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Prevention of the injurious effects of excessive cod-liver oil by its fortification with vitamin E

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The injurious effects of dietary excess of cod-liver oil, and the role of the oil both as a source and antagonist of vitamin E, have been discussed in previous communications (Moore, Sharman & Ward, 1958*a*, *b*, 1959). According to chemical estimation a specimen of medicinal cod-liver oil contained about 10 mg α-tocopherol/100 g. This finding implied that if rats were to be given the oil as 10% of their diet they would receive about four times their minimum requirement of vitamin E, as far as could be judged from the results of previous measurements of the requirements of other rats which had received a basal diet containing lard. Support for the chemical result was obtained in a biological test, in which the unsaponifiable fraction of the cod-liver oil was given to rats fed upon a diet containing lard. When the whole oil was included in the diet, however, most of the signs of avitaminosis E were not prevented. This effect was presumably due to the presence in the cod-liver oil of highly unsaturated fatty acids, which are known to be antagonistic to vitamin E. The ill effects of the cod-liver oil were prevented by adequate weekly doses of DL-α-tocopheryl acetate administered separately from the main diet.

The object of this investigation was to find whether the injurious effects of cod-liver oil can be prevented by fortifying it with α-tocopherol and, if so, what concentration of the vitamin is required for this purpose. The need for inquiry into these points was emphasized by the finding of Mackenzie, Mackenzie & McCollum (1941) that α-tocopherol had to be administered to rabbits at an adequate time interval before or after dosing with cod-liver oil in order to exert its action in preventing muscular dystrophy.

EXPERIMENTAL

Cod-liver oil. The oil was supplied by British Cod Liver Oils (Hull & Grimsby) Limited and was described as of 'high-grade medicinal' quality. According to chemical estimations by Dr R. J. Ward it contained 10·1 mg tocopherol/100 g, all in the α form. Its tocopherol content, therefore, was virtually identical with that of the oil used in our former experiments (Moore *et al.* 1959).

Grouping of rats. Weanling female albino rats were fed upon diets similar to those previously described (Moore *et al.* 1959). These diets had the general composition: casein (vitamin-free) 25, sucrose 50, fat 10, dried brewer's yeast 10 and minerals 5%. Supplements of vitamin K, as 50 μg 2-methyl-1,4-naphthaquinone weekly, were given

to all the rats. Those that had lard as their fat also received adequate doses of vitamins A and D.

Group 1 (five rats) received their fat as lard, without supplement of α-tocopherol. Experience has shown that lard from our usual source contains negligible amounts of vitamin E. In group 2 (five rats) the fat was also lard, but was fortified with DL-α-tocopheryl acetate at a level calculated to supply 20 mg α-tocopherol alcohol/100 g lard. Group 3 (ten rats) received their fat as cod-liver oil, without any additional source of vitamin E. Groups 4, 5, 6 and 7 (five rats each) also received cod-liver oil, but it was fortified with DL-α-tocopheryl acetate in amounts calculated to supply 5, 10, 20 and 40 mg, respectively, extra α-tocopherol/100 g.

Observations on virgin rats. The criteria for the detection of vitamin E deficiency were mostly those used in our previous work, and our scales for grading the severity of the various abnormalities have already been described (Moore *et al.* 1958*a–c*, 1959). The haemolysis test with dialuric acid was applied to most of the rats after they had received their diets for 68–97 days. The upper incisor teeth were inspected for their degree of depigmentation regularly throughout the experimental period, and their final state at the end of the experiment was recorded. After 97 days all the rats in groups 1, 4 and 5 were killed, and also half those in group 3. Observations on the brown discoloration of the uterus and on post-mortem renal histolysis were made as previously described. Gradings for the brown discoloration of the adipose tissues were only recorded for the intraperitoneal fat, since the subcutaneous fat appeared to be virtually normal in colour even in those rats given cod-liver oil without additional tocopherol. During inspection we noticed that the fat was less plentiful in those rats with brown fat than in those with normal white fat. The intraperitoneal fat deposits from each animal were therefore dissected out and weighed.

