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EXPERIMENTAL ANAPHYLAXIS IN PIGS

THE INFLUENCE OF THE VITAMIN E CONTENT IN THE FEED

By

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TEIGE, J. jr. and K. NORDSTOGA: *Experimental anaphylaxis in pigs. The influence of the vitamin E content in the feed.* Acta vet. scand. 1977, *18*, 210—220. — An anaphylactic reaction was provoked in pigs by two intravenous injections of chicken egg albumin given approx. 2 weeks apart; some of the animals were in a state of vitamin E deficiency. The second injection of albumin was followed by vomiting, forced respiration, severe cyanosis and distinct edema in the stomach, larynx and gall bladder. These pathomorphological changes are in accordance with those regarded as typical for edema disease. Both the clinical symptoms and the pathomorphological alterations were most evident in animals with vitamin E supplementation, whereas vitamin E deficient animals showed a more moderate response. The anaphylactic reaction was not accompanied by evident changes resembling Mulberry heart disease, either in normal pigs or in vitamin E deficient animals.

edema disease; vitamin E; pig; anaphylactic reaction; Mulberry heart disease.

An anaphylactic reaction, accompanied by lesions similar to those occurring in edema disease has previously been produced in pigs by *Thomlinson* (1963). In some animals hemorrhages and microscopical myocardial lesions corresponding to the alterations observed in Mulberry heart disease (MHD) were also seen. Many years ago, *Thomas* (1952) noted similar changes in the heart muscle of rabbits after intravenous injections of streptococcal filtrates in animals previously sensibilized to the same microbe (Streptococcus, group A). Some of the experimental rabbits also developed the generalized Shwartzman reaction, including bilateral cortical necrosis.

It seems generally accepted that vitamin E deficiency is involved in the etiology of MHD as a predisposing factor (*Grant*

1961, *Nafstad & Tollersrud* 1970), but the exact mechanism eliciting the myocardial lesions remains obscure.

The present investigation was conducted in order to study the possible influence of vitamin E deficiency in experimental anaphylactic reaction. An additional aim was to investigate if myocardial lesions similar to MHD could be provoked by an anaphylactic mechanism.

MATERIALS AND METHODS

Experimental animals

Experiments I and II included 12 and 10 pigs, respectively. The average weight of the pigs in Exp. I was 25 kg when the experimental feeding period started; in Exp. II the corresponding weight was 17 kg. In Exp. I, the pigs were divided into three groups, (groups 1, 2 and 3), each of four pigs. Exp. II consisted of two groups (groups 1 and 2), each of five pigs. The animals in both experiments were given consecutive numbers (Nos. 1—22).

Experimental diets

Exp. I.

Group 1. Casein 20 %, cod liver oil 10 %, sugar 15 %, potato meal 46 %, straw meal (coarsely ground) 5 %, and mineral mixture 4 %*. This feed was supplied with vitamins**.

Group 2. The same feed was used as in group 1. In addition the diet was supplemented with 200 mg α-tocopherol acetate per pig per day.

Group 3. The diet consisted of commercial pig feed***, with a supplement of α-tocopherol.

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* Contents in %: 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, 0.5; potassium iodide, 0.05; zinc oxide, 0.2.

** The following were added, in mg per 100 kg feed: Ascorbic acid, 500; nicotinic acid, 1600; calcium pantothenate, 1100; inositol, 400; choline chloride, 35,000; riboflavin, 200; biotin, 20; folic acid, 70; pyridoxine chloride, 130; thiamine hydrochloride, 200; vitamin B_{12}, 1. 200,000 i.u. vitamin A and 200,000 i.u. vitamin D were also added per 100 kg feed.

*** "Svinefôr III" produced by Møllesentralen, Oslo.

Exp. II.

Group 1. The diet contained the same ingredients as in group 1, Exp. 1, except for the straw meal which was replaced by a corresponding increase in the potato meal (51 %).

Group 2. The pigs were fed a commercial pig feed* with an addition of 100 mg α-tocopherol per pig per day.

The cod liver oil used in both experiments was subjected to a silica earth absorption method in order to reduce the vitamin E content. After this procedure the cod liver oil contained 170 and 45 mg α-tocopherol per g in Exps. I and II, respectively**.

Feeding procedures

All the pigs were handfed twice daily. The cod liver oil was added to the feed at the time of feeding. The experiments were conducted in closed buildings, and the pigs were confined in a pen with arrangements for individual feeding.

Laboratory tests

Blood samples were taken, at two weeks intervals, from the anterior vena cava for the following examinations: hematocrit value, hemoglobin concentration, glutamate-oxalacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK)***.

