THE CAUSE OF THE MYOCARDIAL 'HYALINE DEGENERATION' INDUCED IN RATS FED PREDOMINANTLY MAIZE DIETS SUPPLEMENTED WITH EITHER THE NNRI FOOD FORMULAE OR SKIMMED-MILK POWDER*

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A long-term investigation carried out recently at this Institute[1] into the question of the mineral efficiency of the Institute's high-protein food formulae and of skimmed-milk powder in a predominantly maize dietary context, has revealed that, in spite of attempts at making the experimental diets 'complete' in respect of those vitamins known to be required by the experimental animals (albino rats), the animals developed a lesion designated at that time as 'myocardial hyaline degeneration'. Since hyalinization of muscular tissue had previously been associated with vitamin-E deficiency, the possibility of the above phenomenon being due to a deficiency of this vitamin was considered; but as the experimental diets contained vitamin-E bearing ingredients, such as wheat germ oil and low-extraction grade maize meal, it did not appear that vitamin-E deficiency could have been the cause of the tissue changes observed.

It was nevertheless considered necessary to reinvestigate this matter, particularly in the light of the possibility of destruction of the vitamin-E contents of the diets under the prevailing experimental conditions.

The stability of vitamin E in foods depends very much on the absence of factors which promote rancidity,[2] such as unchelated pro-oxidants (iron, copper and manganese) and adverse temperatures and humidities. It is therefore possible that part of the vitamin E in the diets tested was oxidized, the experiment having been conducted in an environment in which the room temperature was 28°C and relative humidity 50%.

It has also been observed[3] that when a diet low in vitamin-E content is supplemented with cod-liver oil, as was done in the study under consideration, there is a preponderance among the experimental animals towards developing signs of vitamin-E deficiency. According to Brown,[4] who has tested a number of fish oils, such oils do contain vitamin E, but they also contain a number of highly-unsaturated fatty acids which are capable of inducing vitamin-E deficiency symptoms; Witting and Horwitt[5] have shown that the creatinuria of the vitamin-E deficient rat is inversely related to the degree of saturation of the dietary fat. It is conceivable that, in view of the proneness of unsaturated fatty acids to peroxidize, the conditions prevailing in diets high in unsaturated fatty acids are conducive to the destruction of vitamin E.

Another factor which has been associated with the utilization of vitamin E is the selenium content of the diet. Having reviewed the subject, Witting[6] concluded that tissue damage caused by peroxidized lipids may possibly be minimized by several non-lipid factors such as selenium and sulphur-containing amino acids. Desai and Scott[7] have established that selenium acts as a carrier of vitamin E and may thus function in the absorption and retention of the vitamin, in prevention of its destruction and perhaps also in the transfer across membranes, thereby enhancing the biological activity of this vitamin. However, according to the results of Nesheim and Scott,[8] it appears that, at least as far as its growth effects are concerned, selenium is not effective in diets containing vitamin E, unless these diets are high in Torula yeast content.

In the study to be reported here I have investigated the effect of a number of materials, each of which could directly or indirectly be associated with the destruction and/or utilization of dietary vitamin E, on the incidence of 'hyaline degeneration' of the type observed in my previous study.

MATERIALS AND METHODS

The general plan of the experiment entailed the feeding of 5 groups of 10 young rats each (5 males and 5 females per group) for 140 days with a diet consisting of cooked and dried maize meal and fixed proportions of fat and NNRI Food Formula V, treatment of the rats varying

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from group to group only in respect of (*a*) the source of the fats included in a given diet and (*b*) the nature of the test materials added to each diet (Table I).

TABLE I. COMPOSITION OF EXPERIMENTAL DIETS

Constituent	*Weight in G/100 G of air-dry diet*				
	Diet I	*Diet II*	*Diet III*	*Diet IV*	*Diet V*
Maize meal (cooked, roller-dried)	84·0	84·0	84·0	84·0	84·0
NNRI Food Formula V	14·0	14·0	14·0	14·0	14·0
Coconut oil	2·0	2·0	2·0	2·0	—
Cod-liver oil	—	—	—	—	2·0
Sodium selenite + dextrin	—	0·1*	—	—	—
Vitamin E acetate	—	—	0·03 ml.†	—	—
β-hydroxy-anisole	—	—	—	0·01	—
β-hydroxy-toluene	—	—	—	0·01	—

*Selenium content of diet approximately 4 mcg./100 G diet.
†Vitamin-E content of diet approximately 30 mg./100 G diet.

