EFFECT OF VITAMIN-B COMPLEX ON HEALTHY PEOPLE IN A WARM CLIMATE

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The question whether there is an increased need for vitamins of the B complex among people living in a warm climate has not been clearly answered as yet, but such vitamins are often prescribed in the tropics and elsewhere to people who show no signs of vitamin deficiency and whose diets are apparently adequate. More thiamine is excreted in the urine during hot than during cold weather (Holt, 1943), and this could be interpreted to mean that increased amounts of the vitamin should be taken in warm climates; but sweating does not increase the losses of thiamine through the skin (Lugg and Ellis, 1954), and there is no evidence of any prevalence of anaemia in an equatorial climate when economic conditions are good (Wadsworth, 1952a, 1952b, 1954). Since, however, the deep body temperature of healthy people at rest tends to be a little higher in a warm than in a cool climate (Adam and Ferres, 1954), while the rise of the deep body temperature which takes place during exercise is also enhanced in a warm environment (Martin, 1930), it is possible that the increase in enzyme activity resulting from such a raising of the body temperature would increase the demands for various vitamins, especially those of the B complex, and that mild symptoms of lassitude would follow which could explain the syndrome of tropical fatigue (Macpherson, 1949).

Mild general symptoms associated with slight vitamin deficiencies were, indeed, observed among soldiers serving overseas during the last war (Glaser, 1953b). A further possibility is that some inhabitants of the tropics may be suffering from undiagnosed sprue-like conditions, and that they could thus benefit from vitamin-B supplements.

In the absence of conclusive findings, it seemed important to ascertain whether there was any need for the excessive prescription of vitamin preparations prevalent in many tropical and equatorial areas, so that wasteful therapy should be avoided if it was useless or its general application ensured if it was of value. Since the level of serum cholinesterase is a sensitive indicator of the state of nutrition (McCance *et al.*, 1948; Hutchinson *et al.*, 1951), it was of interest to estimate at the same time the levels of cholinesterase in the blood.

Methods

In order to assess whether vitamins altered the incidence of mild subjective symptoms, a technique was modified which had been previously shown to allow unbiased assessment of subjective responses and their variations (Glaser, 1953a; Glaser and Whittow, 1954; Glaser and Newling, 1955). A class of 79 medical students took part in the experiment. Their ages ranged from 19 to 23 years and nine of them were women. Two-thirds of the subjects were Chinese and the rest were Indians, Sinhalese, Malays, or Eurasians. About half the students lived in university hostels and the other half in their own homes. The diet taken

by the middle-class population of Singapore and by students living in hostels contains a variety of lightly cooked fresh vegetables as well as salads, fruit, fresh meat (mostly pork), and some vitamin-enriched bread, so that it is likely to be adequate in vitamins of the B complex, even though polished rice forms the basis of most meals.

The subjects were told nothing about the purpose or the design of the investigation, except that they would be given vitamins, that they should take one tablet daily, and that they should take no other vitamin supplements of any kind before and during the experiment. They were also casually told that lost or damaged tablets would be replaced and that mutual borrowing of tablets was not a good thing. Previous experience with similar classes of students has shown that Singapore students co-operate most conscientiously in such experiments (Glaser, 1953a; Glaser and Whittow, 1954; Glaser and Newling, 1955) and there was every evidence that they did so in the present investigation. The students were divided at random into two groups, and the experiment was conducted in two periods of eight weeks separated by an interval of six weeks. At the beginning of each experimental period every subject received one dummy tablet daily for 14 days, after which half the subjects continued to receive dummy tablets and the other half were given one vitamin-B complex tablet daily, so that each subject took vitamins for the last six weeks of one experimental period and dummy tablets throughout the other period.

The vitamin tablets were made by a well-established firm, and their composition was claimed to be:

Thiamine hydrochloride	..	..	..	2	mg.
Riboflavin	..	..	..	2	mg.
Niacinamide	..	..	..	10	mg.
Pyridoxine hydrochloride	..	..	..	0.1	mg.
Calcium pantothenate	..	..	..	3	mg.
Folic acid	..	..	..	0.2	mg.
Vitamin B ₁₂	..	..	..	1	µg.
Insoluble liver fraction	..	..	..	250	mg.

The dummies were indistinguishable and contained nothing but lactose. All tablets were sugar-coated and were stored in airtight containers at 21° C. They were issued weekly in screw-top bottles labelled with each subject's name. Known dummy tablets were available to us while all subjects were taking dummies, but when vitamins were being taken all issuing of tablets was done from coded containers, so that we did not know which were the vitamins until all the results had been calculated.

The sensations and illnesses of the subjects were assessed before each experimental period and at intervals of one week thereafter, always at the same time of the day. Questionnaires were similar to those described before (Glaser and Whittow, 1954), and they were designed to avoid errors so far as was possible. Questions were included about skin lesions and illnesses, both at the moment of filling in the questionnaires and during the preceding six days.

Estimations of Cholinesterase

At the end of the experiment 35 subjects were asked to provide a sample of finger blood. In order to obtain a homogeneous group, these subjects were selected at random from among the largest single group in the experiment, Chinese men. Thirty-three of these subjects agreed to participate, and after the results had been worked out it was found that 15 had been taking vitamins for the last six weeks.