Experimental schedule

The pigs were sensitized by intravenous injection of crystalline chicken egg albumin**** dissolved in saline. The injections were given 10 and five weeks after the beginning of the feeding period in Exps. I and II, respectively. The doses of albumin used are shown in Table 2. The time between the sensitizing and challenging injections was between 13 and 15 days except for pig

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* "Svinefôr III" produced by Møllesentralen, Oslo.

** Tests performed by Vitaminlaboratoriet, Bergen.

*** The serum enzyme tests were performed at the Research Station, Kjeller. Analytical procedures were according to Sigma Technical Bulletins (STB): for GOT and GPT: STB No. 505, 1964, Sigma Frankel (S-F) units; for LDH: STB No. 500, 1960, Berger-Broida (B-B) units; for CKP: STB No. 520, Sigma (S) units of CPK.

**** Produced by Koch-Light laboratories Ltd., England.

Table 1. Concentrations of serum enzymes and values of hematocrit and hemoglobin measured in each group before the second albumin injection. Group mean ± s.

Exp. No.	Group No.	LDH (B-B units)	GOT (S-F units)	GPT (S-F units)	CPK (S-units)	Hematocrit (%)	Hemoglobin (g/100 ml)
I	1 (cod liver oil)	1534 ± 1678*	83 ± 72.8*	30 ± 13.3	365 ± 1657*	36 ± 2.6	11.6 ± 0.3
	2 (cod liver oil + vit. E)	683 ± 56	52 ± 35.7	21 ± 6.9	75 ± 49	38 ± 0.8	12.3 ± 0.6
	3 (commercial feed)	863 ± 245	29 ± 6.2	28 ± 7.9	17 ± 0	40 ± 0.8	12.8 ± 0.6
II	1 (cod liver oil + vit. E)	6540 ± 1106	147 ± 61.8	78 ± 16.4	—	33 ± 2.4	10.3 ± 0.7
	2 (commercial feed)	1510 ± 290	34 ± 7.9	40 ± 2.9	—	37 ± 1.5	10.9 ± 0.6

* Pig No. 4 had the following values: LDH:4050, GOT:188 and CPK: 1350. The other pigs in the same group had serum enzyme values on the same level as in the remaining two groups of Exp. I.

No. 17, in which it was 11 days. The second injection of albumin was also given intravenously. In Exp. I, one pig in each of the three groups was given an additional injection of 1 g albumin subcutaneously in order to increase the antigenic stimulation. In Exp. II the first injection of albumin took place when the enhancement of the serum enzymes and the drop in the hematological values, indicating a deficiency of vitamin E were observed in group 1 (Table 1). This procedure was not accomplished in Exp. I as only one pig (No. 4) developed a manifest vitamin E deficiency, according to the serum enzyme values, during the feeding period.

The pigs were killed between ¾ and 24 hrs. after the second injection of albumin (Table 2). Necropsies were performed immediately after death except for pig No. 12, where there was a 12 hrs. delay between death and necropsy. Samples from the kidneys, liver, spleen, lungs, heart and skeletal muscles, intestine and stomach were fixed in 10 % formalin and embedded in paraffin. The sections were routinely stained with hematoxylin and eosin (H & E). Lendrums acid picro-Mallory method (*Lendrum et al.* 1962) was used on sections from the myocardium, kidneys, lungs and stomach.

RESULTS

None of the pigs showed any symptoms after the first injection of albumin.

Clinical observations after the second injection

Exp. I

The respiration frequency increased rapidly just after the second injection. In pig No. 2 this symptom was the only one observed. Most of the other animals had transient muscular tremors, they vomited and developed a distinct cyanosis in the abdominal region. This discoloration was most prominent after 20 min. and usually disappeared within ½ hr. A white to reddish foam appeared in the mouths of two of the pigs, and in two other animals soft, red stools were observed.

Exp. II

Group 1. In three pigs (Nos. 14, 15 and 17), the only symptoms observed after the second injection were a moderate cyanosis and a slight increase in the respiration frequency. In the remaining two pigs these symptoms were somewhat more prominent. Vomiting was also seen in the same two animals about 10 min. after the injection.