Breeding tests. It had been our intention, if necessary, to kill all the rats while they were still virgins. The two lower levels of the additional α-tocopherol to the cod-liver oil, however, were found adequate to give partial or complete protection against vitamin E deficiency as judged by the criteria used up to this stage. We decided, therefore, to use the remaining rats for breeding tests. On the 104th day of the investigation, each surviving female was paired with an albino male, and vaginal smears were taken until the presence of spermatozoa was established. The male was then removed, and the female was weighed daily, and kept under observation until a litter had been born or the normal gestation period had elapsed without the birth of a litter. Those rats that failed to have litters were examined at autopsy for the presence or absence of placental sites.

RESULTS

Experiments on virgin rats. From Table 1 it will be seen that the rats of group 1, given a diet deficient in vitamin E and containing lard, showed the usual signs of avitaminosis E. Thus the erythrocytes were almost completely haemolysed in the three rats tested, and some degree of dental depigmentation was seen in four out of the five rats. In all five rats the uterus was decidedly brown. Rapid post-mortem kidney histolysis was observed in one rat, and very slight histolysis in three others. As was

Table 1. *Signs of vitamin E deficiency in virgin female rats, kept for 97 days on diets containing cod-liver oil or lard, with or without fortification with* DL-α-*tocopheryl acetate*

Group no.	Fat in diet	α-Tocopheryl acetate added (mg/100 g fat)	Rat no.	Body-weight			Haemolysis (%)	Dental pigmentation (score)*	Brown uterus (score)*	Intraperitoneal fat		Renal histolysis (score)*
				Initial (g)	Final (g)	Gain (g)				Brown colour (score)*	Weight (g)	
1	Lard	0	1	87	193	106	88	1	2·5	0	5·0	0·25
			2	43	207	164	84	4	2	0	7·5	0
			3	86	206	120	85	1	2·5	0	12·3	2
			4	45	200	155	N.E.	2	2	0	7·1	1
			5	57	230	173	N.E.	3	2	0	10·9	0·5
	Mean			64	207	144	86	2·2	2·2	0	8·6	0·8
3	C.L.O.	0	11	72	198	126	96	2	0·5	3	6·5	2·5
			12	50	215	165	89	0	0	4	5·7	3
			13	72	191	119	87	2	0	2	3·0	2
			14	51	221	170	87	0	0	2	4·5	3
			15	70	214	144	93	0	0	3	4·2	2·5
	Mean			63	208	145	90	0·8	0·1	2·8	4·8	2·6
4	C.L.O.	5·5	21	67	244	177	84	4	0	0·5	10·5	0
			22	53	244	191	18	4	0	0	9·2	0
			23	67	228	161	2·3	4	0	0·25	12·0	0
			24	53	216	163	0·8	4	0	0·25	8·7	0
			25	60	258	198	0·9	4	0	0·5	8·0	0
	Mean			60	238	178	21·2	4	0	0·3	9·7	0
5	C.L.O.	11	26	64	239	175	0·3	4	0	0	9·7	0
			27	54	269	215	0·4	4	0	0	13·7	0
			28	65	257	192	0	4	0	0	9·8	0
			29	55	214	159	0	4	0	0	9·5	0
			30	59	256	197	0	4	0	0	11·5	0
	Mean			59	247	188	0·1	4	0	0	10·8	0

N.E., not examined; C.L.O., cod-liver oil. * See p. 298.

to be expected from the absence of cod-liver oil from the diet, the intraperitoneal fat deposits in all the rats of this group were normal in colour.

In the rats in group 3, which received cod-liver oil without α-tocopherol, the results for the haemolysis test were the same as in group 1. Dental depigmentation was perhaps more pronounced as the result of giving cod-liver oil. The uterus was normally coloured in four of the rats, and only slightly abnormal in one rat. In contrast, the intraperitoneal fat was decidedly brown in all the rats. Renal histolysis was rapid in all the animals (Pl. 1).