The red-cell ("true") cholinesterase was estimated as acetylcholinesterase and the plasma ("pseudo") cholinesterase as butyrylcholinesterase on samples of whole blood. Preliminary tests had shown that 87% of the activity of whole blood with regard to acetylcholine perchlorate was due to red cells and 82% of the activity with regard to butyrylcholine chloride was due to plasma. For estimations of the acetylcholinesterase, samples of 20 µl. of blood were run from the finger into 0.98 ml. of a 0.01% solution of saponin, identical samples being used for duplicate and control determinations. To duplicate diluted blood samples,

Symptoms and Illnesses Reported After Taking Dummy Tablets and After Taking Vitamin-B Complex Supplements in a Crossed-over Experiment

	No. Completing Questionnaires	Minor Illnesses During Last 6 Days		Condition at Moment of Completing Questionnaires											
				At Least One Symptom		Unhealed Cuts or Abrasions		Sleepiness		Fatigue		Feeling Better		Feeling Worse	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Two weeks on dummy tablets ..	79	8	10	49	62	8	10	31	39	34	43	7	9	2	3
Eight weeks on dummy tablets ..	70	2	3	28	40	2	3	22	31	19	27	7	10	3	4
Six weeks on vitamin tablets ..	73	8	11	49	67	6	8	21	29	28	38	6	8	1	1

1 ml. of 0.004 M acetylcholine perchlorate in 0.134 M phosphate buffer (pH 7.2) was added and the solutions were then thoroughly mixed and incubated in a water-bath at 30° C. ($\pm 0.1^\circ$ C.) for 10 minutes. A separate tube containing 1 ml. of the acetylcholine solution was incubated at the same time, and at the end of 10 minutes the control blood solution was poured into this tube.

The three solutions were now assayed for residual acetylcholine according to the method of Hestrin (1949), with the following modifications. Double the volume of the recommended reagents was used and the ferric chloride solution was 0.74 M. Protein precipitated in the final stages of the estimation was removed by filtration through Whatman No. 40 filter paper. Estimations were made in a Spekker photoelectric absorptiometer, using an OGI filter. The same technique was used for the estimation of butyrylcholinesterase, except that the substrate used was 0.004 M *n*-butyrylcholine chloride in 0.134 M phosphate buffer. Preliminary experiments had shown that no measurable non-enzymic hydrolysis occurred in the control tubes, that under the experimental conditions employed the amount of acetylcholine and butyrylcholine hydrolysed by the whole blood was linearly related to the time of incubation, and that in both estimations the results obtained with whole blood compared well with the summation of the results obtained when plasma and cells were separated in the normal way. The packed red-cell volumes were estimated from finger blood in duplicate heparinized haematocrit tubes and spun at 3,000 r.p.m. for 30 minutes. The results were expressed as μ M ester hydrolysed by 20 μ l. of whole blood in 10 minutes at 30° C. and at a pH of 7.2, and the values were corrected to a packed red-cell volume of 50%.

Results

There were no consistent differences between the incidence of subjective symptoms or of minor illnesses when the subjects were taking dummies and when they were taking vitamins. Indeed, slightly more symptoms and illnesses were recorded by the same subjects after taking vitamins than after taking dummies (see Table). It was impossible also to demonstrate any advantages of the vitamin supplements when comparisons were made between the responses of different subjects who had been taking vitamins and dummies at the same time. There were no illnesses involving hospital treatment, or absence from classes for more than one week. Mild symptoms were regularly reported, however (see Table), and this conforms with previous observations when subjects were given questionnaires but no pharmacologically active substances (Glaser, 1953a; Glaser and Whittow, 1954; Glaser and Newling, 1955). The incidence of symptoms gradually diminished when all subjects were given dummies only, and this also conforms with previous experience. Nevertheless, in the present investigation fewer symptoms were reported by subjects receiving dummies than in previous experiments, presumably because the subjects knew that they were to be given vitamins, whereas in the earlier experiments they were expecting to take drugs, so that they may have expected fewer symptoms in the present investigation than in previous ones. This aspect of the problem has been discussed elsewhere (Glaser and Whittow, 1957).

In 15 subjects who had been taking vitamins for six weeks the mean acetylcholinesterase level was 1.54 μ M (S.E., ± 0.05)

of ester hydrolysed, and in 18 subjects who had had dummies for three months the mean figure was 1.55 μ M (± 0.05). The mean butyrylcholinesterase level of the 15 subjects who had had vitamins was 0.74 μ M (± 0.05) and that of the 18 subjects who had had no vitamins for three months was 0.54 μ M (± 0.04), and the difference was statistically highly significant ($t=3.24$; $P<0.01$). Further control samples of 10 normal people working in the Faculty of Medicine of the University of Malaya gave average levels of 1.50 μ M (± 0.05) for acetylcholinesterase and 0.60 μ M (± 0.03) for butyrylcholinesterase.

Discussion

The results clearly show that supplements of vitamins of the B complex do not improve the health or enhance the subjective sense of well-being in normal people who do not know when they are taking vitamins. Since the method used to assess subjective responses is very sensitive, while the present results did not even indicate the possibility that the vitamins might have caused any improvement, it is improbable that larger doses of these vitamin supplements would have had a better effect. The findings that the plasma cholinesterase level was significantly raised by the giving of a vitamin-B complex preparation are difficult to explain at the moment. Davis (1946) found that folic acid and liver extracts raised the cholinesterase activity of dogs' serum, both when these substances were given by injection *in vivo* or added to the serum *in vitro*, but Hawkins (1948) was unable to confirm this with regard to folic acid. It will be necessary to investigate in detail which of the components of the tablets given in the present investigation caused or aided the increase of the plasma cholinesterase level and what were the underlying mechanisms of action.

Summary

In a tropical climate supplements of vitamin-B complex and insoluble liver fraction produced no improvement of the subjective sense of well-being and no decline in the incidence of minor illnesses among apparently healthy people on an adequate diet. Such supplements of vitamins raised the serum cholinesterase level significantly above normal.

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