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CONGENITAL ASCORBIC ACID DEFICIENCY IN PIGS

By

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THODE JESEN, P., A. BASSE, D. HALD NIELSEN and H. LARSEN: *Congenital ascorbic acid deficiency in pigs.* Acta vet. scand. 1983, *24*, 392—402. — In a swine production herd, spontaneous scurvy was observed among piglets 2-3 weeks after weaning. All affected pigs had the same boar as both maternal and paternal grandfather. The affected pigs had only traces of ascorbic acid in blood and tissues as compared to litter mates and other normal pigs. The ratio between the total numbers of normal and affected pigs in the 4 litters concerned was in agreement with a 3:1 segregation, which is characteristic of simple autosomal recessive inheritance in matings between non-affected carriers.

Two affected pigs were restored to normal when given ascorbic acid in the diet. Without vitamin C supplement affected pigs died or had to be euthanized.

Liver microsomes from an affected pig were unable to synthesize ascorbic acid in vitro with l-gulonolactone as a substrate, unlike microsomes from normal control pigs.

scurvey; vitamin C; congenital; inbreeding; pigs.

Most animal species, excepting man, other primates, and guinea pigs, are presumed to be able to synthesize vitamin C with l-gulonolactone oxidase (E.C. 1.1.3.8) as the critical enzyme in the biogenesis. In pigs as in other mammals this enzyme is localized in the liver microsomes (*Burns et al.* 1956, *Grollman & Lehninger* 1957) and normally pigs will produce sufficient amount of vitamin C to meet their demand.

A feed supplement of vitamin C has at least in one case been reported to prevent navel bleeding in new-born piglets (*Sandholm et al.* 1979) and in another case vitamin C supplement apparently reduced the incidence of leg-weakness in growing pigs (*Nielsen & Vinther* 1982). To the authors' knowledge clinical cases in pigs

of vitamin C deficiency with scurvy have not been described previously. In the following, an outbreak of scurvy among pigs, cauced by a hereditary vitamin C deficiency, will be described.

MATERIALS AND METHODS

In February 1982 three euthanized young pigs were received for post mortem examination at the State Veterinary Serum Laboratory. The pigs originated from a herd raising pigs for slaughter (Landrace-Yorkshire crossbreed). While the mother sows were raised within the herd, the breeding boars were usually purchased from special breeding herds. The clinical symptoms were described as unthriftiness, unwillingness to move, swelling around joints, and signs of pain on touching.

During the following weeks a further 4 affected pigs were observed in the herd and submitted to the laboratory for examination. One of the pigs was dead on arrival and one, with severe symptoms, was euthanized after blood sampling. After blood sampling and radiological examination the other 2 affected pigs, a barrow and a gilt, were used in a reconstitution experiment and fed 0.5-1 g vitamin C per day each.

For comparison, blood and tissue samples from pigs of the same age, including an unthrifty pig with chronic diarrhoea, were examined for vitamin C content. All blood samples were drawn just before feeding.

During the treatment experiment the condition of the pigs was followed by clinical examinations and by blood vitamin C determinations. Also the influence of a short period without vitamin C was investigated. At 7 months of age the reconstituted barrow was fed without vitamin C supplement for 2 weeks and then killed for biochemical examination. The gilt continued on vitamin C and was kept for breeding experiments.

Both in the herd of origin and during the treatment experiment the pigs were fed a standard barley-soybean based diet supplied with macro- and microminerals and vitamins A, D and E.

When the barrow was killed the capability of its liver microsomes to synthesize vitamin C in vitro was investigated, as was the capability of liver microsomes from 2 normal slaughter pigs, 2 rabbits, and 2 guinea pigs.

Duplicate determinations of vitamin C in blood plasma and in tissues were made by the 2,6-dichlorophenol-indophenol tech-

nique (*Bessey* 1938) modified by *Lund* (1980). Blood samples were stabilised by K_2EDTA.

In vitro vitamin C synthesis by liver microsomes was tested as described by *Chatterjee et al.* (1958) with l-gulonolactone as substrate. Immediately after slaughter, liver homogenate was prepared in ice-cold 0.01 mol/l phosphate buffer, 1.15 % KCl, pH 7.2 by means of a Potter Elvehjem homogenizer. The homogenate was spun at 10,000 × g for 20 min. After recentrifugation the post mitochondrial supernatant was spun at 87,000 × g for 90 min to sediment the microsomes. The microsome preparation was washed once and then dispersed in cold buffer to a concentration equivalent to 1 g of wet tissue per ml for the immediately following enzyme assay. The enzyme test system contained: 20 mmol/l sodium phosphate buffer, pH 7.2, 5 mmol/l l-gulonolactone, and 0.75 ml microsome dispersion. Total test volume was 3.75 ml; the mixture was incubated in air for 90 min at 37°C. Since the 2,6-dichlorophenol-indophenol technique, measuring only the reduced ascorbic acid, is not suitable for measuring ascorbic acid in this mixture, "total" ascorbic acid was estimated by the method of *Roe & Kuether* (1943) which includes oxidation. The test was made in triplicate with and without substrate.

