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From the Department of Pathology and the Department of Animal Husbandry and Genetics, Veterinary College of Norway, Oslo.

THE VITAMIN E-DEFICIENCY SYNDROME IN PIGS

I. PATHOLOGICAL CHANGES*)

By

Inger Nafstad and *Sverre Tollersrud*

Vitamin E-deficiency has been described in most animal species and in man. The deficiency state has been associated with infertility, encephalopathia, myocardial and skeletal muscle degeneration, vascular injury, liver necrosis, brown discoloration of fat depots and haematologic disorders. The experiments described in this paper were performed to obtain further informations about pathology of the vitamin E-deficiency syndrome in pigs. Further, our intention was to determine the susceptibility of the different lesions within the syndrome to supplements of selenium and sulphur-containing amino acids, respectively. The morphologic changes described in this study demonstrate that some of the above mentioned conditions, including myocardial injury identical to the changes which occur in "mulberry heart disease", show a great tendency to occur simultaneously. The pathologic lesions described herein were produced by diets containing unsaturated fatty acids in excess. However, gross and histologic lesions appeared identical to their respective counterparts occurring during natural conditions.

Some aspects of the experimental work have been published previously. The haematologic alterations in experiments 1 and 2 were described in a light microscopic (*Nafstad* 1965) and an

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electron microscopic study (*Nafstad & Nafstad* 1968). The experiments here designated 1 and 4 were included in the material used for studies of gastric ulcers in swine (*Nafstad & Tollersrud* 1967, *Nafstad et al.* 1967). The results of the examinations of serum and tissue enzymes are treated in a separate paper.

Parts of the vitamin E-deficiency syndrome in pigs have been described by several investigators, with the importance stressed upon one or another of the diseases involved.

In a morphological and experimental study of hepatosis diaetetica (HD), *Obel* (1953) gave a detailed description of the pathologic changes which are characterized by excessive necrosis of the liver tissue. The experimental disease, which corresponded to the spontaneous one, was produced by *Obel* with diets containing 18 % dried brewer's yeast and 6 % cod liver oil. Protection was obtained with supplement of 0.5 % methionine or cystine or with addition of 150 mg alpha-tocopheryl acetate twice a week when lard was simultaneously substituted for cod liver oil. No liver injury occurred when the experimental diet contained 15 % casein, even though 12 % cod liver oil was included in the ration. The reason why this diet failed to produce the disease was, according to the author's explanation, that the diet was sufficiently rich in protein. In the same way, the supplement of sulphur-containing amino acids made the yeast protein adequate and thus was able to prevent the disease.

Waxy degeneration of the skeletal muscles occurred with or without simultaneously liver injury, as did also, but less frequently, waxy degeneration of the myocardium. In both spontaneous and experimental cases there was a high frequency of fibrinoid degeneration of the arterial walls, especially in cases with repeated attacks of the disease. A close correlation between vascular changes and degeneration of the myocardium was observed. In the experimental cases described by *Obel*, a yellowish discoloration of fat was observed when the animals survived for a sufficient time. This condition has been reported in its experimental and natural occurrence in pigs from several countries (*Gorham et al.* 1951 a, *Davis & Gorham* 1954) and is comparable to steatitis in fur-bearing animals (*Ender & Helgebostad* 1944, *Gorham et al.* 1951 b) and yellow discoloration of body fat in rats (*Dam & Granados* 1945), which have been repeatedly produced on vitamin E-deficient diets with high contents of unsaturated fatty acids (*Mason et al.* 1946).

Davis & Gorham described the microscopic findings as deposition of acid-fast pigment in the interstices of subcutaneous and visceral adipose tissue, sometimes accompanied by scarce cellular reaction. The precursors of the yellow-brown pigment are generally suggested to be fat peroxides formed from highly unsaturated fatty acids. The staining differences between the various forms of the pigment observed in histochemical studies (varying affinity to fat stains, inconstantly PAS-positive reaction, variously autofluorescence intensity), are evidently a function of the length of the time that they have been deposited in the tissue (*Porta* 1963).

The literature concerning nutritional muscular dystrophy (MD) in pigs until 1955 was reviewed by *Blaxter & McGill* (1955—56). Most experiments performed in order to provoke MD were conducted with fish-liver oil or lard and a deficiency of vitamin E. The lesion, both during experimental and natural condition, is a hyaline degeneration of the muscle cells. *Blaxter & McGill* also directed the attention to the association between MD and the muscular damage in the myocardium occurring in "fatal syncope" or "plötzlicher Herztod", earlier described by *Hupka* (1939). Later on, MD in pigs has been produced with diets containing cod liver oil (*Lannek et al.* 1961), lard (*Hill & Larsson* 1955/56), maize oil (*Thafvelin* 1960, *Grant* 1961) or vitamin E-stripped cottonseed oil (*Lindberg & Orstadius* 1961). *Tanhuanpää* (1965) in his investigation on fatty acid composition of tissue lipids in pigs, produced MD by increasing the amount of dietary C 18:2 in relation to dietary tocopherol. The author concluded that experimental MD occurred when diets with a tocopherol/polyunsaturated fatty acids ratio of 0.4—0.6 or less were given. In one outbreak of spontaneous MD, however, the ratio between tocopherol and polyunsaturated fatty acids according to the author's opinion, was within favourable limits, and the investigator directed the attention to a possible selenium deficiency. Using diets with equal parts of heated barley and oats and 1.5 % methyl ester of linoleic acid (MELA), the same investigator induced HD together with MD in his experimental pigs. When 8 % of casein or 17.6 % of skim milk powder was included in the grain diets, no liver damage occurred even though MELA was added. MD, however, occurred independently of an increase in the casein content up to 20 %.

In a later report *Lindberg & Tanhuanpää* (1966) claimed that

selenium deficiency is obviously the most important factor in spontaneous MD. This view was based upon selenium analyses showing very low amounts of selenium in Swedish grain as compared with grain grown in other countries.

In the experiments performed by *Grant*, which have been referred to earlier, MD produced by feeding diets containing unsaturated and oxidatively unstable fats was not prevented by addition of selenium, neither when the fat was supplied as cereals nor in the form of other vegetable or marine oils. The hepatic lesions characteristic for HD which occurred in the same animals, could be prevented by selenium supplementation. Selenium also protected against the mycocardial damage designated in *Grant*'s investigation as "dietetic microangiopathy", a condition identical with what in Britain and Canada has been described as "mulberry heart" (MH). MH was described from Ireland (*Lamont et al.* 1950) and from Canada (*Geib* 1959). Apart from myocardial lesions, leucoencephalomalacia predominantly affecting the cerebrum, was encountered. The descriptions of the disease given by British investigators are surveyed by *Harding* (1960), who described the pathologic changes to include haemorrhages in the myocardium, signs of circulatory failure, and vascular lesions characterized by endothelial swelling, thrombosis, and degenerative mural changes. The condition known as "plötzlicher Herztod", which bears several points of similarity to MH, has been known for a long time, and was first described in German literature about 1930. Since then numerous papers have dealt with the disease (reviewed by *Maas* 1958, *Matthias* 1962 and *Grant*). Briefly, the morphological changes of "plötzlicher Herztod" is characterized by circulatory failure, myocardial haemorrhages and degenerative changes, and generally some secondary inflammatory reaction.

The observation that the skeletal muscles were commonly involved has already been mentioned. Later on, *Ludvigsen* (1957) proposed "Herztod" to be a severe form of MD.

