Commercial breads as sources of vitamin E for rats as determined by the haemolysis test

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Previous work in this and other laboratories has shown that the tocopherols present in wheaten flour are largely destroyed on treatment with chlorine dioxide (Moran, Pace & McDermott, 1953; Moore, Sharman & Ward, 1957). It has also been shown (Moore *et al.* 1958) that when rats are fed upon bread specially made from flour known to have been treated with chlorine dioxide they develop signs of vitamin E deficiency. It was thought that it would be of interest to examine other breads available commercially and to assess their adequacy, or otherwise, as sources of vitamin E for rats.

Various lesions are known as criteria of vitamin E deficiency in rats. Many of these, e.g. the classical manifestation of foetal resorption, the browning of the uterus and the degeneration of the testes, take several months to develop. Some are also irreversible, which makes them valueless as a basis for curative tests. For purposes of expediency, therefore, the present experiments have been based on the dialuric-acid haemolysis test (György & Rose, 1949). Rats may be prepared for this test by a short deprivation of vitamin E, and cures may be effected even more rapidly. A further advantage of this technique is that only a few drops of blood, from the end of the tail, are required for each examination, so that it is not necessary for the animals to be killed. It is thus possible to carry out several tests on the same rat.

The present paper describes, in greater detail, results already given in a preliminary communication (Sharman & Richards, 1959).

EXPERIMENTAL

Breads tested. The commercial breads, all purchased locally, investigated were:

A, a typical white loaf, which had reputedly been made from flour treated with chlorine dioxide;

B, a well-known proprietary germ-enriched bread;

C, a wholemeal bread, also a proprietary brand;

D, a typical 'brown' loaf, said to be prepared from a 93–95% extraction flour without the addition of improvers; baked by the same firm as bread A.

Through the kindness of our colleague, Dr R. J. Ward, tocopherols were estimated in all the breads by the usual method with ferric chloride and 2,2′-dipyridyl, applied after the separation of the various forms by paper chromatography.

Preparation of rats. Piebald rats, of both sexes, about 90 g in weight, were first fed on the white bread A only. After the animals had received the bread for about 7 weeks their erythrocytes were examined by the haemolysis test. This preparation of the animals may have been unnecessarily long, but it was inconvenient to begin feeding with the different breads any earlier. As the erythrocytes of all the rats were then found to haemolyse, the animals were placed in a pool, from which members were taken, as required, for the tests. Many of the animals were used serially, i.e. after having been used to test one bread they were fed on bread A again; after their erythrocytes had become once more liable to haemolysis, the rats were used to investigate a second bread, and so on.

The rats received bread and water *ad lib.* The only supplement was one drop, 20 mg, of halibut-liver oil given weekly, to make good the deficiency of vitamin A. Other rats, used as controls, were given our standard basal diet deficient in vitamin E, either with or without the addition of α-tocopheryl acetate. The standard basal diet contained sucrose 50; casein (vitamin-free) 25; dried brewer's yeast 10, lard 10 and salt mixture 5%.

Dialuric-acid test. A modification of the method of György & Rose (1949) was used. Our technique, which included collecting blood from the tail of each rat under examination, has already been described (Moore *et al.* 1957). The erythrocytes were subjected to the action of dialuric acid in phosphate-buffer solution. The degree of haemolysis was estimated by comparing the red colour remaining after the removal of intact erythrocytes by centrifugation with that of a solution obtained by completely haemolysing an equivalent number of cells with distilled water. In each series of tests negative and positive controls were included to ensure that the test was working correctly.

Expt 1. Five rats, whose erythrocytes showed almost complete haemolysis on being tested with dialuric acid, were fed for 14 days on bread B, a germ-enriched bread, after which the haemolysis test was again applied. The bread was changed once more, this time back to the original A variety, and after a further 29 days the test was again applied.

Expt 2. Six rats, whose erythrocytes were extensively haemolysed in the test, were divided into two equal groups, the first group continued on bread A and the other was given the wholemeal bread C. After 14 days the haemolysis test was again applied, and the breads given to the two groups were reversed. Liability to haemolysis was again investigated after 5, 10, 14 and 34 days.

Expt 3. For this experiment an ordinary brown loaf, designated D, was investigated. Six rats were fed on this bread for 14 days, while another two animals continued to receive bread A. Haemolysis tests were performed at the beginning and end of the trial.

RESULTS

Expt 1 (*germ-enriched bread*). In the first test, when bread B was compared with bread A, extensive haemolysis occurred in the test on all rats after receiving bread A for 7 weeks, as will be seen from Table 1. However, after they had been given bread B

for 14 days there was virtually no haemolysis. When the erythrocytes were re-examined after the rats had reverted to bread A there was again extensive haemolysis.

