled and the cooked material, there was hardly any possibility that the results had been influenced by the variable factors of enzymic release or bacterial synthesis or both.

SUMMARY

1. To ascertain the distribution of vitamin B_{12} in Indian diets, several varieties of fish, goat muscle and liver, egg yolk and cow's milk were assayed. The effect of boiling and cooking on the availability of the vitamin was also investigated.

2. The material was homogenized in the natural raw state, or after boiling for 15 min or after cooking (frying and boiling). All three homogenates were digested with papain and sodium cyanide and extracted with acetate buffer. The vitamin B_{12} activity of the extracts was determined microbiologically with *Euglena gracilis*.

3. The largest amount of vitamin B_{12} activity was found in goat liver; most of the fish contained a fair amount.

4. Considerable amounts of vitamin B_{12} activity were destroyed by cooking. The loss, as a percentage of the activity after boiling, varied from 23·7 to 89·6%.

5. The results of the study are discussed in relation to other published work.

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REFERENCES

Antener, I. (1958–9). *Int. Z. Vitaminforsch.* **29**, 357. Quoted in *Nutr. Abstr. Rev.* (1960), **30**, 92.
Banerjee, D. K. & Chatterjea, J. B. (1960). *Brit. med. J.* ii, 992.
Banerjee, D. K., Hosain, F. & Chatterjea, J. B. (1959). *Bull. Calcutta Sch. trop. Med.* **7**, 147.
Barrett, I. M. & Widdowson, E. M. (1960). In *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 297, p. 161. [R. A. McCance and E. M. Widdowson, editors.]
Braekkan, O. R. (1959). *Fiskeridir. Skr. TechUndersøk.* **3**, 42. Quoted in *Nutr. Abstr. Rev.* (1960), **30**, 71.
Collins, R. A., Harper, A. E., Schreiber, M. & Elvehjem, C. A. (1951). *J. Nutr.* **43**, 313.
Gregory, M. E., Ford, J. E. & Kon, S. K. (1958). *J. Dairy Res.* **25**, 447.
Hartman, A. M., Dryden, L. P. & Riedel, G. H. (1956). *J. Nutr.* **59**, 77.
Lichtenstein, H., Beloian, A. & Murphy, E. W. (1961). *Res. Rep. U.S. Dep. Agric. Home Econ.* no. 13. Quoted in *Nutr. Abstr. Rev.* (1962), **32**, 431.
Love, R. M. (1961). In *Biochemists' Handbook*, p. 769. [C. Long, E. J. King and W. M. Sperry, editors.] London: E. and F. N. Spon Ltd.

Macy, I. G., Kelly, H. J. & Sloan, R. E. (1953). *Publ. nat. Res. Coun., Wash.*, no. 254. Quoted in *Biochemists' Handbook* (1961), p. 897. [C. Long, E. J. King and W. M. Sperry, editors.] London: E. and F. N. Spon Ltd.
Ross, G. I. M. (1952). *J. clin. Path.* **5**, 250.
Schweigert, B. S., Scheid, H. E. & Marquette, M. M. (1951). *Fed. Proc.* **10**, 394.
Shenoy, K. G. & Ramasarma, G. B. (1954). *Arch. Biochem. Biophys.* **51**, 371.
Sreenivasamurthy, V., Nambudripad, V. K. N. & Iya, K. K. (1953). *Indian J. Dairy Sci.* **6**, 105.
Sreenivasamurthy, V., Swaminathan, M. & Subrahmanyan, V. (1955). *Indian J. Physiol.* **9**, 33.
Teeri, A. E., Loughlin, M. E. & Josselyn, D. (1957). *Food Res.* **22**, 145.
Ueno, S. (1956). *Vitamins, Japan*, **9**, 45. Quoted in *Chem. Abstr.* (1956), **50**, 15619*b*.

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