As to vascular lesions accompanying "Herztod" and "mulberry heart", hyaline thrombi in myocardial vessels were described in "plötzlicher Herztod" by *Renk* (1951), and degenerative mural changes in arteries and arterioles of some organs were mentioned by *Kersten & Beck* (1960) in a paper dealing with "Herztod" and oedema disease. The observation of vascular changes in the material of *Harding* has already been mentioned.

Geib in his study of MH, noticed the presence of hyaline thrombi and mural degeneration in the vessels of myocardium and brain.

The investigation performed by *Grant* upon dietetic microangiopathy (MAP) dealt predominantly with the vascular changes, which he regarded as the primary lesion within the syndrome. The changes were localized in the capillaries and smaller vessels of the myocardium and might be found in other tissues. Associated with the vascular changes in the myocardium were degenerative changes and haemorrhages, and when localized in other organs, local oedema and haemorrhages. The usual autopsy appearance of MAP in pigs was described to include vivid red mottling of the myocardium, transudation to the serous cavities and signs of circulatory failure. Both macroscopically and histologically the pattern agreed with that of "mulberry heart". Histological examination of the vascular MAP lesions revealed accumulation of amorphous, PAS-positive material in or under the endothelial lining, resulting in mural thickening and various degree of obstruction of the lumen; PAS-positive material of the same character as that situated in the wall also appeared in the vessel lumen. Experimentally induced MAP was observed in pigs fed diets containing unsaturated and oxidatively unstable fat, and was then accompanied by MD and frequently by HD and yellow fat. Tocopherol and sodium selenite were effective in preventing MAP in the experimental pigs. According to these findings *Grant* proposed MAP to be added to the diseases which can be influenced by selenium or tocopherol.

As to earlier records concerning "mulberry heart" and "plötzlicher Herztod", numerous causes have been postulated. *Maas* reviewed most of these hypotheses suggested by different authors until 1958, including endocrine disturbances, infection, nutritional deficiencies of various kinds, and hereditary defects. What appears reasonable from the present knowledge about the matter is that an association between diet and the disease seems probable.

MATERIALS AND METHODS

Four experiments including 112 Norwegian Landrace pigs of both sexes were conducted with diets containing unsaturated fat to produce vitamin E-deficiency. The variable factors in the diets were vitamin E, selenium, and some essential amino acids,

probably being limiting in the relatively protein-low diets used in some of the experiments. Vitamin E was tested at two dose levels, the amount of casein was tested at the 10 %, 16 % and 22 % levels, and in experiment 4 a partial or wholly substitution with soybean meal for casein was tested. Because the number of animals in each group was relatively small, repeated experiments were carried out.

The casein used in all experiments was manufactured by Norsk Kasein A/L, and had an average protein content of 90.9 % based on dry matter. The soybean meal was extracted and heated. It contained 51 % of protein based on dry matter and was manufactured by Norsk Soyamelfabrik A/S. The cod liver oil was produced by J. C. Martens and Co. Ltd., Norway. Excess of vitamin A was removed by silica earth absorption before it was delivered from the manufacturer. This procedure also reduced the vitamin E content to an amount of 87—95 μg/g. The average peroxide value of the cod liver oil during the experiments was 8 meq/1000 g according to Wij's method, and the average iodine number was 140. The trials were conducted in confined quarters with concrete floors within a closed building. The pigs were divided into lots according to sex and weight and were individually hand-fed twice a day with adequate water supply.

At 2-week intervals the pigs were weighed and blood samples were obtained from the anterior vena cava for haematologic and clinical chemical examinations.

At the end of each experiment, tissues were collected for microscopic examination. Formalin fixed tissues were processed by conventional paraffin embedding. For particular purposes Helly's solution or Carnoy's solution were employed, or frozen cryostat sections were used. Tissues for electron microscopy were fixed in glutaraldehyde and post-fixed in osmium.

Experiment 1

Thirty-two pigs with an average body weight of 23.3 kg were divided into eight lots as follows:

Lot 1. Basal ration of Table 1.
Lot 2. Basal ration plus 100 mg dietary D-L-alphatocopheryl acetate per pig daily.
Lot 3. Basal ration plus additional sodium selenite to provide 0.25 p.p.m. in the diet.
Lot 4. The ration of lot 3 plus 100 mg dietary D-L-alphatoco-acetate per pig daily.

Lot 5. Basal ration plus additional amino acid supplement to provide 0.2 % methionine, 0.2 % lysine and 0.1 % threonine in the diet.

Lot 6. The diet given to lot 5 plus 100 mg dietary D-L-alpha-tocopheryl acetate per pig daily.

Lot 7. The ration containing 16 % casein of Table 1.

Lot 8. The ration of lot 7 plus 100 mg dietary D-L-alphatocopheryl per pig daily.

Table 1. Basal rations in experiments 1, 2 and 3.

Dietary ingredients %	Experiment 1		Experiment 2	Experiment 3
	lot 1	lot 7		
Casein	10	16	10	10
Cod liver oil	10	10	5	10
Sugar	15	15	15	38
Starch	61	55	66	37.5
Minerals[a]	4	4	4	4
Vitamins[b]	0.04	0.04	0.04	0.04

[a] Contained in per cent: Dicalcium phosphate, 68; sodium chloride, 15; potassium chloride, 10; magnesium carbonate, 5; ferrous sulphate, 1.5; manganese sulphate, 0.25; copper sulphate, 0.1; cobalt chloride, 0.05; potassium iodine 0.05; zinc oxide, 0.05.

[b] Supplied the following in mg per 100 kg ration: Ascorbic acid, 500; nicotinic acid, 1600; calcium pantothenate, 1100; inositol 400; choline, 25,000; riboflavin 200; biotin, 20; folic acid, 70; pyridoxine, 130; thiamine hydrochloride, 200; vitamin B_{12}, 1.

Experiment 2

Twelve pigs divided into two lots were fed the ration given in Table 1. The basal ration was supplemented with amino acids to provide 0.2 % methionine, 0.2 % lysine and 0.1 % threonine in the diet. Vitamin E in an amount of 500 mg D-L-alphatocopheryl acetate was supplied to each pig of lot 2 per day. Two pigs from each lot were fed a commercial swine food the first fourty days of the experiment and then fed the experimental diet for the remaining two months.

Experiment 3

Thirty-six pigs with an average body weight of 22.5 kg were divided into eight lots. Two lots were fed the diet with pyridoxin omitted from the vitamin mixture to test a possible effect on the blood vessels. The lots were provided with the following rations:

Lot 1. Basal ration of Table 1.
Lot 2. Basal ration plus 100 mg dietary D-L-alphatocopheryl acetate per pig daily.
Lot 3. Basal ration with pyridoxin omitted.
Lot 4. Basal ration without pyridoxin plus 100 mg D-L-alphatocopheryl acetate per pig daily.
Lot 5. Basal ration plus sodium selenite to provide 0.25 p.p.m. in the diet.
Lot 6. The diet of lot 5 plus 100 mg D-L-alphatocopheryl acetate per pig daily.
Lot 7. Basal ration plus additional amino acids to provide 0.2 % methionine and 0.2 % lysine in the diet.
Lot 8. The diet given to lot 7 plus 100 mg D-L-alphatocopheryl acetate per pig daily.

The tocopheryl acetate was applied per os.

