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EXPLANATION OF PLATE

1. Skin sore, characteristic of prolonged deficiency of vitamin E, observed in a rat (rat no. 1, Table 1) fed for 484 days on a diet containing flour treated with chlorine dioxide.
2. Typical photomicrograph of the kidney cortex of a rat receiving the diet containing untreated flour for 510 days (rat no. 6, Table 2). Kidney fixed 3 h after death. Note virtual absence of autolysis in the tubules.
3. Typical photomicrograph of the kidney cortex of a rat (rat no. 9, Table 2) receiving the diet containing ClO_2-treated flour for 510 days. Kidney fixed 3 h after death. Note extensive autolysis in the tubules.

The biological activities of ε- and ζ-tocopherols

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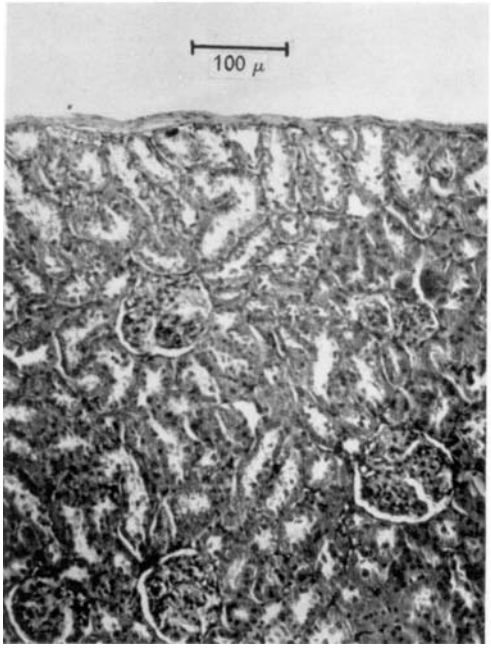
Assessment of vitamin E potency of untreated flour, and of bread baked from it without the use of destructive improvers, has been complicated by the presence of ε-tocopherol (5-monomethyl tocol) and ζ-tocopherol (5:7-dimethyl tocol). The same problem arises over British margarine, which also contains these tocopherols. According to analyses carried out in this laboratory, flour of 80% extraction contains about 23% of α-, 16% of β-, 5% of ζ- and 56% of ε-tocopherol, with a total tocopherol content of 1·82 mg/100 g dry weight (Moore, Sharman & Ward, 1957). Palm oil is used in the manufacture of British margarine and contains about 50% α-, 34% ζ- and 16% η-tocopherol, with a total tocopherol content of 32·7 mg/100 g (Ward, 1958). ε- and ζ-tocopherols are also present in the rations of farm animals, particularly in those containing barley meal or wheat middlings.

In order to estimate the vitamin E activity of any food, it is necessary to have a knowledge of both the relative biological activities and the individual amounts of the various forms of tocopherol present. Although ε-tocopherol has been synthesized (Karrer & Dutta, 1948), there is no published information about its biological activity. It has been suggested by Eggitt & Norris (1956), however, that from its chemical constitution it should have the activity of β-tocopherol or about 30% of that of α-tocopherol. ζ-Tocopherol was synthesized nearly 20 years ago by Karrer & Fritsche

1



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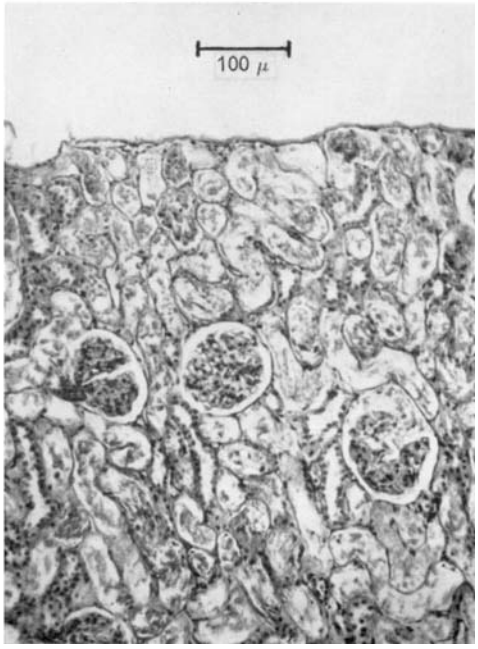

3

(*Facing p.* 226)

(1939), who claimed that it had about 50% of the activity of α-tocopherol. This finding has been supported by Bunyan, Green, Mamalis & Marcinkiewicz (1957), but Jacob, Sutcliffe & Todd (1940) reported that ζ-tocopherol was equal to the α- form in activity.