The fat source was either coconut oil (diets I-IV) or cod-liver oil (diet V), the unsaturated fatty acid contents of which, according to available figures,[9] are approximately 8% and 81% respectively. The supplements were: diet II, sodium selenite; diet III, vitamin E (tocopherol acetate oil); and diet IV, a 50 : 50 mixture of two anti-oxidants, β-hydroxy-anisole (BHA) and β-hydroxy-toluene (BHT). The anti-oxidant levels were in accordance with those permitted by the Food, Drugs and Disinfectants Act of the Republic of South Africa;[10] the vitamin-E level was 5 times greater than that recommended and the selenium level equal to that recommended for the rat by the NRC.[11]

The rats were housed individually in screen-bottomed cages in an air-conditioned room at 28 ± 2°C and 50 ± 5% relative humidity.

During storage all diets were kept in a refrigerator. Rations and drinking water were supplied *ad libitum* daily, for the duration of the experiment; each of the rat groups received one of the diets listed in Table I throughout the experiment.

Body-weights were recorded twice per week.

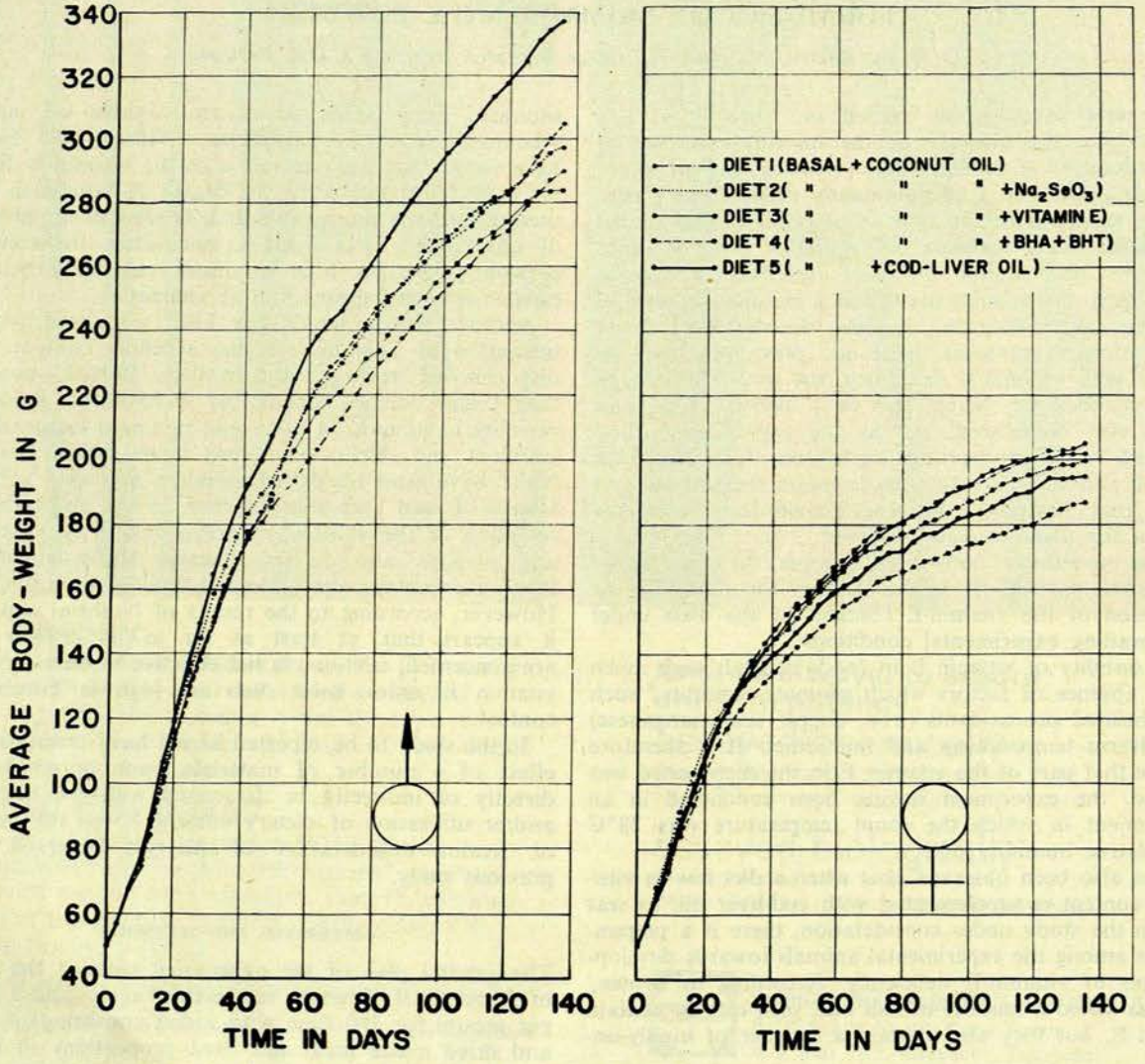

Fig. 1. Growth of male and female rats relative to time during course of trial.

At the end of the 140-day feeding period the rats were anaesthetized with ether for the removal of the myocardium and the femoral muscle tissues. The tissues were fixed in buffered formalin and stained in haematoxylin-erythrosin solution.

RESULTS AND DISCUSSION

The growth rates of the rats (group means of body-weights relative to time in days) are shown in Fig. 1.

Generally speaking the various experimental groups grew fairly uniformly in the case of both male and female rats, with the exception of those male rats which received cod-liver oil (diet V). The heterogeneity in the weights of the males at the end of the experiment (due to the intake of cod-liver oil by one of the experimental groups) was statistically almost significant (P very close to 5% according to the Kruskal-Wallis one-way analysis of variance).