RESULTS

In all affected pigs the symptoms started at 9-10 weeks of age, i.e., 2-3 weeks after weaning. Before the start of the present study, one pig from each of 2 litters had shown symptoms as described above. Those 2 pigs had been killed without being examined post mortem.

The principal radiological and pathoanatomical findings in the dead or euthanized pigs were in good agreement.

Radiologically, the most pronounced changes were the presence of a transverse, radiolucent band within the relatively radiodense metaphyses of the long bones and the ribs, a widening of the metaphyses and a "lipping" over the epiphyseal plates. Furthermore, the compact substance of the diaphyses seemed rather thin (Fig. 1).

The pathoanatomical changes were mainly in the skeletal system: subperiosteal haemorrhages, lesions of the metaphyses, reduction of the compacta, high fragility of the bone tissue and fractures. The subperiosteal haemorrhages were most pronounced

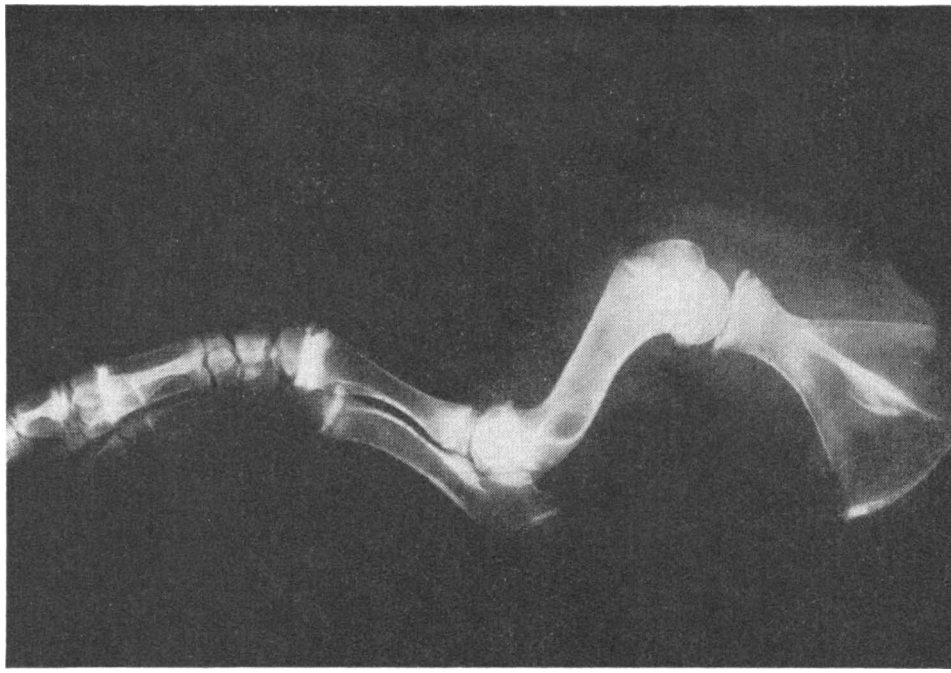

Figure 1. Forelimb from a 3-month-old scurvy pig. Note the transverse radiolucent bands within the radiodense metaphysis of the long bones and the widening of the metaphysis with a "lipping" over the epiphyseal plates.