All these workers used for assessment of biological activity the conventional method depending on preventing foetal resorption in the rat. In my work, however, the activities of ε- and ζ-tocopherols have been compared by somewhat simpler methods, based on the prevention of two other lesions typical of vitamin E deficiency, namely, degeneration of the testes and pigmentation of the uterus. Herraiz & Radice (1949) have already suggested that the prevention of testicular degeneration could be used as a biological method for the assay of vitamin E, but this suggestion does not seem previously to have been put to test. About 20 years ago Martin & Moore (1939) first described the pigmentation of uteri in rats given a deficient diet, but there are no earlier accounts of using this lesion for assay purposes.

When a preliminary communication on this work was presented at the 4th International Congress of Nutrition held in Paris (Ward, 1957), Bunyan, Green, Mamalis, Marcinkiewicz & McHale (1957) stated that preliminary biological tests on synthetic ε-tocopherol had indicated it to have an activity under 10% of that of α-tocopherol.

EXPERIMENTAL

Preparation of test solutions

ε-Tocopherol. Bran oil was obtained by cold percolation of ground wheat bran with light petroleum in a column 3 in. in diameter and 36 in. long. About 80 g of the oil were saponified with potassium hydroxide in the presence of pyrogallol (Tosic & Moore, 1945). The sterols were removed from the unsaponifiable fraction by crystallization from methanol at $-10°$ and the carotenoids by adsorption on a column of Floridin earth (Emmerie & Engel, 1939). The ε-tocopherol fraction was separated from the other tocopherols by chromatography on a column of liquid paraffin supported on non-wetting Celite (Johns-Manville) (Eggitt & Norris, 1956). Purification of the ε-tocopherol was achieved by adsorbing the isolated tocopherol from light petroleum on a column of Floridin earth. After washing the column with light petroleum to remove paraffin, the tocopherol was eluted with benzene. The purity was estimated by two-dimensional chromatography (Green, Marcinkiewicz & Watt, 1955) and was generally about 88%. The impurities in all batches were non-reducing.

ζ-Tocopherol. DL-ζ-Tocopherol was synthesized from *m*-xylohydroquinone and phytol by the method of Karrer & Fritsche (1939). The crude tocopherol was purified by chromatography on alumina and distillation under reduced pressure. The resultant tocopherol was found to be 96% pure by paper chromatographic assay.

Biological tests

Groups of two male and two female weanling piebald rats of about 50 g weight were given a vitamin E-free diet of the composition, vitamin-free casein 25, sucrose 50, dried brewer's yeast 10, lard 10, minerals 5%. Supplements of vitamins A, D and K

were given weekly. The weekly doses of the test tocopherols were given in equally divided portions on Tuesdays and Fridays for a period of 15 weeks. The animals were then killed. In the males the testes were examined for degeneration. Generally the weight of the organ was sufficient indication of its state: the testes from a few animals less palpably deficient were examined histologically. In the females the uteri were examined in daylight and graded for pigmentation on an arbitrary scale (Moore *et al.* 1958). All the uteri were also examined under ultraviolet light (365 mμ) in order to detect traces of the pigment, which are clearly shown by their yellow fluorescence. The biological activity was estimated by comparing the amount of α-tocopherol with the amount of ε- or ζ-tocopherol necessary to prevent the lesion and expressing the result as a percentage.

RESULTS

Males. The mean testes weights of rats receiving graded doses of D-α-, D-ε- or DL-ζ-tocopherols are given in Table 1. With α-tocopherol, doses of 0·25 mg and above resulted in normal testes. With ε-tocopherol even the highest dose given (5 mg) gave no protection. ζ-Tocopherol, however, was more active, with protection at doses of 0·875 mg/week or more.