[32] The above tendency in the growth rate of the males on the diet containing cod-liver oil was probably due to the fact that the essential fatty acid requirement of a male rat is higher than that of a female rat;[33] the essential fatty acid content of the basal diet was probably too low for optimal growth in the case of the male rats, but when the diet was supplemented with essential fatty acids from cod-liver oil it was possible for the growth to proceed at a faster rate.

The incidence rating of hyalinization in the heart and skeletal muscles of the rats is shown in Table II. From

TABLE II. INCIDENCE RATING OF HYALINIZATION IN HEART AND SKELETAL MUSCLES OF RATS FED BASAL DIET CONSISTING OF MAINLY FORMULA V AND COOKED MAIZE PLUS ONE OF A NUMBER OF OTHER DIETARY SUPPLEMENTS

Diet and test material	*Tissue*	*Incidence rating (%)*
I: Basal + coconut oil	Heart	50*
	Skeletal muscle	70
II: Basal + coconut oil + Na_2SeO_3	Heart	30*
	Skeletal muscle	20
III: Basal + coconut oil + vitamin E	Heart	0
	Skeletal muscle	0
IV: Basal + coconut oil + BHA + BHT	Heart	10*
	Skeletal muscle	40
V: Basal + cod-liver oil	Heart	100†
	Skeletal muscle	100

*In a zone close to endocardium in the vicinity of the left ventricle.
†In two zones in the vicinity of the left ventricle, one close to the endocardium and the other close to the epicardium.

the data presented it will be seen that, compared with other groups, the cod-liver oil group was most severely affected as regards 'hyaline degeneration' in both of the two types of muscular tissue investigated. At the other end of the scale, the vitamin-E group showed no signs of the type of degeneration under consideration. It is also interesting to note that not only was the incidence rating highest in the cod-liver oil group but the degeneration was also more extensive in the sense that two foci in the wall of the left ventricle, one in the vicinity of the endocardium and the other in the vicinity of the epicardium, were affected. It appears therefore that the cod-liver oil introduced factors which caused a rise in the incidence and severity of the tissue changes.

