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Observations on feeding pigs on a low-fat diet with and without supplementary tocopherol

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The objects of this investigation were twofold, first, to investigate the possible effects of dietary tocopherol on composition of depot and liver fat in pigs, and secondly, to attempt to induce clinically recognizable signs of tocopherol deficiency by the prolonged feeding of a tocopherol-deficient diet.

Bratzler, Loosli, Krukovsky & Maynard (1950) reported that supplementing a fat-deficient, tocopherol-deficient ration with mixed tocopherols led to the formation of depot fat containing more oleic acid than was found in pigs not given the supplement. In these experiments Bratzler *et al.* (1950) fed five weanling male pigs to 75 lb. live weight on the basal ration supplemented, for three of the animals, with 2·87, 55·12 and 110·2 mg mixed tocopherols/kg body-weight/day, giving at slaughter depot fat of iodine value 64·0, 65·8 and 61·0 compared with 57·1 and 55·0 for two unsupplemented (control) pigs. In addition, the work of Hove & Seibold (1955) suggested that the amount of liver fat and the proportions of its component polyethenoid fatty acids may be affected by the absence or presence of dietary tocopherols.

The problem of muscular dystrophy in pigs has recently been reviewed by Blaxter & McGill (1955) who concluded that the aetiology of the many field cases described in

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the literature was obscure, though the abundant clinical evidence suggested that tocopherol was involved. This suggestion is supported by the finding of muscular degeneration in piglets born to tocopherol-deficient sows (Adamstone, Krider & James, 1949).

EXPERIMENTAL

Animals and diet

Exp. 1. Twelve pigs (numbered 1–12) were randomized, according to weaning weights at 7–8 weeks of age, into three groups each of four pigs all of which were fed on a slightly restricted feeding scale (Lucas & Calder, 1955) on the basal diet given in Table 1.

Table 1. *Percentage composition of the low-fat, low-tocopherol basal diet*

Dried skim milk	5·0
Dried yeast	5·0
Extracted white-fish meal	7·5
Extracted groundnut meal	15·0
Sucrose	35·0
Maize starch	26·0
Oat feed	5·0
Sodium chloride	0·5
Ground limestone	1·0

To this mixture was added a virtually fat-free concentrate of vitamin A (6200 i.u./g) and vitamin D_3 (2100 i.u./g) at the rate of 10 oz./1000 lb. diet.

The complete diet contained 16·5 % crude protein, 0·73 % ether-extractable matter and 0·05 mg/100 g total tocopherols. It was purposely designed to be of low fat content to obviate possible effects of dietary fat on depot-fat composition (cf. Garton & Duncan, 1954).

One group of pigs (group A) served as controls, whilst the animals in the other two groups (groups B and C) were given, respectively, 5 mg and 10 mg DL-α-tocopheryl acetate/kg body-weight/day. The pigs were individually fed daily, at 7.0 a.m. and 4 p.m., and the tocopheryl acetate in the form of Rovimix E (Roche Products Ltd) was mixed daily with the diet which was fed as a wet mash. Rovimix E is a free-flowing powder containing 10 % of DL-α-tocopheryl acetate B.P.C. adsorbed on a silica base, marketed as a supplement for animal feeding-stuffs.

The pigs were weighed weekly and, on this basis, the amount of the tocopheryl-acetate preparation required/pig/day for the ensuing week was calculated. After 12–13 weeks on the experimental diets the animals were slaughtered. Table 2 shows the allocation of the pigs to the different groups, their breed and sex and their weights at the beginning and end of the experiment.

Initially nearly all the pigs scoured, but this was controlled by the administration of Sulphamezathine. Unfortunately, two of the animals in group A were lost; one (no. 12) died during the 4th week of the experiment and the second (no. 4) was slaughtered *in extremis* during the 8th week. Examination of the carcasses revealed a gastric haemorrhage in pig no. 12 and an enlarged spleen, pyloric oedema and excess

pericardial fluid in pig no. 4. The aetiology of both conditions was obscure but did not appear to be related to the experimental treatment. These two animals were replaced by other (spare) pigs which had been similarly treated; it is these replacements that are referred to throughout as pigs nos. 4 and 12.