Experiment 4

Thirty-two pigs initially weighing 21.1 kg on an average were divided into eight lots. The rations used in this experiment are shown in Table 2. Each of the four experimental groups was controlled with groups which were treated orally with 100 mg D-L-alphatocopheryl acetate per pig per day.

Table 2. Composition of rations in experiment 4.

Dietary ingredients %	Lot 1 (basal)	Lot 3 (casein and soya)	Lot 5 (high casein)	Lot 7 (high soya)
Casein*	16	16	22	—
Soybean meal**	—	10	—	36
Cod liver oil	10	10	10	10
Sugar	15	15	15	15
Starch	55	45	49	35
Minerals[a]	4	4	4	4
Vitamins[b]	0.04	0.04	0.04	0.04

[a, b] To supply the same amounts as in experiments 1, 2 and 3.

* Contained 2.2 p.p.m. selenium and 5 μg alphatocopherol per 100 g.

** Contained no traceable selenium or alphatocopherol. (Selenium analyses were kindly carried out by Statens Lantbrukskemiska Laboratorium, Helsingfors, and tocopherol analyses by Vitaminlaboratoriet, Bergen).

Table 3. Major necropsy findings of experiment 1.

Lot	Pig	Survival, days	Av. daily gain, kg	Dominant pathol. findings	Myocardial changes				MD	YF
					degen.	haemor-rhages	vascular injury	scar		
	3	37		MH	++	++	++	—	++	—
1	5	39	0.174	HD	+	+	+	+	++	+
(basal)	13	47		„	++	+	+	+	++	+
	35	78		„	+	—	+	++	+	+
	1	96 (killed)			—	—	—	—	—	—
2	14	96 „			—	—	—	—	—	—
(basal +	33	97 „	0.226		—	—	—	—	—	—
vit. E)	7	97 „			—	—	—	—	—	—
	24	79		*	—	—	—	—	+	+
3	32	82	0.167	MH+HD	++	++	++	+	++	+
(basal + Se)	26	96 (killed)			+	—	—	—	—	+
	20	96 „			+	—	—	—	++	+
	18	97 (killed)			—	—	—	—	—	—
4	11	97 „	0.264		—	—	—	—	—	—
(basal + Se	22	98 „			—	—	—	—	—	—
+ vit. E)	112	98 „			—	—	—	—	—	—
	8	36		MH	++	++	++	—	—	—
5	21	46	0.284	„	++	++	++	—	+	+
(basal +	95	62		„	++	++	++	+	+	+
amino acids)	28	74		MH+HD	++	++	++	+	+	+
6	15	95		**	—	—	—	—	—	—
(basal +	30	98 (killed)	0.303		—	—	—	—	—	—
amino acids	17	98 „			—	—	—	—	—	—
+ vit. E)	19	98 „			—	—	—	—	—	—
	27	51		HD	++	+	+	+	+	+
7	10	78		„	+	—	+	++	++	+
(16 % casein)	29	89	0.151	„	++	+	+	++	+	+
	31	99		„	+	—	+	+	++	+
	4	99 (killed)			—	—	—	—	—	—
8	16	99 „	0.373		—	—	—	—	—	—
(16 % casein	12	99 „			—	—	—	—	—	—
+ vit. E)	9	96 „			—	—	—	—	—	—

* Gastric ulcer haemorrhages.
** Intestinal volvulus.

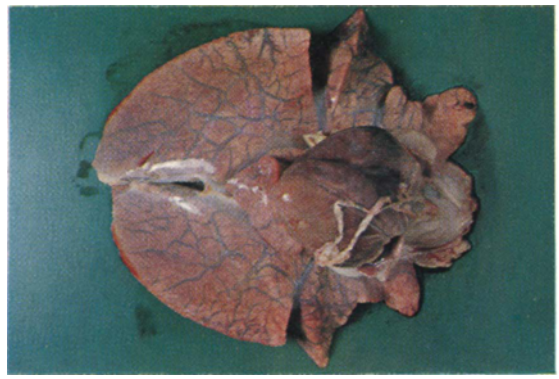

Fig. 1 A.

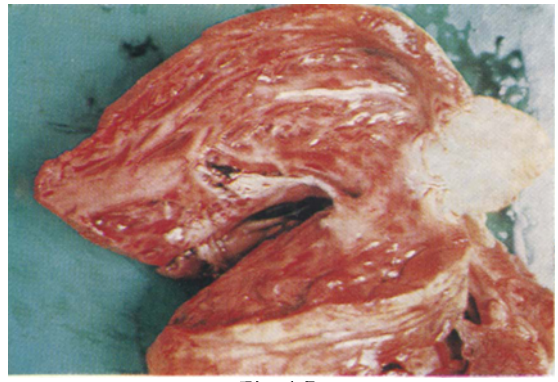

Fig. 1 B.

Figure 1. Gross changes in the heart.

A. Subepicardial haemorrhages and right ventricular dilatation. The pericardial sac, which was distended by transudate, has been opened. Fibrin strands have been left on the epicardium. Interstitial lung oedema is visible. Exp. 1, lot 5, pig no. 28.

B. Subendocardial haemorrhages and mottling of the myocardium ("mulberry heart"). Exp. 1, lot 5, pig no. 8.

RESULTS

Experiment 1

Fourteen of the pigs fed the tocopheryl-deficient diets, two of which were selenium supplemented, died between day 36 and day 99 in experiment. One of the pigs receiving tocopherol died due to intestinal volvulus. The remaining animals were killed at the end of the experiment and necropsies performed. A summary of the results is presented in Table 3. The diagnosis MH, HD, MD and YF were used according to the criteria of the diseases given in the literature. As will be seen from Table 3 no clear-cut limits could be drawn between the different conditions. In fact, MD and YF accompanied both MH and HD, and the pigs which had HD as major necropsy finding commonly had some degree of myocardial changes similar to those seen in MH.

"Mulberry heart". The diagnosis was determined according to the gross lesions and microscopic examinations. The gross changes included a pale cadaver with subcutaneous, intermuscular and pulmonary oedema. Hydropericardium, hydrothorax, and ascites were common. The amounts of transudates varied from 10 to 250 ml in the pericardium, 40—500 ml in the pleural cavity and 50—1000 ml in the abdominal cavity. The transudates were fluid, straw-coloured accumulations containing fibrin dots, and coagulation commonly occurred when exposed to air. The transudates contained 3.9—5.6 % protein. Venous engorgement was observed in all pigs showing the pathologic appearance of MH, with congestion of liver, kidneys, in the great veins, and in the intestinal tract. Coagulated blood was usually present in both heart ventricles, the right ventricle was dilated in some of the pigs.