Table 2. *Results of breeding experiments with rats reared and mated on diets containing cod-liver oil or lard*

Group no.	Fat in diet	α-Tocopheryl acetate added (mg/100 g fat)	Rat no.	Result of mating
2	Lard	22	6	Litter of ten
			7	Litter of eight
			8	Litter of eleven
			9	Litter of ten
			10	Litter of seven
3	C.L.O.	0	16	Resorption
			17	Resorption
			18	No implantation
			19	Resorption, slight haemorrhage
			20	Resorption
6	C.L.O.	22	31	Litter of ten
			32	Litter of eleven
			33	Litter of eight
			34	Litter of eleven (six dead)
			35	Litter of eleven (one dead)
7	C.L.O.	44	36	Litter of eleven
			37	Litter of eight
			38	Litter of eleven
			39	Litter of eight
			40	Litter of six (two dead)

C.L.O., cod-liver oil.

In group 4, in which the cod-liver oil was fortified with 5 mg α-tocopherol/100 g, the signs of avitaminosis E were almost completely prevented. No evidence was found of dental depigmentation, discoloration of the uterus or renal histolysis (Pl. 1). Protection in the haemolysis test was virtually complete in three rats, and partial in another, but in the remaining rat haemolysis was almost complete. In four of the animals there was very slight discoloration of the intestinal fat. In group 5, given cod-liver oil fortified with 10 mg α-tocopherol/100 g, all signs of avitaminosis E were prevented.

Comparisons between the various groups of the amounts of intraperitoneal fat indicated that the rats of group 3, which had received unfortified cod-liver oil, had much less fat than the rats of any other group. Between group 3 and group 4, for which the cod-liver oil was fortified with α-tocopherol at the lowest level, this difference was highly significant ($P < 0.001$).

Breeding experiments. From Table 2 it will be seen that litters were produced

successfully by all the rats in group 2 (diet with lard fortified with α-tocopherol) and groups 6 and 7 (diets with cod-liver oil fortified with the two higher levels of α-tocopherol). In group 3 (diet with unfortified cod-liver oil) four of the rats became pregnant, but resorbed their foetuses. In one instance the resorption was accompanied by slight haemorrhage at the vagina. The remaining rat in this group gained no weight after spermatozoa had been detected in the vaginal smear. No implantation sites were found at autopsy after the normal gestation time had elapsed. In this animal, therefore, fertilization was ineffective.

DISCUSSION

Experiments on virgin rats. Our results on virgin females show clearly that the toxic effects produced by cod-liver oil in the rat may be counteracted by the additon of vitamin E to the oil, in the form of DL-α-tocopheryl acetate. Even the addition of an amount of DL-α-tocopheryl acetate equivalent to 50% of the α-tocopherol naturally present in the oil was sufficient, apart from a few exceptions, to prevent or minimize all the indications of avitaminosis E. When the level of fortification was raised so as to bring the concentration to double that of the α-tocopherol naturally present, no signs of avitaminosis E were observed in any of the rats.

Contrary to the early finding of Mackenzie *et al.* (1941) the administration of α-tocopherol dissolved in cod-liver oil did not in our observation cause its inactivation. A point still unsettled is whether α-tocopheryl acetate, used in our experiments, may be more stable in the presence of cod-liver oil than free α-tocopherol. It is well known that α-tocopheryl acetate cannot act as an anti-oxidant, and, as a corollary, acetylated tocopherol might be expected to be more stable than free α-tocopherol up to that site in the intestinal contents or walls where tocopheryl esters are hydrolysed.

Apart from the question of the mechanism of action of DL-α-tocopheryl acetate, it seems remarkable that such small additions in relation to the amount of α-tocopherol already present in the cod-liver oil should have such decided effects in preventing signs of avitaminosis E. We suspect that a physiological relationship may hold between the concentrations of polyunsaturated acids and α-tocopherol naturally present in the oil At the low body temperatures at which the cod-fish normally lives, the tocopherol in its liver may be fully adequate to balance the amount of polyunsaturated acids contained in it. Hove & Harris (1951) have drawn attention to a significant correlation between the linoleic acid and total tocopherol contents of animal and vegetable fats and oils.