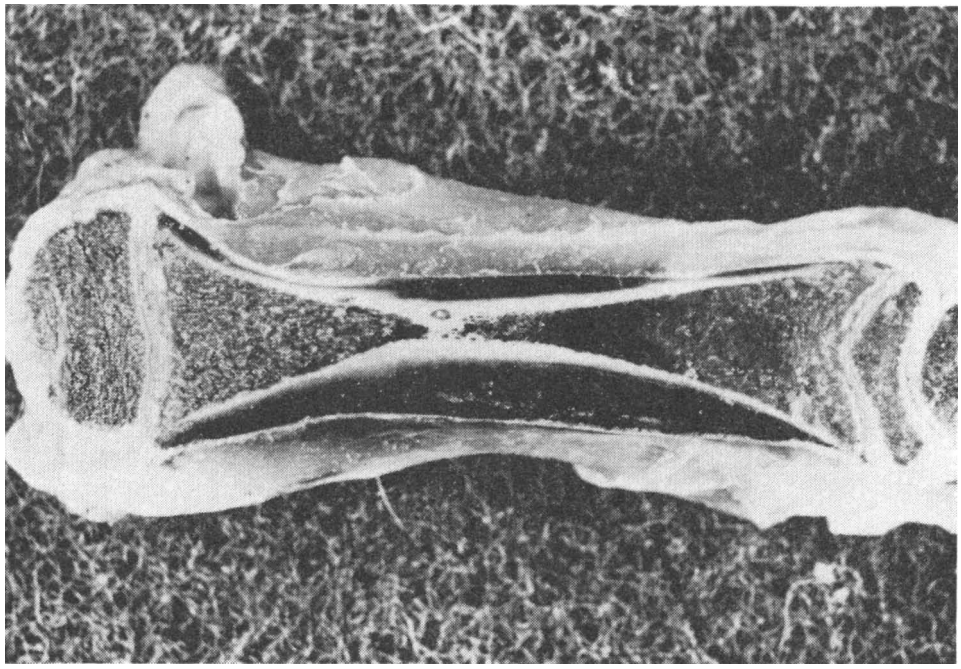

Figure 2. Longitudinal section of the tibia. Subperiostal haemorrhage with dislocated periosteum. "Scorbutic lattice" in the metaphysis.

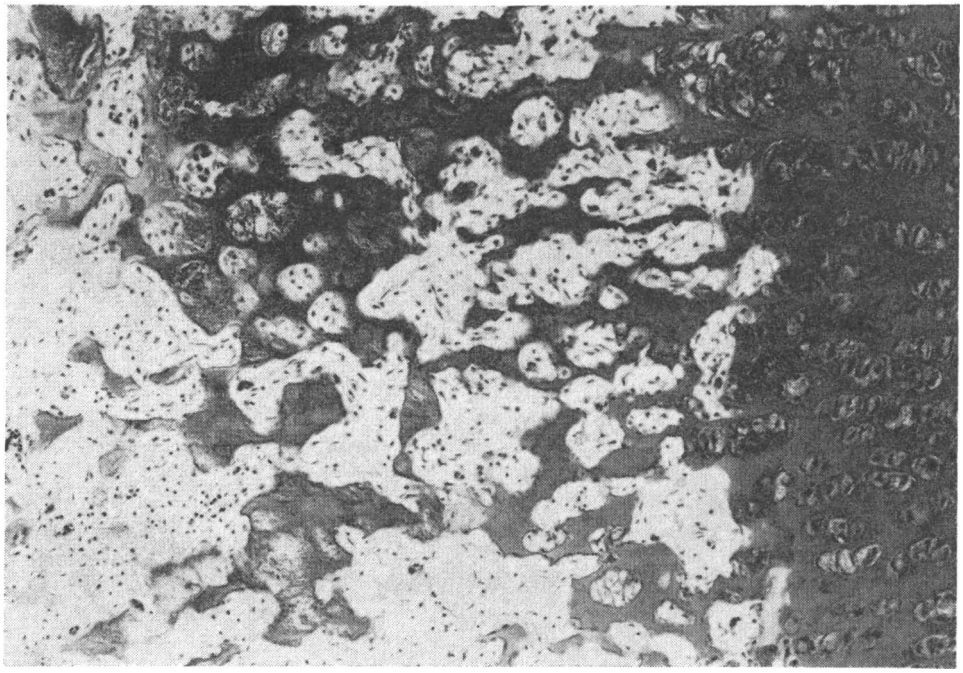

Figure 3. Costa from a scorbutic pig. Growth plate. Reduced amount of osteoid and interruption of the orderly palisade structure of the cartilage. HE, × 100.