Subepicardial haemorrhages, usually most evident over the right ventricle and atrium, were present (Fig. 1), as were subendocardial and myocardial haemorrhages. The changes in the right ventricle usually extended through the whole ventricular wall, while in the left ventricular wall the discoloured areas appeared as mottling, particularly in the subendocardial regions. Microscopical examination revealed varying degrees of myocardial injury. Haemorrhages and oedema were constantly present. Degenerative changes in the myocardial fibres varied from the appearance of swollen fibres containing a granular sarcoplasm to completely necrotic and calcified areas (Fig. 2), with the

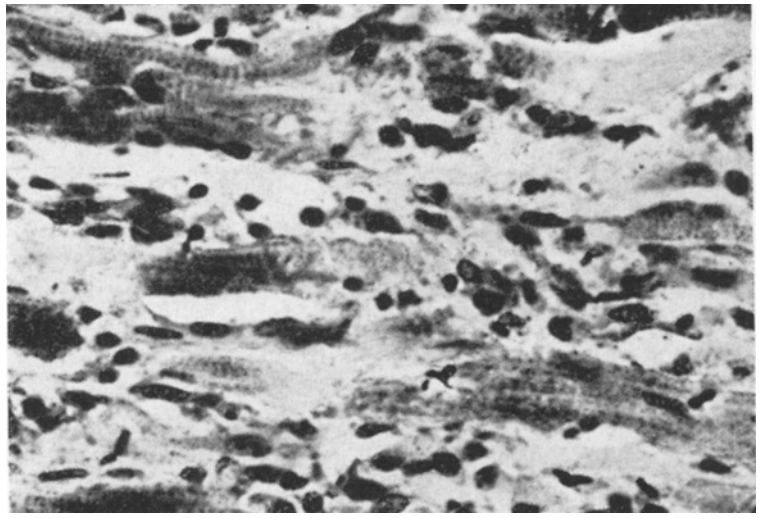

Figure 2. Myocardial changes. Area from the right atrium with degenerated fibres with deposition of calicum salts. Some inflammatory reaction is present. Exp. 1, lot 1, pig no. 3. H & E, × 480.

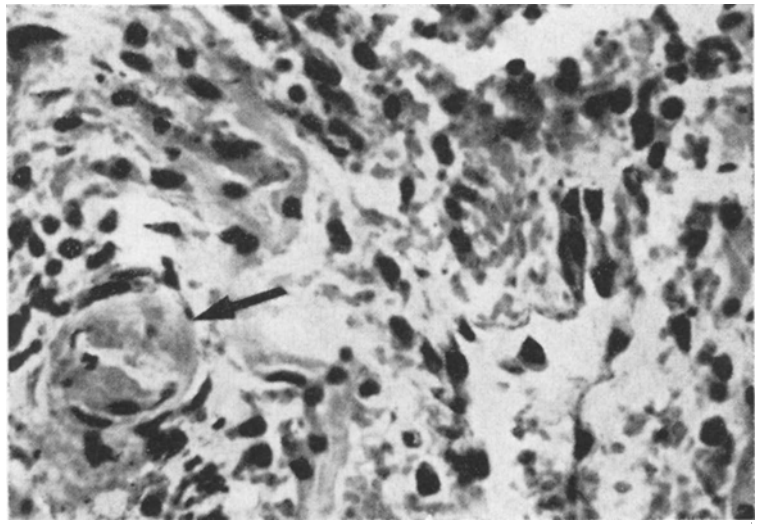

Figure 3. Myocardium with haemorrhages and myolysis. A partially thrombosed arteriole is demonstrated (arrow). Exp. 1, lot 3, pig no. 32. H & E, × 480.

severest altered areas usually situated around injured blood vessels. Other changes displaying myocardial damage were cellular inflammatory reactions in and around severely altered myocardial spots, and, in some cases, myocardial scars. Acute degenerative changes and chronic fibrosed areas could sometimes be found in the same hearts. The vascular changes which appeared in arterioles and capillaries exhibited the same morphological pattern as the microangiopathy described by *Grant* (1961). The principal manifestations were swelling of the wall with PAS-positive material deposited within the vessel wall and on the luminal side of the endothelial lining, resulting in partially or completely obstruction of the lumen (Fig. 3). The homogenous or finely granular luminal material sometimes could be mixed up with erythrocytes and a few leucocytes. More detailed histochemical and electron microscopic studies of the composition of this material will be described in a separate paper. In capillaries and arterioles without deposition of PAS-positive material swelling of the endothelial cells was a common finding.

PAS-positive material also appeared in small arteries, where it was located on the luminal side of the endothelium and under the endothelial lining. In affected arteries the internal elastic lamina appeared fragmented, and media displayed degenerative changes (Fig. 4).

Hepatosis diaetetica. Both gross and microscopic findings were considered when the diagnosis was decided. The gross changes included a pale-looking cadaver, usually with prominent subcutaneous, intermuscular and pulmonary oedema. Transudations into serous cavities were not so extensive as in pigs with typical MH, the protein content of the transudates was of the same amount.

The morphologic alterations in the liver have been documented by others and will not be repeated here. The changes conformed well with previous descriptions (Fig. 5). The livers of pigs with MH as a dominant pathological finding showed no other changes than congestion and centrolobular necrosis. Two pigs, however, (see Table 3) displayed the typical pattern of HD together with gross and microscopic myocardial changes corresponding to MH. One pig in the selenium supplemented group and one in the amino acid supplemented group developed HD. As will be seen from

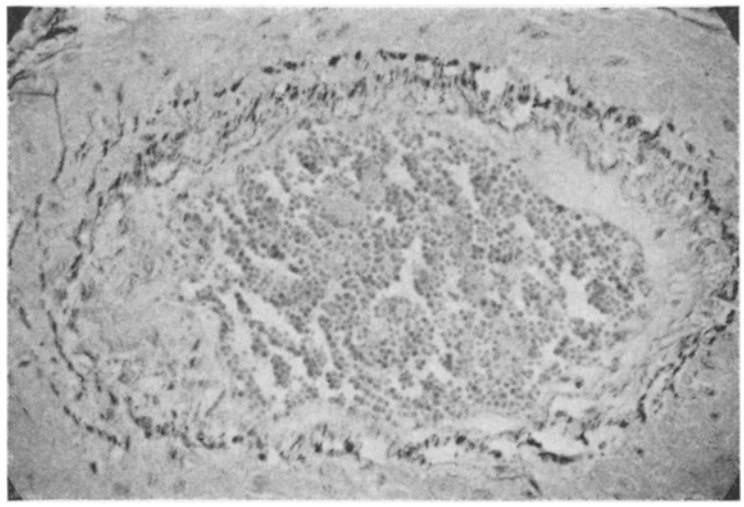

Figure 4. Fragmentation of the internal elastic lamina and degenerative changes in the media of a myocardial artery. Endothelial deposits of homogenous material. Exp. 1, lot 3, pig no. 26. Elastin-van Gieson, × 300.

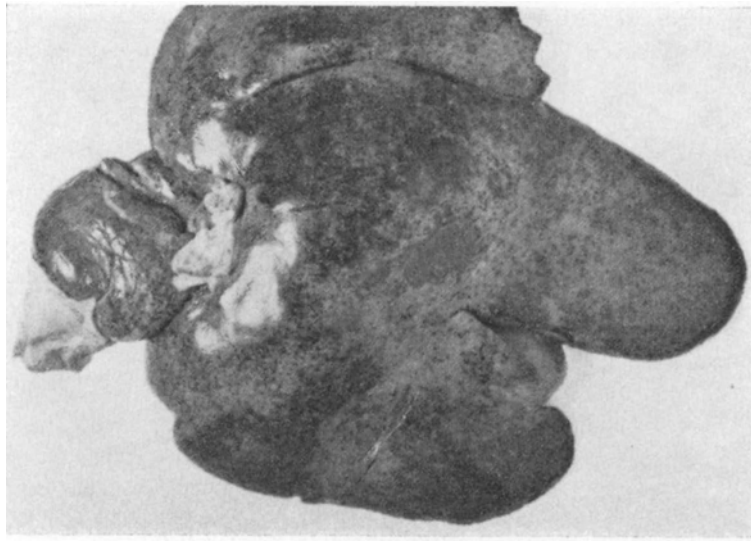

Figure 5. Liver with hepatosis diaetetica of characteristic type. The mosaic-like pattern is typical for the lobular distribution of the necrosis. Exp. 3, lot 7, pig no. 43.