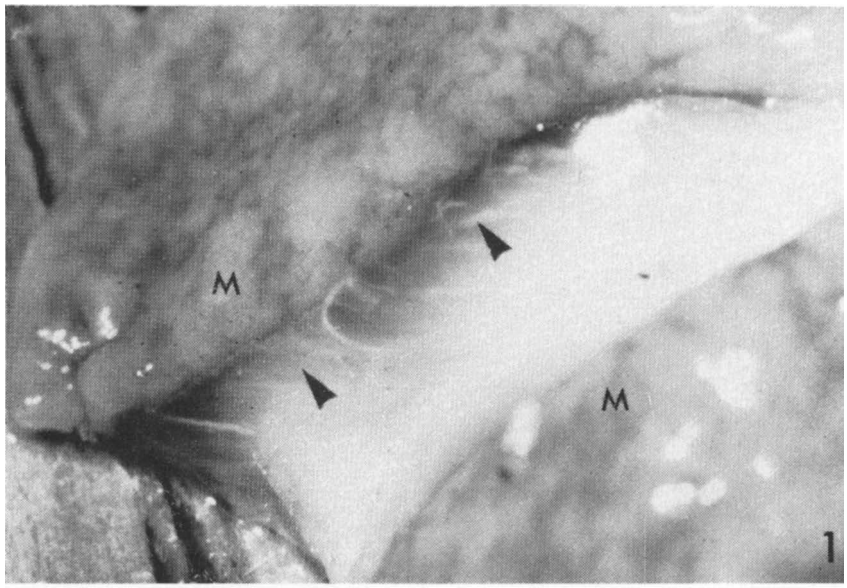
Group 2. In one pig (No. 19), an increase in the respiration frequency and a moderate muscular tremor were the only symptoms observed. In the other pigs the symptoms were prominent, and repeated vomiting was seen from one to 10 min. after the second injection; other symptoms for the same pigs were in accordance with the observations described for the majority of the pigs in Exp. I.

Gross lesions

Edematous changes of the stomach, larynx and gall bladder of the animals of both experiments are recorded in Table 2.

Exp. I

Edema was seen in the submucosa of the stomach and gall bladder (Fig. 1). In some of the pigs these edematous lesions had a maximal thickness of about 1 cm. Edema of the stomach was found in the fundic portion and in the vicinity of pars oesophagea. Hyperemia of the mucosa was observed in the jejunum



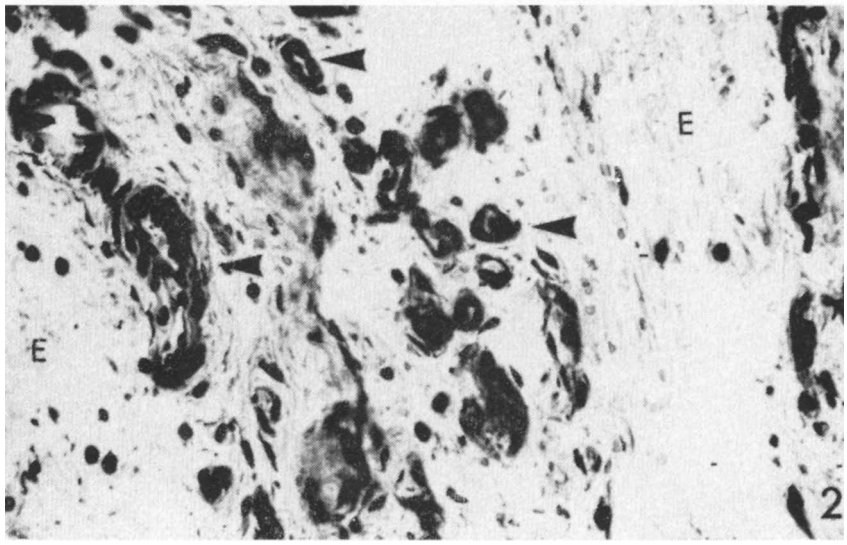


Figure 1. Incision through the stomach wall showing edema in the submucosa (arrows); the mucosal surface is indicated by M. Pig No. 22.

Figure 2. Submucosa of the stomach with edema (E) and vessels with proliferation of endothelial cells and mural thickening (arrows). Pig No. 22. H & E, × 100.

Table 2. Weights of the pigs at necropsy, amounts of albumin administered, time between 2nd injection of albumin and sacrifice and grading of the edematous lesions.