Exp. 2. Nine Large White pigs (numbered 13–21), from two litters, were weaned at 10 days of age and reared for a further 10 days on a proprietary sow's-milk substitute. They were subsequently fed to appetite on the same basal diet as that used in Exp. 1 (Table 1) for 29 weeks, by which time they had reached bacon weight (200–210 lb.) and were slaughtered. For 28 days before slaughter two of the pigs (nos. 18 and 19) were each given 1 oz. cod-liver oil daily, and two others (nos. 20 and 21) were each given 2 oz. daily; for the last 14 days of the experiment pigs nos. 20 and 21 were given forced exercise in a large open run.

Table 2. *Plan of Exp.* 1, *showing breed, sex and weight of pigs used*

(Group A, controls; group B received 5 mg DL-α-tocopheryl acetate/kg body-weight/day; group C received 10 mg DL-α-tocopheryl acetate/kg body-weight/day)

Pig no.	Sex	Breed	Allocated to group	Weight at weaning (lb.)	Weight at slaughter (lb.)
1	F.	Wessex	A	36	128
2	F.	Wessex	B	41	136
3	F.	Wessex	C	37	131
4	M.	Large White	A	39	123
5	M.	Large White × Wessex	B	38	137
6	M.	Large White × Wessex	C	45	133
7	F.	Large White × Wessex	B	43	130
8	F.	Large White × Wessex	A	43	132
9	F.	Large White × Wessex	C	48	132
10	M.	Large White × Landrace cross	B	30	130
11	M.	Large White × Landrace cross	C	22	128
12	M.	Large White	A	30	123

Examination of tissues

Tissue samples. At slaughter all the carcasses and organs were examined for abnormalities, and the livers and samples of blood and of back tissue were obtained. The back tissue was taken as pieces about 7 × 4 × 2 in., in such a way that the complete depth of back fat was included. It was taken from the same place (between the shoulders) in each animal, since the iodine value of the back fat of pigs is known to vary along the length of the back, both inner and outer back fat being less unsaturated at the tail end than at the head end (Shorland, Hansen & Hogan, 1944). The back-fat tissue was carefully dissected into its inner and outer layers which, after being finely chopped, were exhaustively extracted with boiling acetone. The acetone solutions were taken to dryness and the fat was extracted with light petroleum (b.p. 40°–60°). The light-petroleum solution was dried over anhydrous Na_2SO_4, filtered and the solvent removed to yield the fat.

In Exp. 1 liver fat was extracted from 50 g samples of tissue with boiling ethanol–ether (3:1 by vol.). The extract was taken to dryness under nitrogen and the crude fat was extracted with warm light petroleum. The light-petroleum solution was

treated as described above to obtain the fat. In Exp. 2 liver tissue from each pig was examined histologically.

The back fat and liver fat were stored under nitrogen at $-1°$ for a few days until they were examined chemically. Blood samples were allowed to clot and the serum so obtained was taken for tocopherol estimation.

Chemical methods. Iodine values were determined by the Wijs method as described by Hilditch (1949). The method of Wiese & Hansen (1953) was employed to determine the proportions of polyethenoid components present in the total fatty acids of some of the liver fats. Total serum tocopherols were estimated according to the method of Nair & Magar (1956).

RESULTS

Experiment 1

No signs that might have suggested tocopherol deficiency (e.g. weak leg muscles or oedema) or fat deficiency (cf. Witz & Beeson, 1951) were observed in any of the pigs. At slaughter no abnormalities of any kind were visible in any of the carcasses or organs; the back fats were firm and not discoloured and the muscles, hearts and livers appeared completely normal. The carcasses, livers and kidneys were accepted for human consumption.

In Table 3 the serum-tocopherol values and the iodine values of the inner and outer back fat are recorded, and Table 4 gives the proportions of fat in the livers, the iodine values of the component fatty acids and the polyethenoid fatty-acid content of the total fatty acids of the liver fats of five animals.

Table 3. *Serum-tocopherol values and iodine values of the back fats of the pigs in Exp.* 1

Group	Pig no.	Serum tocopherol (μg/100 ml.)	Iodine value	
			Inner back fat	Outer back fat
A	1	170	52·5	58·7
	8	140	53·1	60·1
	4	n.d.*	47·3	55·8
	12	n.d.*	51·7	58·9
B	2	225	55·9	60·9
	5	145	53·2	59·7
	7	105	50·6	59·1
	10	150	54·3	60·4
C	3	120	53·7	59·9
	6	130	55·9	61·6
	9	130	53·3	59·7
	11	120	53·8	60·0
		Mean	52·9	59·6

* n.d. = not determined owing to experimental loss.