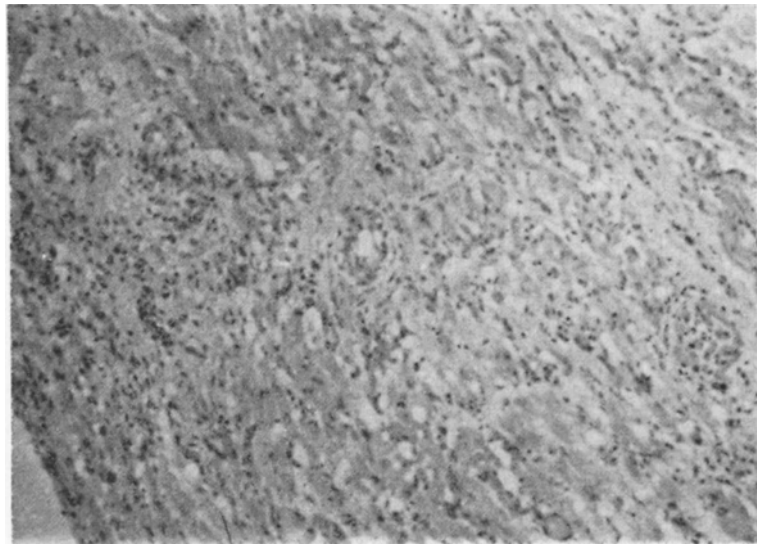

Figure 6. Myocardial tissue largely replaced by scar tissue. Exp. 1, lot 7, pig no. 10. H & E, × 120.

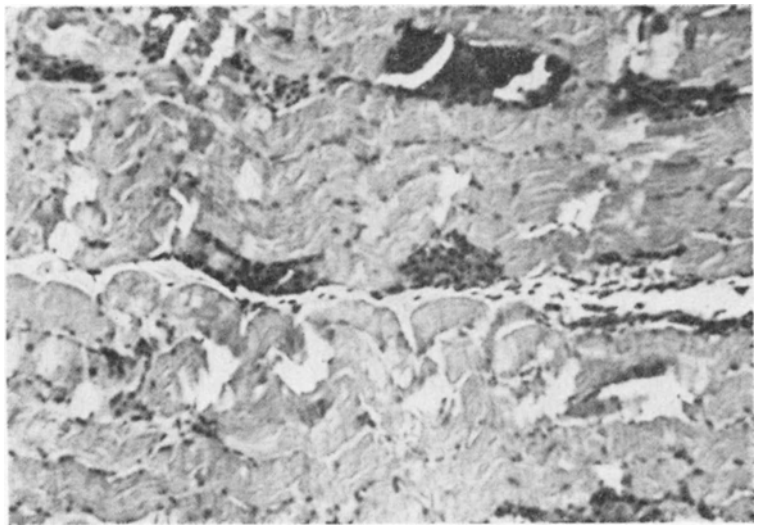

Figure 7. Skeletal muscle degeneration with calcium deposits in necrotic fibres. Exp. 3, lot 1, pig no. 33. H & E, × 120.

Table 3, the pigs with HD invariably had degenerative myocardial lesions. These lesions differed from the typical MH in that the myocardial injuries were less severe. Right-side dilatation was common, as were subendocardial haemorrhages, which in some cases were extensive over the left ventricle. Microscopic examination revealed degenerative changes of the same type as seen in MH-pigs, including vessel injury of the microangiopathic pattern. The atrial walls and the wall of the right ventricle commonly revealed areas of young collagenous tissue or extensive fibrous scarring (Fig. 6).

Muscular dystrophy. Degenerative muscular lesions developed in all the non-tocopherol groups. The gross appearance of altered muscles included a greyish colour, sometimes interspersed with whitish streaks which corresponded to calcified foci when examined microscopically. Intermuscular and interstitial oedema was common, and the oedema fluid could be blood-tinged. Microscopic changes consisted of hyaline degeneration and necrotic fibres accompanied by inflammatory cellular infiltration and calcification of necrotic areas (Fig. 7). Selenite supplementation had no effect on the development of MD in these experiments.

"Yellow fat" was present in all non-tocopherol pigs except two, which died with the typical appearance af MH after 36 and 37 days in experiment. The morphologic changes corresponded to earlier descriptions. No preventing effect of selenium supplementation on this disease was observed.

Experiment 2

A summary of the results is presented in Table 4. Three non-tocopherol pigs died during the experiment. No clinical symptoms were observed, except for refusal of food the last day before death of pig no. 5. Pig no. 3 escaped from his pen and came to a fight with another pig the night before death. Transudations into serous cavities were obvious in the three pigs that died spontaneously and most prominent in pig no. 3, which showed the pathologic pattern of MH with no liver changes except congestion. The amounts of transudative fluid in this pig was 600 ml in the abdominal and 200 ml in the thoracic cavity, while in pigs no. 11 and 5 showing typical HD, the amounts of transudates in the different cavities varied from 5 to 25 ml. The total protein content of the transudates varied from 2.5 to 6.85 %, with an albumin/

Table 4. Major necropsy findings of experiment 2.

Lot	Pig	Survival, days	Av. daily gain, kg	Dominant pathol. findings	Myocardial changes				MD	YF
					degen.	haemorrhages	vascular injury	scar		
	11	56		HD	+	+	+	—	+	+
	5	58	0.253	HD	++	+	+	+	++	+
1	3	91		MH	++	++	+	+	++	—
(basal +	2	109 (killed)		HD	++	—	+	—	++	+
amino acids)	8*	106 „			+	—	+	—	++	+
	10*	106 „			+	—	+	+	++	+
	1	109 (killed)			—	—	—	—	—	—
2	7	109 „			—	—	—	—	—	—
(basal +	12	109 „	0.240		—	—	—	—	—	—
vit. E +	4	109 „			—	—	—	—	—	—
amino acids)	6*	106 „			—	—	—	—	—	—
	9*	106 „			—	—	—	—	—	—

* Fed a commercial swine food until day 40 of experiment.

globulin ratio from 0.73 to 1.27. As will be seen in Table 4, three of the non-tocopherol pigs kept on the vitamin E-deficient diet from the start of the experiment developed HD and myocardial lesions similar to those of MH. Pigs nos. 8 and 10 which got a commercial swine food for the first 40 days in experiment did not develop HD in the course of the remaining two months. Degenerative myocardial changes were present also in these two pigs, and MD appeared in all the non-tocopherol pigs. Serum protein analysis of blood samples drawn at the end of experiment showed no significant differences between groups. The mean total serum protein content was 6.9 g% and 6.6 g% for controls and non-tocopherol pigs, respectively. The albumin/globulin ratio was about 0.90 for all pigs.

Experiment 3

The results of gross and microscopic examinations are given in Table 5. No difference in clinical symptoms or pathological findings were observed between groups fed the diets with or without pyridoxin included in the vitamin supplementation. Two tocopherol-supplemented pigs, in lots 4 and 8 respectively, died from gastric ulcer haemorrhages during the experiment, and one pig in lot 2 died in connection with blood sampling. The necropsy

Table 5. Major necropsy findings of experiment 3.