The other supplements, Na_2SeO_3 and anti-oxidants did not afford complete protection against the degeneration, but the incidence ratings were generally lower in the selenium and anti-oxidant groups than in the group fed the basal diet. It seems probable that, although vitamin E was clearly the most effective protecting agent, the vitamin-E content of the basal diet was too low for the rats to benefit much from consumption of substances which could improve either the utilization of vitamin E (selenium) or the stability in the diet of this vitamin (anti-oxidants).

In the light of the above results there can be little doubt that the cause of the muscular degeneration considered here was vitamin-E deficiency, and that, as was put forward by Brown,[4] cod-liver oil contains substances which jeopardize either the stability or the utilization of the vitamin.

Although there is as yet no evidence regarding the incidence of hyaline degeneration among individuals subsisting on predominantly cereal diets—unsupplemented or supplemented with high-protein foods—it appears that the incorporation of vitamin E in high-protein food formulae would be a good precautionary measure. Sandstead *et al.*[34] found evidence suggesting that vitamin-E deficiency may be important in the macrocytic anaemia of patients suffering from protein-calorie malnutrition.

According to a calculation based on data compiled by Dicks,[35] the maximum vitamin-E content of a diet consisting of maize porridge plus NNRI Formula V, fed in the quantities recommended for children in the kwashiorkor age-group,[36] would furnish about 30 mg. of vitamin E. If we assume a body-weight of 13 kg. and a minimum requirement of 0·5 mg. vitamin E/kg. body-weight,[37] this would amount to a daily intake of more than 4 times the minimum requirement. It is nevertheless possible that the vitamin-E content of maize meal, and that of air-dry food mixtures such as the NNRI formulae, is reduced considerably during preparation and storage. Kodicek *et al.*[38] have shown, for example, that the vitamin-E potency of ground maize is decreased by about 30% after storage for 6 months.

In view of the present uncertainty regarding the vitamin-E intake of individuals in the age-group susceptible to protein-calorie malnutrition, and the finding in the present investigation that the NNRI Food Formula V was not capable of fully protecting the experimental animals against tissue changes which appeared to have been due to a vitamin-E deficiency, it seems advisable to recommend that, as a preliminary measure, the NNRI formulae should be fortified with vitamin E. Although a recommendation regarding the appropriate fortification level involves the making of assumptions in respect of matters on which information is as yet not available, viz. the daily vitamin-E requirements of individuals and the stability of vitamin E in the NNRI food formulae, it seems that there can be no harm in making at least a tentative suggestion. Majaj *et al.*[39] gave kwashiorkor children intramuscular injections of 100 mg. alpha-tocopherol phosphate plus 280 mg. alpha-tocopherol acetate *per os* for 5 days, with only beneficial effects. A critical upper limit in the dosage level is therefore not indicated. Thus it would be quite safe to ignore the vitamin E al-

ready present in the diet, and to make allowance for storage losses to the extent of using 4 times the minimum daily requirement of 6·5 mg. This would amount to the addition of close on 100 mg. of tocopherol to 100 G of the NNRI formulae.

SUMMARY

In an experiment with 5 groups of young rats, each group receiving a basal diet of cooked and dried maize meal plus NNRI Food Formula V and certain supplements for 140 days, it was found that: the basal diet plus coconut oil induced hyaline degeneration in myocardium and skeletal muscle; the incidence rating of the hyaline degeneration was raised to 100% and the degree of tissue change was enhanced by supplementation of the basal diet with cod-liver oil; supplementation of the basal diet with vitamin E afforded complete protection against hyalinization; and supplementation with selenium and anti-oxidants afforded only a limited degree of protection against the muscular tissue changes.

The above results suggest that the tissue changes were due to vitamin-E deficiency and that cod-liver oil contains substances (probably peroxidized fatty acids) which cause destruction of vitamin E. It is tentatively recommended that the NNRI food formulae should be fortified with vitamin E to the extent of 100 mg. alpha-tocopherol per 100 G.

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