Experiment 2

As in Exp. 1, no overt signs suggesting tocopherol or fat deficiency were seen in any of the pigs, though during the last 6 weeks before slaughter the skin and bristles of the animals turned noticeably reddish brown. The carcasses were all accepted for

human consumption though, as a precaution against possible taint, only those from the five pigs that had not received cod-liver oil were cured for bacon. The back fats were all firm and not discoloured, and all the organs, with the exception of some of the livers (see below), appeared normal. Table 5 shows the serum-tocopherol values and the iodine values of the inner and outer back fat.

Table 4. *Characteristics of the liver fats of the pigs in Exp.* 1

		Liver fat						
		As percentage of wet weight of liver	Iodine value of total fatty acids	Polyethenoid fatty acids (as percentage of total fatty acids)				
Group	Pig no.			Di-ethenoid	Tri-ethenoid	Tetra-ethenoid	Penta-ethenoid	Hexa-ethenoid
A	1	3·2	124·4	8·4	2·2	3·5	11·7	9·9
	8	3·1	116·9	—	—	—	—	—
	4	3·8	120·9	—	—	—	—	—
	12	3·8	123·1	6·3	4·1	4·3	14·3	12·5
B	2	3·5	110·9	—	—	—	—	—
	5	3·6	120·9	6·9	2·3	3·5	10·9	9·7
	7	3·6	120·5	—	—	—	—	—
	10	3·0	120·9	6·3	2·9	4·2	11·1	10·7
C	3	3·1	125·1	—	—	—	—	—
	6	3·1	117·9	—	—	—	—	—
	9	3·4	126·7	7·2	3·2	4·3	12·3	10·7
	11	4·4	119·9	—	—	—	—	—

Table 5. *Serum-tocopherol values, and iodine values of the back fats, of the pigs in Exp.* 2

	Serum tocopherol	Iodine value	
Pig no.	(μg/100 ml.)	Inner back fat	Outer back fat
13	40	48·5	58·5
14	30	53·7	59·6
15	50	50·9	59·4
16	25	51·8	59·8
17	165	53·0	61·1
18*	40	51·1	61·0
19*	120	55·7	62·0
20†	60	52·9	61·3
21†	80	52·1	61·2
	Mean	52·1	60·4

* Received 1 oz. cod-liver oil/day for 28 days before slaughter.
† Received 2 oz. cod-liver oil/day for 28 days before slaughter.

Lesions were visible in the livers of five of the pigs (nos. 13, 14, 15, 16 and 18); only one of these (no. 18) had received cod-liver oil at the end of the experiment. Macroscopically the lesions consisted of a mild, irregularly distributed cirrhosis with no evidence of parasitic invasion and, in animals nos. 15 and 16, some of the liver lobules showed unusually red against the generally pale background.

In the following description of the histological findings livers nos. 13–17 were from the animals fed on the basal diet alone, livers nos. 18 and 19 were from the animals

given 1 oz. cod-liver oil/day for 28 days before slaughter, and livers nos. 20 and 21 were from the pigs given 2 oz. cod-liver oil/day for 28 days before slaughter.

Liver no. 13. Occasional local areas of portal fibrosis, mild in degree, were present with no evidence that the process was an active one. No other abnormalities were detected.

Liver no. 14. In the sections stained with haemotoxylin and eosin, the centrolobular parenchymal cells stained less deeply and the cytoplasm appeared slightly swollen and showed occasional vacuoles. These changes may represent mild degeneration of the cloudy swelling–hydropic degenerative type. A few eosinophils were observed in the portal tracts.

Liver no. 15. A diffuse mild portal fibrosis was present tending to be more severe in the subcapsular areas. In the more severely affected areas, occasional remnants of lobules were observed which appeared to be undergoing atrophic changes. Moderate numbers of eosinophil leucocytes were present in the portal tracts.