From these results it appears that ε-tocopherol has an activity of less than 5% of that of α-tocopherol and ζ-tocopherol has about 29% of the activity of α-tocopherol, with possible limits of 20–33%, arising from the unavoidable intervals between the doses.

Table 1. *Mean weight of testes of pairs of male rats in relation to tocopherol dose*

α-Tocopherol		ε-Tocopherol		ζ-Tocopherol	
Dose (mg/week)	Mean testes weight (g) (per pair)	Dose (mg/week)	Mean testes weight (g) (per pair)	Dose (mg/week)	Mean testes weight (g) (per pair)
0	1·283	1·0	1·150	0	1·112
0·175	1·065	2·0	0·967	0·500	1·123
0·250	2·556	3·0	1·280	0·625	1·078
0·375	2·803	4·0	1·193	0·750	0·968
0·500	2·864	5·0	1·118	0·875	2·641
1·000	2·930	—	—	1·000	2·761

Table 2. *Colour of uterus of pairs of female rats in relation to tocopherol dose*

α-Tocopherol		ε-Tocopherol		ζ-Tocopherol	
Dose (mg/week)	Uterus colour	Dose (mg/week)	Uterus colour	Dose (mg/week)	Uterus colour
0	Brown+++	1·0	Brown+++	0	Brown++
0·250	Brown++	2·0	Brown+++	0·500	Brown++
0·375	Normal	3·0	Brown+++	0·625	Brown+
0·500	Normal	4·0	Brown+++	0·750	Brown+
1·000	Normal	5·0	Brown+++	0·875	Normal
				1·000	Normal

Females. The colour of the uteri of rats on doses of tocopherols similar to those given to the males is indicated in Table 2. With α-tocopherol a dose of 0·375 mg prevented the formation of brown pigment in the uterus. With ε-tocopherol, as in the males, the highest dose of 5 mg was completely inactive. ζ-Tocopherol proved to be active at doses of 0·875 mg and above.

Thus for females, ε-tocopherol proved to have an activity less than 7·5% of that of α-tocopherol, and ζ-tocopherol had about 43% of the activity of α-tocopherol, with possible limits from 29 to 50%.

DISCUSSION

ε-Tocopherol. The lack of activity of natural ε-tocopherol found in this work is in agreement with the low activity reported by Stern, Robeson, Weisler & Baxter (1947) for δ-tocopherol (8-monomethyl tocopherol) and also with the preliminary results of Bunyan, Green, Mamalis, Marcinkiewicz & McHale (1957) for synthetic ε-tocopherol. The suggestion of Eggitt & Norris (1956) that ε-tocopherol should be as active as β-tocopherol does not take into account any differences that may exist in the absorption of the dimethyl and monomethyl tocopherols by the rat. Lundberg, Barnes, Clausen, Larson & Burr (1947) reported that feeding equal amounts of α-, β- and γ-tocopherols to rats, led to deposition in the body fat in the ratio of 100:73:13, and on the basis of their results suggested that a relationship might exist between the biological potencies and relative amounts of tocopherols deposited in the various body fats and tissues.

When Dju, Quaife & Harris (1950) gave equal amounts of α-, γ- and δ-tocopherols to hens, the amounts of γ- and δ-tocopherols found in the eggs were only one-fourth and one-tenth of that of α-tocopherol, respectively. Bolliger & Bolliger-Quaife (1956) showed that rats that ingested D-γ- or DL-ζ-tocopherols had increased blood tocopherol levels about one-half that caused by the ingestion of DL-α-tocopherol, but the liver uptake of ζ-tocopherol was about six times as great as that of γ-tocopherol and about one-half that of α-tocopherol. It thus appears most likely that the absorption of the various forms of tocopherol greatly affects their biological activity.

Rodnan, Chernick & Schwarz (1956) claimed that intraportally injected γ-tocopherol was nearly as active as α-tocopherol in reversing respiratory decline during necrotic liver degeneration. However, β-tocopherol and δ-tocopherol had only 36 and 17%, respectively, the activity of α-tocopherol.