Lot	Pig	Survival, days	Av. daily gain, kg	Dominant pathol. findings	Myocardial changes					
					degen.	haemor-rhages	vascular injury	scar	MD	YF
	40	75		HD	+	—	—	—	+	+
1	14	77	0.302	HD	++	—	+	+	++	+
(basal)	2	114 (killed)			++	—	—	—	+	+
	33	114 „		HD	++	—	+	+	++	+
	36	49		**	—	—	—	—	—	—
2	8	114 (killed)			—	—	—	—	—	—
(basal	9	115 „	0.419		—	—	—	—	—	—
+ vit. E)	39	115 „			—	—	—	—	—	—
	3	22		HD	+	+	+	—	+	—
3	11	45	0.289	HD	+	+	—	—	++	+
(basal)*	41	62		***	++	—	—	+	++	+
	37	81		HD	++	+	+	—	++	+
	17	57		***	—	—	—	—	—	—
4	6	115 (killed)	0.352		—	—	—	—	—	—
(basal* +	34	116 „			—	—	—	—	—	—
vit. E)	42	120 „			—	—	—	—	—	—
	26	84		MH	++	++	++	+	++	+
5	16	86		MH	++	++	++	—	++	+
(basal	32	120 (killed)	0.351	HD	+	—	—	—	++	+
+ Se)	12	120 „		HD	+	+	—	—	+	+
	1	112 (killed)			—	—	—	—	—	—
6	5	112 „			—	—	—	—	—	—
(basal + Se	13	112 „	0.396		—	—	—	—	—	—
+ vit. E)	38	112 „			—	—	—	—	—	—
	29	86		MH+HD	++	++	++	—	++	+
	43	104		MH+HD	++	+	++	—	++	+
7	22	121 (killed)		HD	+	—	—	+	+	—
(basal +	7	121 „	0.522	HD	+	—	—	—	++	+
amino acids)	44	121 „		HD	++	+	+	+	++	+
	24	121 „		HD	++	+	—	+	++	+
	31	85		***	—	—	—	—	—	—
	18	136 (killed)			—	—	—	—	—	—
8	25	136 „			—	—	—	—	—	—
(basal +	27	136 „	0.444		—	—	—	—	—	—
amino acids	28	136 „			—	—	—	—	—	—
+ vit. E)	30	136 „			—	—	—	—	—	—

* Pyridoxin was omitted from the vitamin mixture.
** Died after blood sampling.
*** Gastric ulcer.

showed haemorrhagic infiltration and haematomas along the jugular vein, arteria carotis and the vagus nerve. Ten of the non-tocopherol pigs died between day 22 and day 104 of experiment, one because of a perforation of this gastric ulcer (lot 3) and the others with HD or MH, or a combination of these lesions as dominant pathological findings.

Clinical symptoms were lacking, or, when present, included refusal for the last feed, poor appetite for a couple of days and some dullness and reluctance to moving. Transudations into serous cavities occurred only in pigs which died spontaneously. The amounts of fluid varied from 50 to 500 ml. The vitamin E-deficient pigs which survived until the experiment was completed gained weight poorly and were in a bad health condition.

The gross and histological changes were of the same nature as described for Experiment 1. In pigs showing HD, both acute and chronic lesions were commonly found in the same livers. Degenerative myocardial lesions, as revealed by microscopic examination, developed invariably in HD pigs. Of the four pigs showing the gross and microscopic pattern of MH, two had developed HD simultaneously. MD and YF were obvious in nearly all of the non-tocopherol pigs.

Experiment 4

In this experiment, which was performed mainly to compare the effect of different protein sources on the development of gastric ulcers (*Nafstad et al.* 1967), the most prominent degenerative myocardial and hepatic lesions developed in the non-tocopherol casein groups. Seven pigs (from lots 1, 2, 3 and 5) died during experiment, the one in lot 2 as a result of gastric ulcer haemorrhages. The others showed the usual pattern of HD and MH lesions, with prominent transudations. The results of gross and microscopic examinations are given in Table 6. The pigs in the 36 % soya groups survived until the experiment was completed after 15 weeks. Histologically, the right atrial and ventricular walls of the non-tocopherol soya pigs were scarred, but with only small acute degenerative changes. The livers were normal or slightly scarred, and MD occurred in only one of the 36 % soya pigs (lot 7). Yellow fat, however, occurred in all non-tocopherol pigs. A few foci of degenerated muscle fibres were also found in pig 459 of lot 8. Average total serum proteins at the end

Table 6. Major necropsy findings of experiment 4.

Lot	Pig	Survival, days	Av. daily gain, kg	Dominant pathol. findings	Myocardial changes				MD	YF
					degen.	haemor-rhages	vascular injury	scar		
	437	44		MH+HD	++	++	++	+	++	+
1	440	50	0.292	MH	++	++	++	+	++	+
(basal)	480	51		HD	++	+	+	+	+	+
	516	81		HD	+	—	—	++	++	+
	468	57		*	—	—	—	—	—	—
2	463	101 (killed)	0.425		—	—	—	—	—	—
(basal +	491	101 „			—	—	—	—	—	—
vit. E)	475	102 „			—	—	—	—	—	—
	478	92		**	++	+	+	++	+	+
3	452	101 (killed)	0.441		—	—	—	—	+	+
(casein	370	101 „			—	—	—	—	—	+
and soya)	472	102 „			++	+	—	—	+	+
4	395	101 (killed)			—	—	—	—	—	—
(casein,	450	101 „	0.530		—	—	—	—	—	—
soya +	453	102 „			—	—	—	—	—	—
vit. E)	496	102 „			—	—	—	—	—	—
	474	68		HD	++	+	++	+	+	+
5	477	108 (killed)	0.349	HD	++	—	+	+	+	+
(22 %	489	108 „		HD	++	—	—	+	++	+
casein)	424	108 „			+	—	—	—	++	+
6	457	107 (killed)			—	—	—	—	—	—
(22 %	443	107 „	0.471		—	—	—	—	—	—
casein	473	107 „			—	—	—	—	—	—
+ vit. E)	495	108 „			—	—	—	—	—	—
	390	105 (killed)			—	—	—	+	—	+
7	458	105 „	0.513	***	—	—	—	+	—	+
(36 %	460	106 „			—	—	—	+	—	+
soya)	470	106 „			+	—	+	+	+	+
	476	106 (killed)			—	—	—	—	—	—
8	459	106 „	0.547		—	—	—	—	+/—	—
(36 % soya	469	106 „			—	—	—	—	—	—
+ vit. E)	479	107 „			—	—	—	—	—	—

* Gastric ulcer.
** Myocardial fibrosis.
*** Postnecrotic scarring in the liver.

of experiment was 7.7 g% and 7.3 g% for non-tocopherol and control pigs, respectively. The albumin/globulin ratio was about 0.7 for all pigs.

Other changes. Experiments 1, 2, 3 and 4

Anaemia. The non-tocopherol pigs in all experiments developed a moderate to severe anaemia with morphologic changes in blood and bone marrow cells (*Nafstad* 1965, *Nafstad & Nafstad* 1968).

Kidneys. The kidney of non-tocopherol pigs, especially those which died spontaneously, were enlarged and soft in consistency. Histologically, there were degenerative changes in the tubular epithelium with proteinaceous casts in the lumen, accompanied by some swelling and proliferation of glomerular epithelium.

Intestine. No significant lesions except from congestion were observed. A mild catarrhal enteritis sometimes occurred. Intestinal contents from several pigs were sent for bacteriological examination, no considerable isolations of haemolytic E. coli were reported.