Liver no. 16. A mild diffuse portal fibrosis was present with locally heavy infiltrations of the portal tracts by eosinophil leucocytes. Occasionally this infiltration also involved adjoining areas of the lobular parenchyma. In four lobules focal haemorrhagic lesions were present with varying degrees of eosinophil infiltration resembling the lesions associated with ascarid larval migration.

Liver no. 17. The liver was normal, except for one very small portion in which a diffuse mild portal fibrosis was present with, in one area, the remnant of a lobule undergoing atrophy. In another lobule a small group of hypertrophied cells was observed.

Liver no. 18. A diffuse fibrosis, mild to moderate in degree, was present and in a few areas lobular remnants, apparently undergoing atrophy, were noted.

Livers nos. 19, 20 *and* 21. No significant abnormalities were observed.

DISCUSSION

Serum-tocopherol values

In Exp. 1 it was expected that, at slaughter, the serum-tocopherol values of the animals in the groups which had received supplementary dietary tocopherol would be greater than those of the control group. However, it was not so and all the values can be considered as being of the same low order of magnitude, with no essential difference between the groups. These findings may reflect impaired intestinal absorption of the dietary tocopherol, possibly related to the low fat content of the diet. However, if it be so, the absorption of vitamin E and that of vitamin A, both fat-soluble, appear to differ in some fundamental respect, since Witz & Beeson (1951) reported significantly higher plasma-vitamin A values in pigs reared on a diet containing 0·06% fat than on a similar diet containing 5% lard, though the amounts of dietary vitamin A were the same.

In the experiment referred to earlier, Bratzler *et al.* (1950) found no measurable amount of plasma tocopherol in two pigs fed on a low-fat, tocopherol-deficient ration, though the plasma values of three pigs given the same diet with about 3, 55 or 110 mg

mixed tocopherols/kg body-weight/day for 75 days were 248, 668 and 594 μg/100 ml., respectively. These results contrast with our findings and with those of Hove & Seibold (1955) who fed pigs on a low-protein, tocopherol-deficient diet containing 6% protein, 6% lard and 2% cod-liver oil and found that supplementing this ration with amounts of DL-α-tocopheryl acetate corresponding to 150 mg/animal/day (or more) for periods of up to 187 days did not significantly affect the plasma-tocopherol values, which were of the same order as those found by us.

In Exp. 2 the serum-tocopherol values were, with two exceptions, lower than those found in the pigs killed at an earlier age in Exp. 1 indicating that depletion of tocopherol had continued in these animals. The feeding of cod-liver oil to four of the pigs during the last 4 weeks of the experiment did not appear to affect the plasma-tocopherol values.

Back fats and liver fats

As mentioned on p. 97, Bratzler *et al.* (1950) reported that supplements of mixed tocopherols caused an increase in the unsaturation (iodine value) of the depot fat of pigs fed on a low-fat, tocopherol-deficient ration. This conclusion, as presented in the paper, is based on the average iodine and thiocyanogen values of five fats from different sites in the body of each animal, namely, leaf (omental), ruffle (mesenteric), jowl (cheek), back and ham-facing fats. On this basis no valid conclusion regarding the effect of dietary tocopherols on the unsaturation of body fats can be drawn. However, in a private communication, Professor Bratzler kindly provided us with the analytical results for each of the depot fats; these values showed a difference of 5–10 units of iodine value between each of the depot fats of the control pigs and those of the tocopherol-supplemented animals. No such difference was apparent in the iodine values of the back fat of the pigs in Exp. 1; prolonged feeding of the tocopherol-deficient diet (Exp. 2) gave back fat of a similar degree of unsaturation which was not affected by the administration of cod-liver oil at the end of the feeding period.

In the experiment to which reference was made on p. 97, Hove & Seibold (1955) found that tocopherol supplementation led to an increase in liver fat from 1·6 to 2·3%, expressed on a wet-weight basis. Although the iodine values of the liver fats did not differ appreciably, the proportions of pentaethenoid and diethenoid acids were increased (mainly at the expense of the oleic and triethenoid acids) in the fat from tocopherol-deficient pigs compared with the fat from the livers of pigs that had been given the dietary supplement of tocopherol. In our Exp. 1 the iodine values of the fatty acids of the liver fats were all of a similar order, and a similar spread of values was evident within each group; no significant differences were apparent in the amounts of liver fat, or in the relative proportions of the component polyethenoid fatty acids in the total fatty acids of the liver fats from animals in the different treatment groups.