ζ-Tocopherol. The activity of ζ-tocopherol I have found in the female (43%) is in good agreement with previously reported values (Karrer & Fritsche, 1939; Bunyan, Green, Mamalis & Marcinkiewicz, 1957) obtained by the rat-antisterility test. The value for males (29%), however, was only about two-thirds of the value given in the tests on females. Because of the small number of animals used in each group, no importance is attached at present to the apparent sex difference, particularly as there is an overlap in the limits. There seem to be no reliable reports on the use of males to estimate the biological activity of the tocopherols other than one by Filer, Rumery & Mason (1946) that γ-tocopherol was only one-tenth as active as α-tocopherol in preventing degeneration of the testes. It should be noted that these workers were giving 20% of the calories in methyl esters of lard as the dietary fat.

Dietetic implications. These results indicate that ε-tocopherol, a major component of the tocopherols of untreated flour, is almost valueless as a source of vitamin E. Normally the ε-tocopherol intake of human beings from flour will be negligible, since most of it is destroyed by the improvers used in the baking of bread (Moran, Pace & McDermott, 1953; Moore *et al.* 1957). A small amount of η-tocopherol is present in margarine (Ward, 1958) from the palm oil it contains. The human intake of ζ-tocopherol in the western hemisphere is also likely to be low. Again the only intake of any importance is from margarine manufactured from palm oil. In the rice-eating countries, however, the intake of ζ-tocopherol will be higher, as 26% of the tocopherols of rice are in the ζ- form (Green & Marcinkiewicz, 1956). The two tocopherols studied by me feature largely in the vitamin E intake of farm animals. In particular, chick mash contains about 28% of its tocopherols in the ε- form (Eggitt & Ward, 1955). The highest consumption of ζ-tocopherol will be by those animals receiving a diet containing barley, in which 44% of the tocopherols are in the ζ- form. Many analyses of farm-animal rations will have to be undertaken to determine the tocopherol pattern before the effect of their different biological activities can be assessed. It should now be clear that direct estimation of total tocopherols in any substance is inadequate to indicate its vitamin E activity.

SUMMARY

1. Natural ε-tocopherol (5-monomethyl tocol), isolated from wheat-bran oil and synthetic ζ-tocopherol (5:7-dimethyl tocol) were given to groups of two male and two female rats in graded doses.

2. Two lesions typical of vitamin E deficiency, pigmentation of the uterus and degeneration of the testes, were used to estimate the biological activity.

3. ε-Tocopherol was found to have less than 7·5% of the activity of α-tocopherol by either method.

4. ζ-Tocopherol had about 40% of the activity of α-tocopherol.

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The vitamin E content of margarine

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The destruction of vitamin E by chlorine dioxide, the agent at present most commonly used in Great Britain for 'improving' flour, is now well established (Moran, Pace & McDermott, 1953, 1954; Frazer, Hickman, Sammons & Sharratt, 1956; Moore, Sharman & Ward, 1957). If vitamin E is required by man, as seems probable from, *inter alia*, its presence in human blood plasma, it may be important to assess the amount of the loss. Information is therefore needed about the proportion of our total intake of vitamin E that could be provided by flour. As a basis for our calculations information must be available about the vitamin E contents of all the other dietary components. According to figures already published, which unfortunately have sometimes been based on obsolete methods of analysis, margarine and cooking fat can make by far the most substantial contributions to our vitamin E supplies. Accurate knowledge of their vitamin contents, of the relative proportions of the various tocopherols present and of the effect of biological differences associated with their different chemical structures, must therefore be obtained.

Up to the present only scanty information is available about margarine manufactured abroad, and none at all about margarine made in Britain. Lundborg (1945) reported from Sweden that margarine derived from sunflower-seed oil, coconut oil, rape-seed oil and melted lard contained 8·9 mg tocopherols/100 g. In clarified American margarine, however, Quaife & Harris (1948) found the much higher content of 28·4 mg α-tocopherol/100 g. Both α-tocopherol (28 mg/100 g) and γ- and δ-tocopherols (amounting together to 26 mg/100 g) were later reported by Harris, Quaife & Swanson