Exp. No.	Group No.	Pig No.	Weight (kg)	Amounts of albumin injected (g) 1st inject.	(g) 2nd inject.	Time between 2nd inj. and sacrifice	Edema** stomach	larynx	gall bladder
I	1 (cod liver oil)	1	76	2	1.5	1 hr.	+++	+	0
		2	74	2	1	3 hrs.	(+)	0	0
		3	74	2	2	3 „	++	+	+++
		4	53	2.5	1.5+1*	23 „	+	+	0
	2 (cod liver oil + vit. E)	5	79	2	1.5	¾ hr.	+++	+	0
		6	87	3	2	3½ hrs.	++	+	+++
		7	81	2	1.5	23 „	+++	0	0
		8	69	2	1.5+1*	23 „	+++	+	+++
	3 (commercial feed)	9	61	2.7	1.5	3 hrs.	+++	+	+++
		10	65	2	—	died***	—	—	—
		11	75	2	2	24 hrs.	+	++	—
		12	77	2	1.5+1*	died***	+	+	—
II	1 (cod liver oil)	13	33	2	1	24 hrs.	—	—	—
		14	32	2	1	24 „	—	—	—
		15	35	2	2	24 „	—	+	—
		16	35	2	2	24 „	—	—	—
		17	38	2	2	24 „	—	—	—
	2 (commercial feed)	18	39	2	2	24 hrs.	+	+	—
		19	38	2	2	22 „	+	(+)	—
		20	37	2	2	22 „	++	+	—
		21	32	2	2	22 „	+++	+	—
		22	32	2	2	20 „	+++	+	(+)

* 1 g of albumin injected subcutaneously.
** Degree of edema (0 to +++).
*** Pigs No. 10 and 12 died of mesenterial volvulus. Pig No. 10 died just before the planned second injection of albumin, and pig No. 12 died 6 hrs. after the second injection.

and caecum, and one pig (No. 9) had a blood stained content in the colon.

Exp. II

Group 1. Edema was not observed except for one pig (No. 15) which had slight edematous lesions in the larynx. The fat tissue

had a yellow discoloration, and the skeletal muscles were pale in all the animals. The myocardium of three pigs (Nos. 13, 14 and 15) had a red, mottled cut surface in the right ventricle. Similar changes, but less pronounced, were observed in the left ventricle. In the liver of one pig (No. 15) a few groups of hemorrhagic lobules were observed.

Group 2. The pigs had similar edematous infiltrations as observed in Exp. I. In addition edema was seen in the subcutaneous and intermuscular tissues of the abdominal wall and in the eyelids (Nos. 21 and 22). The edematous fluid in the stomach wall was, in some parts, mingled with blood. In two pigs (Nos. 21 and 22) hyperemia was found in the mucosa of the caecum, and in one of them (No. 22) hyperemia and erosions were also observed in the fundic portion of the stomach.

Histological lesions

Exp. I

Fatty degeneration and hyperplasia of the reticulo-endothelial system (RES) were observed in the liver. The liver of one animal (No. 4) also contained a few groups of necrotic lobules. Interalveolar septa in the lungs had a moderate infiltration of mononuclear cells. Especially in one pig (No. 6) a fibrinoid material was observed in small pulmonary vessels, including interalveolar capillaries. A similar material could be seen in interstitial capillaries of the kidneys. Edematous infiltrations were observed both in the submucosa and the muscular layer of the stomach, but in the latter location, to a minor degree. In the edema, a moderate infiltration of eosinophilic leukocytes and mononuclear cells was observed. The submucosa of the stomach also contained many small vessels with proliferation of endothelial cells and mural thickening (Fig. 2). In one pig (No. 4) there was incipient proliferation of endothelial cells in some small myocardial arteries.

Exp. II

Group 1. Many small areas with hyaline degenerative lesions were demonstrated in the skeletal muscles. The endothelial cells in small vessels of the myocardium showed proliferative changes. In some parts of the heart muscle the vessels showed widespread congestion and the myocardial fibres exhibited a moderate granular degeneration. In one pig (No. 16), infiltration of eosino-

philic leukocytes and mononuclear cells was found in the submucosa of the stomach. A moderate hyperplasia of the RES in the liver appeared, and in one pig (No. 15) a small area of the liver contained necrotic lobules.

Group 2. Hyperplasia of the RES in the liver and cellular infiltrations of interalveolar septa in the lungs were also observed in these pigs. The changes in the submucosa of the stomach were equivalent to those in Exp. I.

DISCUSSION

The most dominating clinical symptoms in the experimental animals were vomiting, respiratory distress and cyanosis; these signs are in accordance with those observed in other experiments on anaphylaxis in pigs (*Thomlinson & Buxton* 1963). In edema disease, nervous symptoms including ataxia, convulsions and paralysis occur frequently (*Clugston et al.* 1974). Except for slight muscular tremor immediately after the second injection of albumin the pigs showed no nervous symptoms in the present experiment. *Thomlinson & Buxton* were unable to demonstrate nervous symptoms other than muscular tremor in their experiments on anaphylaxis in pigs. In this connection, it seems interesting to note that neurotoxin from certain strains of Escherichia coli (*Schimmelpfenning* 1970) and so-called "edema disease principle" from E. coli. 0 139 (*Clugston et al.*), have produced both clinical and pathological changes typical for edema disease. If one compares these results with the clinical observations in our investigations, it seems likely that also mechanisms other than anaphylaxis are involved in the pathogenesis of edema disease.