Brain. Vascular congestion and perivascular oedema of cerebrum and cerebellum, accompanied by swelling of small arterial walls and luminal deposition of some PAS-positive material appeared in most of the non-tocopherol pigs which died spontaneously, and in some of the others. Degenerated neurons and minute foci of softening were found occasionally without any apparent specific site of location. Small foci of haemorrhages and degenerative lesions of the Purkinje cells appeared in the most severely all-over affected vitamin E-deficient pigs.

Adrenals. In the non-tocopherol pigs congestion and microscopic foci of haemorrhages were commonly observed in the adrenal cortex. The average weight of the adrenals calculated in per cent of the whole body weight was 0.018 in the vitamin E-deficient pigs in Experiment 1 as compared with 0.006 in control pigs of the same experiment. In S III-stained frozen sections, sudanophilic material was very sparsely in the cortex of the non-tocopherol pigs, contrary to the presence of abundant globular sudanophilic particles in the adrenal cortex of control pigs.

Thyroid glands. No abnormalities, other than congestion, and sometimes interstitial haemorrhages were found.

The *spleen* might be markedly congested, the *lymph nodes* were commonly enlarged and oedematous, and the *thymus* might be scattered with multiple petecchiae.

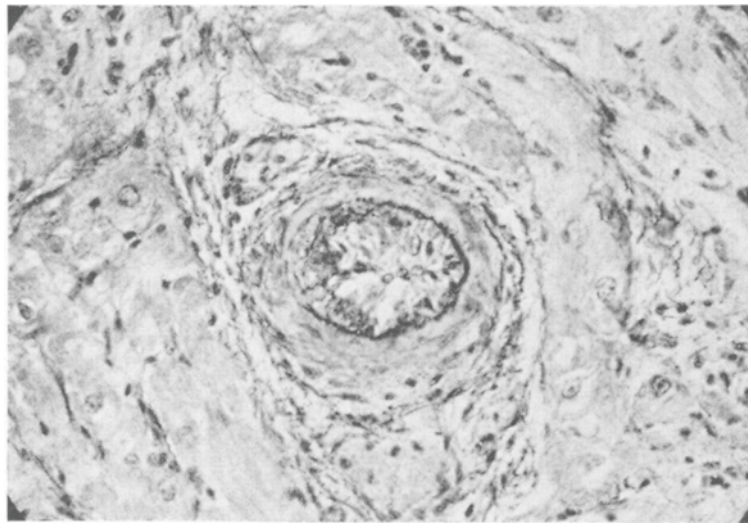

Figure 8. Fibromuscular intimal thickening of myocardial artery. The vascular lumen is obliterated predominantly by vacuolated smooth muscle cells. Note fragmentation of the internal elastic lamina and adventitial fibrosis. Exp. 5, lot 7, pig no. 24. Elastin-van Gieson, × 300.

Two types of pathological changes were frequently found in both non-tocopherol pigs and controls. Among these, the distribution of gastric ulcers has been described earlier (*Nafstad & Tollersrud* 1967, *Nafstad et al.*). The other, the fibromuscular thickening of intima in small and middle-sized intramyocardial arteries are to be mentioned briefly. Morphologically, the deposited material consists of collagen, elastic tissue and smooth muscle cells situated on the luminal side of a fragmented and commonly duplicated internal elastic lamina. The smooth muscle cells, probably migrated from the media, are frequently arranged at right angles to the smooth muscle cells in media (Fig. 8). The result of the intimal thickenings is reduced or completely obliterated vascular lumen. Occasionally, a rim of homogenous or finely granular material limits the obliterating material towards the lumen. This type of vascular changes has been described to occur in normal pigs (*French et al.* 1963, *Luginbühl & Jones* 1965), predominantly in elderly ones. In the present material, however, they appeared to be evidently aggravated in pigs with myocardial injuries, especially associated with fibrous scarring. As to atherosclerotic changes in the aorta, these were occasionally

found as fatty streaks and fibrous plaques with some lipid deposition in the arcus aortae and the lower part of the abdominal aorta in pigs of all groups regardless of dietary manipulations and corresponded to the usual picture of atherosclerosis in pigs as described by *Skold et al.* (1966).

DISCUSSION

Vitamin E-deficiency in pigs is manifested by a wide variety of organ changes.

In the view of earlier investigations and the present experimental findings it seems reasonable to regard the different lesions described as related disorders forming different parts of a syndrome. From the present data, the conspicuous common denominator was vitamin E-deficiency. However, it must be emphasized that to regard the presence of these related disorders as pathognomonic of uncomplicated vitamin E-deficiency is not justified. Improper balance of the ration in respect of different nutritional factors and excess of other ingredients, especially unsaturated fatty acids, because of their antagonistic effects upon the tocopherols, must be considered. It may be useful to compare the vitamin E-deficiency syndrome of pig with that of chicken as described in a recent study of *Cheville* (1966). In an experimental investigation upon vitamin E-deficiency in chicken he demonstrated that the tendency of encephalomalacia, exudative diathesis and muscular dystrophy to occur simultaneously or separate depended to a great extent on the amounts of dietary lipids. Exudative diathesis was the dominant lesion when the diet contained 3 % stripped corn oil, as encephalomalacia dominated in chicks on 9 % dietary lipid. Muscular lesions accompanied both encephalomalacia and exudative diathesis. Moreover, exudative vascular lesions were common even if encephalomalacia or muscular dystrophy was the major lesion. Anaemia resulting from bone marrow depression was an accompanying disorder. 100 mg alphatocopherol per bird per week or a combined tocopherol and selenium treatment effectively prevented the lesions. Animals treated with selenium alone lost weight from the fourth week in experiment and developed muscular dystrophy and, in one animal, encephalomalacia.

No similar systematic study with graded levels of unsaturated fatty acids included into otherwise identical diets has been con-

ducted with pigs. When comparing different experimental studies, it appears that HD is most easily produced on the highest dietary lipid levels when there is a simultaneous lack of tocopherol and, possibly, a faulty protein balance. In the experiments of *Obel* (1953) HD readily developed on the 6 % cod liver oil level when brewer's yeast was used as a source of protein, but less frequent when lard was substituted for cod liver oil, and not at all when casein was used as a source of protein. *Grant* (1961) in his experiments produced HD on diets containing 5 % cod liver oil and 5 % lard or 250 ml heated maize oil per day, but only in one of 20 pigs which were fed grain diets that were otherwise effective in producing other vitamin E-deficiency lesions. *Tanhuanpää* (1965) observed HD in one group which was fed heated barley and oats in equal parts, with 1.5 % of methylated linoleic acid added. As to factors effective in preventing HD, in the experiments performed by *Obel*, the lesions were prevented with methionine or cystine in two groups, with two pigs in each. 150 mg alphatocopherol per pig per week could not protect against the lesions. In view of our present knowledge, one may suppose that this dosage of tocopherol may be considered too low to counteract the effect of the high fatty acid content. *Grant* obtained good protective effect with supplementation of 0.2 mg sodium selenite per kg diet.