The balance between vitamin E and polyunsaturated acids in different tissues. In our previous paper (Moore *et al.* 1959) we pointed out that the outcome of the antagonism between vitamin E and polyunsaturated fatty acids may vary in different tissues of the same rat. Thus the inclusion of cod-liver oil in the diet protected the uterus from pigmentation, presumably because of its content of α-tocopherol, but caused pigmentation in the fat deposits, presumably because of its content of polyunsaturated acids. This variation between tissues of the effect of the cod-liver oil was seen again in the work now presented. Pl. 2 shows photographs of uteri and adjacent fat deposits from rats from groups 1, 3 and 4, respectively. In the rat from group 1 (lard diet without

α-tocopherol) the uterus was deeply pigmented, but the fat was normal in colour. In the rat from group 3 (cod-liver oil without α-tocopherol) the uterus was normal, but the fat deposits were pigmented. In the rat from group 4 (cod-liver oil with 5 mg α-tocopherol/100 g) both the uterus and fat deposits were normal. It must be emphasized that in the rats given unfortified cod-liver oil the ability of the α-tocopherol supplied naturally in the oil to outbalance the polyunsaturated acids from the same source was seen only in the uterus. In their effects on dental depigmentation, haemolysis, and kidney histolysis the polyunsaturated acids predominated over the α-tocopherol. As we have previously reported, the ability of unfortified cod-liver oil to protect against uterine pigmentation may be lost when the oil is given over very long periods.

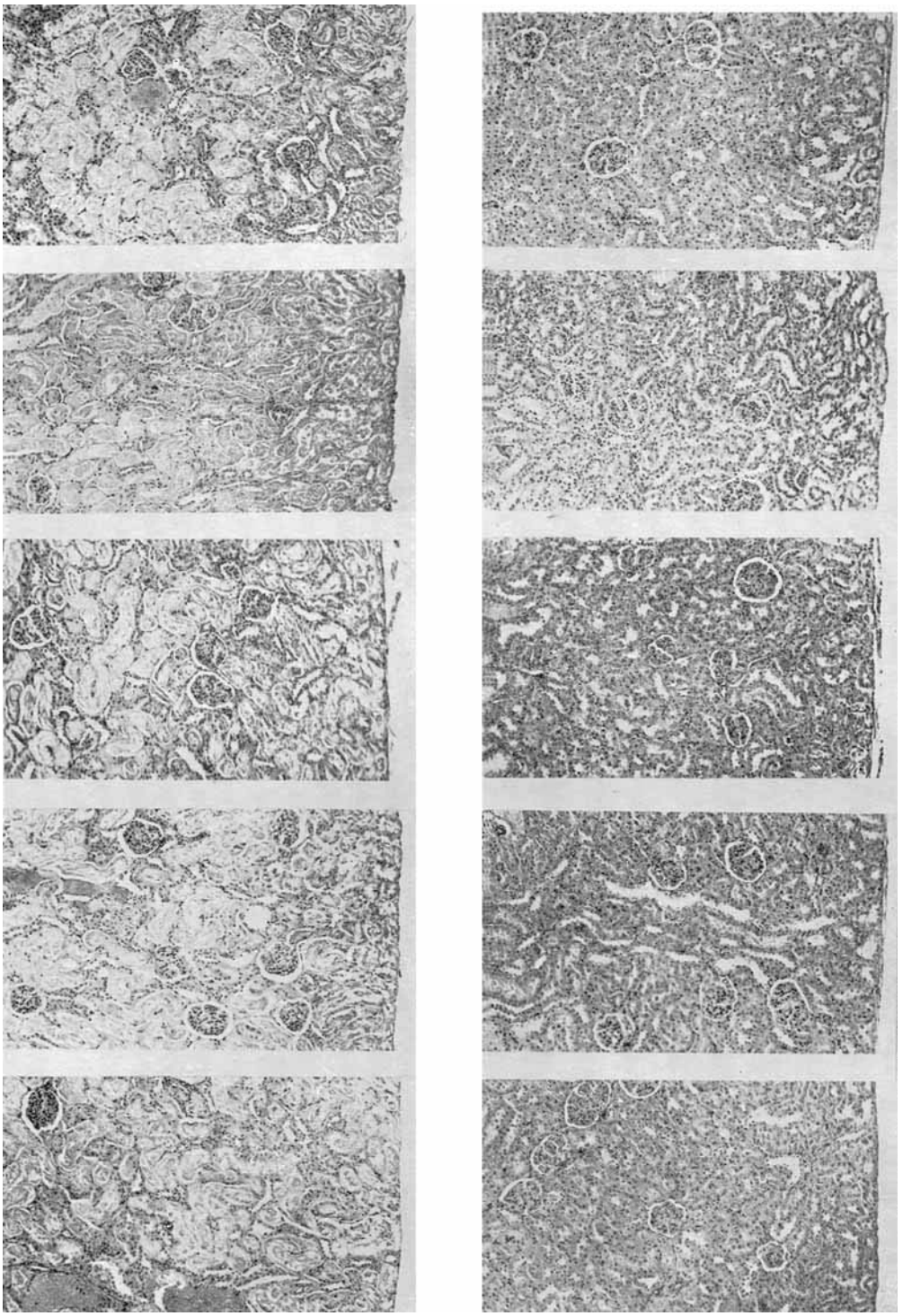
Breeding experiments. The results of these experiments indicated clearly that the amount of vitamin E naturally present in cod-liver oil could not prevent resorption gestations typical of avitaminosis E. Reproduction was normal, however, when the cod-liver oil was fortified with DL-α-tocopheryl acetate. These findings are in broad agreement with the early conclusion of Hartwell (1927) that failure of reproduction in rats given excessive amounts of cod-liver oil is due to lack of vitamin E. We must now conclude, however, that the deficiency of vitamin E is not absolute, but relative to the intake of polyunsaturated acids. It seems noteworthy that the resorption gestation, which may perhaps be regarded as the classical sign of avitaminosis E (Evans & Burr, 1927) may be induced, not only by the absence of vitamin E from the diet, but also by the inactivation, by an excessive intake of polyunsaturated acids, of supplies of vitamin E that would otherwise be adequate.