Expt 2 (proprietary wholemeal bread). Erythrocytes from the rats first given bread C showed almost complete protection after the animals had received the bread for 14 days, whereas those continuing to receive bread A continued to show haemolysis (see Table 2). However, after the treatments were reversed the erythrocytes from those animals that were now receiving bread C exhibited protection, though the speed

Table 1. *Percentage haemolysis of erythrocytes from rats fed first on bread A then on bread B and finally on bread A again*

Rat no.	Bread A, 7 weeks	Bread B, 14 days	Bread A, 19 days
1	83	0	75
2	93	0	90
3	97	0	95
4	92	2·1	98
5	88	0·2	90
Negative control*	100	94	100
Positive control†	2·3	2·6	2·2

A, typical white bread; B, proprietary germ-enriched bread.
* Synthetic diet of sucrose, casein, lard, yeast and salts; no vitamin E supplement.
† Synthetic diet, with 2 mg α-tocopheryl acetate weekly.

Table 2. *Percentage haemolysis of erythrocytes from rats after feeding for various periods on bread A and on bread C, after a preliminary period of 7 weeks on bread A*

Rat no.	At end of preliminary period	After bread A or C for 14 days		After bread C or A for 5 days	10 days	14 days	34 days	
1	100	90	A	69	70	14	0	C
2	100	90	A	85	16	8	0	C
3	98	91	A	76	56	93*	0	C
4	96	2	C	93	86	96	82	A
5	93	9	C	84	83	90	69	A
6	93	3	C	22	76	78	66	A
Negative control†	80	96		81	86	90	87	
Positive control‡	4	0		1	1	3	1	

A, typical white bread; C, proprietary wholemeal bread.
* Animal had lost weight (see below).
† Synthetic diet of sucrose, casein, lard, yeast and salts; no vitamin E supplement.
‡ Synthetic diet, with 2 mg α-tocopheryl acetate weekly.

with which they developed it was not as great as was shown by the first three animals. Rat no. 3, which at first gave no indication of protection, was found to be losing weight, and it was concluded that it was failing to eat its bread. Towards the end of the experimental period it recovered, and at the last examination its erythrocytes showed complete protection.

Expt 3 (ordinary brown bread). Haemolysis values for the rats in this experiment

are given in Table 3. It will be observed that four of the animals showed almost complete protection after receiving brown bread for 14 days, whereas two others did not.

Table 3. *Percentage haemolysis of erythrocytes from rats after feeding for 14 days on bread D or on bread A, after a preliminary period of 7 weeks on bread A*

Rat no.	At end of preliminary period	After bread D or A for 14 days	
1	74	1	
2	80	3	
3	88	81	D
4	88	5	
5	89	43	
6	88	7	
7	88	91	A
8	91	82	
Negative control*	88	92	
Positive control†	1·9	0·7	

A, typical white bread; D, typical brown bread.
* Synthetic diet of sucrose, casein, lard, yeast and salts; no vitamin E supplement.
† Synthetic diet, with 2 mg α-tocopheryl acetate weekly.

DISCUSSION

The white bread designated A must be representative of much the greater part of bread consumed in Great Britain. It did not contain sufficient vitamin E to protect the rats' erythrocytes from haemolysis by dialuric acid. Since much of the flour in this country is treated with chlorine dioxide and since chlorine dioxide is known to destroy tocopherols in wheat and flour this is likely to be the explanation of the lack of vitamin E in the commercial white bread examined. In view of the fact that the rats received bread only, except for one drop weekly of halibut-liver oil, it is remarkable that they survived for so long.

Table 4. *Tocopherol contents of commercial breads**

Bread	Tocopherol content (μg/g) α	ζ	β	ε
B, proprietary germ-enriched	6·90	0·74	4·63	5·03
C, proprietary wholemeal	3·27	0·49	2·32	4·53
D, typical brown	1·43	0·35	1·06	3·70
A, typical white	N.D.	N.D.	0·10	0·35

N.D., not detected, less than 0·05.
* Results kindly supplied by Dr R. J. Ward.

The germ-enriched bread B and the wholemeal bread C contained sufficient vitamin E to reverse the erythrocytes' liability to haemolysis by dialuric acid when those breads were fed to rats deficient in vitamin E. The bread D gave some, but not complete, protection. The results are in agreement with what might be expected from

the tocopherol contents of the breads, as determined by chemical methods (Table 4). Thus if we take the mean bread consumption of the rats as 30 g/day the vitamin E intakes, expressed as mg α-tocopherol weekly, were: bread B 1·90, bread C 0·92, bread D 0·42. These values are based on the assumption that ζ and β tocopherols have 40% of the activity of α-tocopherol and that ε-tocopherol has none. According to findings on the requirements for vitamin E of rats given a standard diet containing 10% of lard (Moore *et al.* 1959), the intakes provided by breads B and C should be adequate to give protection in the haemolysis test, as was demonstrated by the protection observed in all the animals. The intake provided by bread D was marginal, which is consistent with the fact that protection was observed in some animals but not in others.

SUMMARY

1. Weanling rats were first fed on a commercial white bread, which reputedly had been made from flour treated with chlorine dioxide. Supplements of halibut-liver oil were given to make good the known deficiency of bread in vitamin A. The effect of this diet was to give regularly positive results in the haemolysis test with dialuric acid, indicative of deficiency of vitamin E.

2. Selected rats were then given various other commercial breads instead of the white bread. A bread enriched with germ and a proprietary wholemeal bread gave complete protection in the haemolysis test. A non-proprietary 'brown' bread gave protection in some rats, but not in others.

3. These biological results were in agreement with the tocopherol contents of the breads as estimated chemically.

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