The results of the present study differ from earlier studies in that HD occurred almost invariably on casein diets if tocopherol was not supplemented. Addition of sulphur-containing amino acids or 0.25 p.p.m. of sodium selenite under the conditions of these experiments did not protect against HD. The amount of dietary casein did not seem to be of importance for the precipitation of HD within the syndrome. Of 36 non-tocopherol pigs fed the 10 % casein diet in Experiments 1, 2 and 3, 22 pigs (61 %) developed HD, while of 12 non-tocopherol pigs fed diets containing 16 % and 22 % casein in Experiments 1 and 4, 10 pigs (83 %) developed the lesions. The occurrence of HD in pigs fed soya diets was infrequent, in fact, only postnecrotic scarring was observed in the group fed 36 % soybean meal. This difference could not be due to difference in selenium content of the diets because casein contained more selenium than did the soybean meal. Nor is there any evidence for an effect of sulphur-containing amino acids, as casein contains more methionine and cystine than does soybean meal.

An interesting investigation was made by *Todd & Krook* (1966) by reproducing the condition called "sawdust liver" in cattle, which is probably a parallel to the vitamin E-selenium deficiency syndrome in pigs, on a diet rich in polyunsaturated fatty acid and poor in vitamin E and selenium. The hepatic necroses were accompanied by lesions in heart, skeletal muscles and kidneys.

Muscle degeneration

Conflicting results have been reported concerning etiological and preventive factors in MD. Most investigators agree that the experimental myopathies in any animal species induced by feeding diets high in unsaturated fats are prevented by vitamin E administration but are relatively unresponsive to selenium. *Ewen & Jenkins* (1967) demonstrated that the effect of selenium in preventing MD in chicks, induced by a "fat-free", vitamin E-deficient diet, was markedly inhibited when 4 % lard was included in the diet. It is well known that enrichment of unsaturated fats will reduce the protective effect of vitamin E as well. There is, however, one important difference between the two substances concerning their action versus dietary fats, because elevated dietary levels of tocopherol will prevent the disorder, while increased doses of selenium are ineffective (*Jenkins et al.* 1962 a, b). These findings were established in experiments with chicks, but may well be valid for non-avian species too. Previous reports that MD induced by unsaturated fats in lambs (*Welch et al.* 1960), calves (*Maplesden & Loosli* 1960), rabbits (*Draper* 1957) and pigs (*Grant*) cannot be prevented by selenium administration are in good agreement with the findings in the present experiments. In the investigation of *Tanhuanpää* upon the influence of dietary methyl linolate on experimental MD, the correlation between the amounts of dietary tocopherol and dietary unsaturated fats was clearly demonstrated, but a possible effect of selenium in this connection was not studied. However, the author maintained that this rule does not hold true when spontaneous MD is concerned, and that the active factor in field cases is probably selenium deficiency. This point of view was established from the fact that Swedish pigs suffering from MD under field condition have low selenium values in their kidneys (*Lindberg & Tanhuanpää* 1966). Whether this observation justifies the conclusion that selenium deficiency should be the

main factor responsible for outbreaks of spontaneous MD, demands further investigations. In view of the interesting observation reported by *Gardner & Hogue* (1967) that supplementation of selenium to ewes' ration increased the daily yield of tocopherol in milk by one third, one is attempted to ask if the reverse mechanism, an influence of dietary tocopherol content upon the distribution of selenium in tissues, can occur.

In summary, numerous reports concerning different animal species have demonstrated a closely interrelationship between tocopherol and selenium in their protective mechanism against MD. However, unsaturated fatty acids' inhibition of the two nutritients' activity is obviously more pronounced with respect to selenium and is not counteracted by increased levels of the element.

Mulberry heart disease

The proposal of including MH into the vitamin E-deficiency syndrome has already been advanced. In fact, the high frequency of combined MD and myocardial degeneration was reported by German investigators nearly 30 years ago, and *Ludvigsen* (1957) maintained that "fatal syncope" is a severe form of MD. The tendency of MD, HD and myocardial injuries to occur under similar experimental conditions was demonstrated in the studies of *Obel* and *Grant.*

According to the relatively great frequency of mulberry heart disease among the pigs in the present experiments, vitamin E-deficiency must be considered as a predisposing condition for the disease. The tendency to occur together with the diseases generally accepted as tocopherol-selenium sensitive ones, should justify the insert of the disease within this syndrome. However, whether there is need for some particular factor to precipitate the disease in the animals which are "prepared" beforehand by a latent vitamin E-deficiency cannot be answered at present. The possibility exists that different circumstances can influence the precipitation of the disease and that physical stress, which is exemplified by the frequent history of fighting, may be one of them.

Another observation of interest was that most cases of "mulberry heart" occurred in the groups with extra supplementation of amino acids or selenium. A possible explanation for this phenomenon may be the tendency towards a growth-promoting effect of these factors. As will be seen in Tables 3, 4 and 5, the average

daily gain was higher in these groups than in the groups fed the basal diet. This corresponds to field observation that fast-growing pigs are more liable to MD and MH than are more slowly growing litter mates.

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SUMMARY

Four feeding experiments with fat-rich diets were performed to study the gross and histologic pathology of the vitamin E-deficiency syndrome in pigs. The pathologic changes which occurred included skeletal and heart muscle dystrophy with degenerative and microthrombotic vessel injury, anaemia, liver necrosis (hepatosis diaetetica) and yellow discoloration of the fat tissue (yellow-fat disease). In some cases clear-cut pathologic changes corresponding to "mulberry heart disease" or hepatosis diaetetica occurred, but there was an obvious tendency for these pathologic conditions, as well as for muscle dystrophy and yellow fat to occur simultaneously. The changes could be

counteracted with alphatocopherol, while supplement of selenium or sulphur-containing amino acids did not protect against the lesions under the conditions of these experiments. This may be due to the high content of unsaturated fatty acids in the experimental diets. Contradictory to earlier reports, hepatosis diaetetica developed on diets containing casein as a source of protein, and even when extra supplements of sulphur-containing amino acids were given.

In these experiments „mulberry heart disease" was induced experimentally in vitamin E-deficient pigs. The explanation why several of these pigs belonged to groups with extra amino acids or selenium supplement may be a somewhat improved rate of growth in these groups.

SAMMENDRAG

Vitamin E-mangelsyndromet hos gris.

I. Patologiske forandringer.

Det ble utført 4 foringsforsøk for å undersøke de makroskopiske og histologiske forandringer ved vitamin E-mangelsyndromet hos gris. De patologiske forandringer som opptrådte, omfattet skjelett- og hjertemuskel-dystrofi med degenerative og mikro-trombotiske karskader, anemi, levernekroser (hepatosis diaetetica) og gul misfarging av vevsfettet (yellow fat disease). I noen tilfelle opptrådte patologiske forandringer som klart tilsvarte akutt hjertedød eller hepatosis diaetetica, mens det gjennomgående var en tendens til samtidig opptreden av disse sykdomstilstander, så vel som av muskeldystrofi og gult fett. Forandringene kunne forebygges med vitamin E, mens en ikke oppnådde noen beskyttende effekt med tilskudd av selen og svovelholdige aminosyrer under disse forsøksbetingelser. Dette kan muligens tilskrives det høye innhold av poly-umettede fettsyrer i forsøksforet.

I motsetning til tidligere forsøksresultater, fikk en i våre forsøk utviklet hepatosis diaetetica på kasein-holdige dietter og til og med når ekstra tilskudd av svovelholdige aminosyrer ble gitt.

I disse forsøk opptrådte akutt hjertedød hos forsøksgriser på vitamin E-mangel diett. Grunnen til at flere av grisene som døde av akutt hjertedød tilhørte grupper hvor det var gitt tilskudd av selen eller svovelholdige aminosyrer kan kanskje søkes i den noe bedrede tilvekst hos disse gruppene.

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