In Hartwell's experiments the reproductive defect took the form of a failure in parturition, with uterine haemorrhage which was fatal to the mother. Fatal uterine haemorrhage was also observed by Moore & Wang (1945) in one out of six mated female rats which were given excessive amounts of cod-liver oil. They found that the haemorrhage could be provoked more readily, however, by a massive intake of vitamin A, either in the form of pure vitamin A acetate or as halibut-liver oil. In the work now presented slight haemorrhage was noticed in one of the rats given unfortified cod-liver oil. Nevertheless, in most of these rats the defect in reproduction was resorption of the foetuses, as ordinarily seen in rats deficient in vitamin E, without noticeable haemorrhage. Possibly the high concentration of vitamin A in cod-liver oil may sometimes cause haemorrhagic lesions which are superimposed upon the other lesions induced by the high intake of polyunsaturated acids. If the intake of vitamin A does not reach a toxic level, either because the oil contains less vitamin A than usual, or because the percentage of the oil in the diet is not high, then haemorrhagic complications may be avoided.

SUMMARY

1. Groups of weanling female albino rats were fed for about 3 months on a diet containing 10% of cod-liver oil. According to chemical estimation this oil provided much more vitamin E than would have been needed to prevent avitaminosis E in rats given a diet with lard, instead of cod-liver oil, as the fat component.