The amount of dietary fat

Witz & Beeson (1951) reported that obvious signs of fat deficiency such as dermatitis, loss of hair and brown gummy skin exudates were produced in pigs reared on diets containing only 0·06 or 0·12% fat and which were given 50 mg α-tocopherol/kg

body-weight/day; several internal organs were also affected, notably stomach, gall-bladder and kidneys, but no mention is made of any liver damage. Some recession of the skin condition was noted when 1·5% maize oil was included in the ration for 3 weeks. No overt signs of fat deficiency were manifest in our experiments and it must be concluded that the 0·73% of fat present in the diet given to our pigs was adequate to protect them from the effects of fat deficiency.

Pathological findings

No dystrophic muscles were found in any of the pigs fed on the tocopherol-deficient diet; attempts to induce conditions of stress (cod-liver oil feeding and forced exercise) failed to precipitate signs of tocopherol deficiency though it should be noted that dystrophic lesions such as are found in tocopherol-deficient ruminants are usually manifest only in the young growing animal (see Blaxter & McGill, 1955), and the pigs in Exp. 2 were almost mature when stress factors were introduced.

The livers that appeared normal macroscopically were all from animals which, at slaughter, had serum-tocopherol values of 60 μg/100 ml. and more. On the other hand, the livers that were (with one possible exception) histologically abnormal were from pigs having serum-tocopherol values of 25–50 μg/100 ml. The possible exception was the liver of pig no. 17 which, though it appeared to be normal, did show an extremely small area of fibrotic tissue. Thus tocopherol depletion, as reflected by serum values, appears to be accompanied by the onset of liver lesions.

SUMMARY

1. In an attempt to induce clinical signs of tocopherol deficiency and to investigate the possible effects of dietary tocopherol on depot-fat composition, groups of pigs were fed on a basal tocopherol-deficient, low-fat diet with and without supplementary DL-α-tocopheryl acetate.

2. In the first experiment three groups, each of four weanling pigs, were reared to about 130 lb. live weight on the basal diet supplemented in two of the groups with 5 or 10 mg tocopheryl acetate/kg body-weight/day. Compared with the unsupplemented (control) group, the animals given tocopherol did not show any increase in the iodine value of the inner and outer back fat, nor was any change apparent in the amount or composition of the fatty acids in the liver fats. Tocopherol supplementation did not affect serum levels of the vitamin. No liver lesions were observed in any of the pigs.

3. In the second experiment the basal diet was fed to nine pigs from weaning to over 200 lb. live weight. No overt signs of tocopherol or fat deficiency were observed. During the final 28 days of the experiment four of the pigs were given cod-liver oil daily in an attempt to precipitate conditions of tocopherol deficiency, but no signs of such a condition were observed. The back fats of all nine pigs were similar in their degree of unsaturation to those of the animals in the first experiment. The serum-tocopherol levels were, with two exceptions, lower than those found in the pigs killed at 130 lb. live weight. Liver lesions were found in six of the animals, only one of

which had received cod-liver oil during the final stage of the experiment. The liver lesions, mainly portal fibrosis, appeared to be associated with tocopherol depletion.

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Clinical observations on obese patients during a strict reducing regimen

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It is orthodox today to discount the value of physical exercise in treating obesity, and to rely for weight reduction on a diet supplying 1000–1200 Cal./day. The energy expenditure of a sedentary patient is about 2500 Cal./day, giving a negative energy balance of no more than 1500 Cal./day. On such a regimen weight loss is slow and may therefore be discouraging. In this paper we describe a system of management suitable for some obese patients, and show that it is both possible and practicable to accelerate weight reduction by combining an active programme of physical exercise with a diet restricted to 400 Cal./day. Such a regimen can produce a negative energy balance as great as 3000 Cal./day.

It is frequently argued that during a brisk walk lasting an hour a normal subject expends about 350 Cal., which will require the utilization of only about 40 g fat; and further, that such a walk may stimulate the appetite and so an excess of food will be taken. The arithmetic is correct, so far as it goes, but an obese subject expends more energy than a thin subject while walking, on account of the extra work needed to move