In both experiments the anaphylactic reaction was accompanied by edema in the submucosa of the stomach. Edema was also demonstrated in the wall of the gall bladder, larynx, eyelids and in the subcutaneous tissue of the abdominal wall. Histological examination of the stomachs showed, in addition to edema, mural thickening in small vessels, and infiltration of eosinophilic leukocytes and mononuclear cells. These changes are in accordance with previous observations in experimental anaphylactic reactions in pigs (*Thomlinson & Buxton*). Edema of the submucosa in the stomach is also usually accepted as pathognomonic for edema disease (*Emerson* 1967).

In Exp. I the time between the second injection of albumin and sacrifice differed among the pigs; the pathomorphological lesions observed seem not to be influenced by this variation. In both experiments the clinical signs and the edematous lesions were most evident in animals with vitamin E supplementation, whereas vitamin E deficient pigs only showed a slight or moderate response. There was, however, some difference in the response between the two vitamin E deficient groups. This difference may have been influenced by the degree of vitamin E deficiency. According to the serum enzyme value (Table 1) and the pathomorphological lesions, including the necrotic hepatic alterations, the deficiency was distinct in group 1, Exp. II, and more moderate in group 1, Exp. I. These latter observations are perhaps somewhat surprising since the feeding periods for the two groups were 5 and 10 weeks, respectively. However, *Nafstad* (personal communication) has observed that the period of time taken to develop vitamin E deficiency, seems to depend on the weights of the pigs at the start of the feeding period. In this connection it should be noted that the pigs in Exp. I were, on average, 8 kg heavier than those in Exp. II.

Tengerdy et al. (1972, 1973) observed that vitamin E supplementation enhanced the immune response in chickens and mice. *Heinzerling et al.* (1974) demonstrated that vitamin E gave increased protection in experimental E. coli infection in chickens and that the increased length of chick survival was in part due to an immunological response. Supplementation of a combination of vitamin E and selenium, or selenium alone, has been found to enhance the immune response in mice (*Spalholz et al.* 1973). It has also been shown that vitamin E has a similar effect in pigs (*Ellis & Vorhies* 1976). These observations may indicate that the more distinct anaphylactic reaction in pigs with vitamin E supplementation was due to a better immune response. However, in this connection, one must take into account the possibility that the immune response to various antigens may be somewhat different.

Three pigs in group 1, Exp. II, had a red mottled myocardium in the right ventricle; this picture therefore resembled that seen in MHD. The histological examination, however, revealed only hyperemia and no microthrombosis or other changes indicative of MHD. Pathomorphological changes resembling MHD have previously been found in pigs fed an equivalent experimental diet as

that used in group 1, Exp. II (*Grant* 1961, *Nafstad & Tollersrud* 1970). These authors claimed that vitamin E deficiency is an important predisposing factor in MHD. Investigations by *Thomlinson* (1963) indicated that an anaphylactic reaction could precipitate the disease. In our experiments the anaphylactic reaction was, however, not accompanied by obvious morphological lesions resembling MHD, not even in pigs made susceptible to MHD by dietary means. The results seem to indicate that vitamin E deficiency has a retarding effect on the development of anaphylaxis. Furthermore, this observation may indicate that there is no close relationship between anaphylaxis and MHD. This illustrates also that MHD is a multifactorial entity in which many etiological features still are obscure.

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SAMMENDRAG

Eksperimentell anafylaksi hos gris. Betydningen av fôrets vitamin E-innhold.

En anafylaktisk reaksjon ble fremkalt hos gris ved hjelp av to intravenøse injeksjoner av kyllingegg-albumin, gitt med ca. 14 dagers mellomrom. Endel av forsøksdyrene led av vitamin E-mangel. Den andre injeksjonen av albumin ble fulgt av oppkast, respirasjonsbesvær, tydelig cyanose og ødemer i ventrikkel, larynx og galleblære. De patomorfologiske forandringer er i samsvar med det som angis å være typisk for ødemsyke. Både kliniske symptomer og de patomorfologiske forandringer var tydeligst hos de dyrene som fikk vitamin E-tilskudd, mens vitamin E-mangel-dyrene viste en mer moderat reaksjon. Den anafylaktiske reaksjon ble ikke ledsaget av tydelige morfologiske forandringer av den type som sees ved akutt hjertedød, hverken hos normale griser eller hos dyr med vitamin E-mangel.

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