2. When the cod-liver oil contained only its natural concentration of vitamin E,



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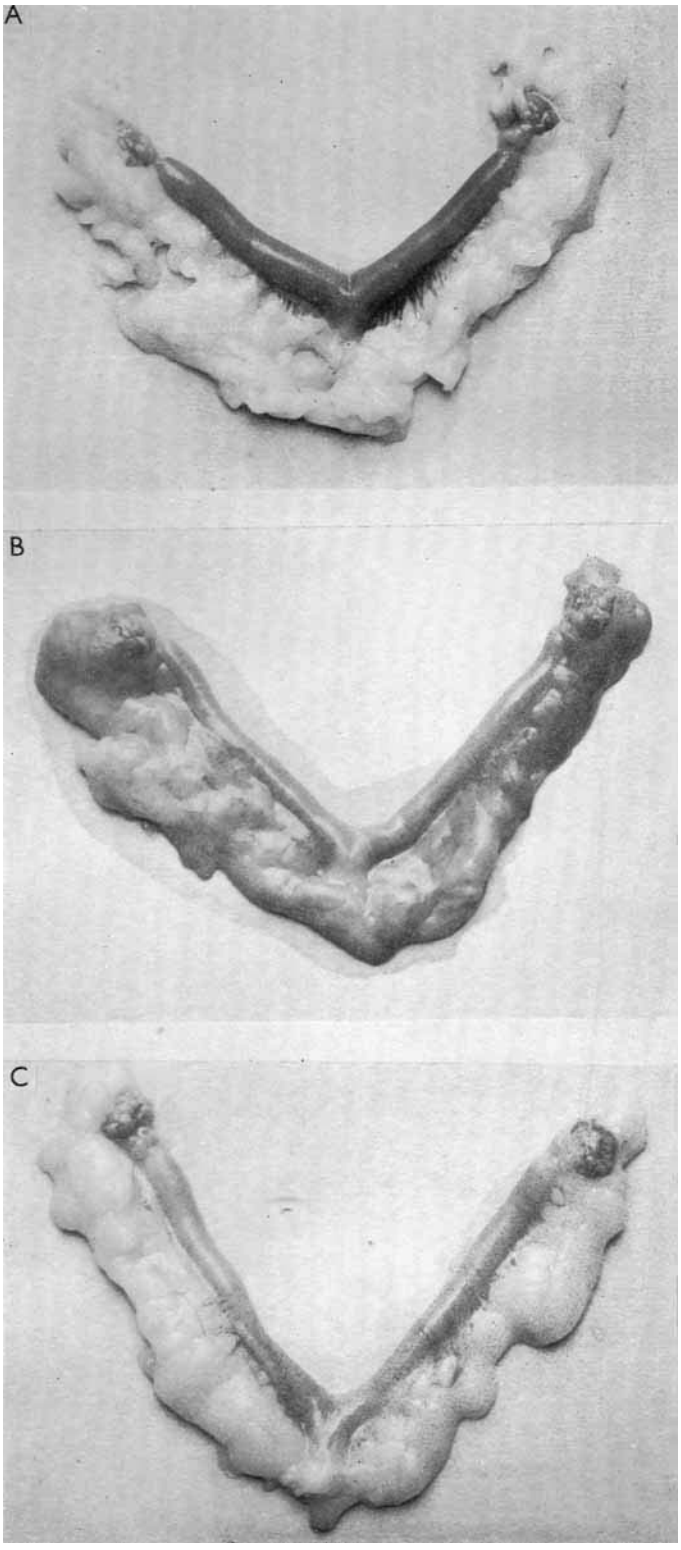

however, the rats developed signs of avitaminosis E. These signs included dental depigmentation, increased liability to haemolysis in vitro, rapid post-mortem renal histolysis and brown discoloration of the body fat. The uterus, however, did not become pigmented.

3. In rats given lard instead of cod-liver oil signs of avitaminosis E also developed, but differed in that the uterus, rather than the body fat, became brown. Renal histolysis was less rapid.

4. When the cod-liver oil was fortified with adequate additions of DL-α-tocopheryl acetate all signs of avitaminosis E were completely prevented. An addition equivalent to 50% of the α-tocopherol originally present in the oil gave almost complete protection.

5. Other rats were reared on similar diets and then used for breeding trials. When the diet contained unfortified cod-liver oil all the rats failed to breed, most of them having resorption gestations typical of avitaminosis E. Rats given cod-liver oil fortified with DL-α-tocopheryl acetate all produced litters successfully.

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EXPLANATION OF PLATES

Plate 1. Photomicrographs of sections from kidneys dissected 3 h after death from rats given a diet containing unfortified cod-liver oil (group 3, rats nos. 11–15) or cod-liver oil fortified with DL-α-tocopheryl acetate, 5·5 mg/100 mg (group 4, rats nos. 21–25). The sections were cut in paraffin, and stained with haematoxylin and eosin. Note the almost complete histolysis in the convoluted tubules in group 3, and the absence of histolysis in group 4.

Plate 2. Photographs of uteri and adjacent adipose tissues of typical rats in (A) group 1 (rat no. 1, diet containing lard without α-tocopherol; uterus brown and fat normal), (B) group 3 (rat no. 12, diet containing cod-liver oil without α-tocopherol; uterus normal and fat brown) and (C) group 4 (rat no. 22, diet containing cod-liver oil fortified with 5·5 mg DL-α-tocopheryl acetate/100 g; both uterus and fat normal).

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