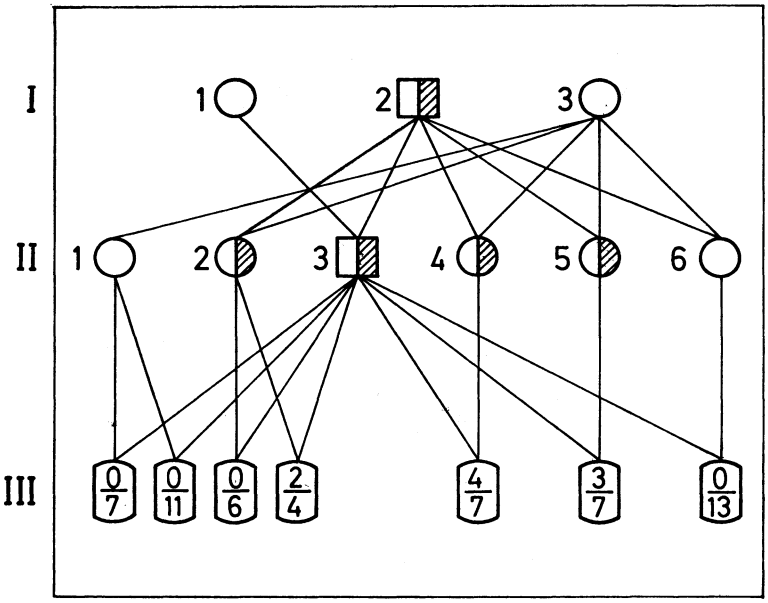

Figure 4. Pedigree for pigs with inborn ascorbic acid deficiency. The symbols mean: square: a male, circle: a female, shaded symbol: a presumed carrier. In the third generation (III) the upper figures indicate the number of affected pigs in the litter and the lower figures indicate the number of unaffected pigs.

around the shafts of the long bones, where the periosteum was often separated from the compacta by an up to 1½ cm thick blood coagulum (Fig. 2). Similar lesions, though less pronounced, were found at the costae, the mandible, the maxilla, and the bones of the skull.

Histologically (Fig. 3), the lesions were characterized by a reduced amount of osteoid in the growth plates, together with haemorrhages and patches of necrosis in the metaphyses.

Fig. 4 gives the genealogical diagram for the affected pigs and the pigs related to them. The syndrome has only been seen in litters with boar No. II-3 as father and boar No. I-2 as both maternal and paternal grandfather. Thus, the parents to affected pigs were half-sibs. The 3 sows producing affected offspring (II-2, II-4, II-5) have previously or later got normal litters sired by boars unrelated to them. The diagram is consistent with a hypothesis of simple recessive inheritance. Both dominant and sexlinked inheritance can be excluded, since the parents of affected animals were all normal, and since both males and females were affected. The observed ratio of 24 normal to 9 affected pigs among offspring of boar No. II-3 by the 3 sows concerned is consistent with a hypothesis of autosomal recessive inheritance with an expected 3 to 1 segretation in matings between non affected carriers.

Tables 1 and 2 give the vitamin C content in blood and tissue from affected pigs as well as from unaffected pigs of the same age. As seen from the tables, only affected pigs had blood ascorbic acid levels below 1 mg per 1 plasma. Also in tissues the affected pigs had definible the lowest levels of ascorbic acid, even compared to an unthrifty pig.

Table 1. Vitamin C levels in blood and tissues from scorbutic pigs (53-4 and 64-5) and from control pigs from other herds.

	Scorbutic pigs		Unthrifty pig	Normal pigs	
	53-4 (3 months)	64-5 (8 months)	(3 months)	(3 months)	(3 months)
Blood (mg/l)	<1.0	<1.0	1.5	2.9	3.6
Liver (mg/g)	<0.01	0.03	0.10	0.27	0.27
Kidney (mg/g)	<0.01	0.01	0.05	0.16	0.21
Adrenals (mg/g)	<0.01	0.60	1.18	1.20	1.66

Table 2. Blood vitamin C levels (mg/l plasma) in normal and scorbutic (*) 9—12-weed-old pigs in the herd concerned. Pigs with same initial number are litter mates.

Pig No.	Vit. C	Pig No.	Vit. C	Pig No.	Vit. C
1-1	3.0	53-4*	<1	64-3	1.9
1-2	4.7	54-1	3.5	64-4*	<1
53-1	5.0	54-2	3.1	64-5*	<1
53-2	4.8	64-1	3.1	98-1	5.0
53-3	4.0	64-2	4.8	98-2	2.1

Within about a week after initiating vitamin C supplementation, the 2 affected pigs were without clinical symptoms and were consuming normal rations. Fig. 5 gives the weekly blood vitamin C levels during the treatment period. Fig. 6 shows the blood vitamin C levels during and after a 6-day-period without vitamin C supplement. After 6 days, weak clinical signs appeared, and the pigs were again given vitamin C. Vitamin C was given only in the morning feed, just after blood drawing. As seen from Fig. 6, the blood samples drawn at the feeding 6 h later and again after another 6 h, i.e. during the digestion period, show a little higher blood vitamin C level than to the sample drawn from the fasting animals in the morning. This difference is assumed to be due to a low but insufficient level of natural vitamin C in the feed. The results of the in vitro vitamin C synthesis test are given in Table 3. As seen from the table, liver microsomes from the

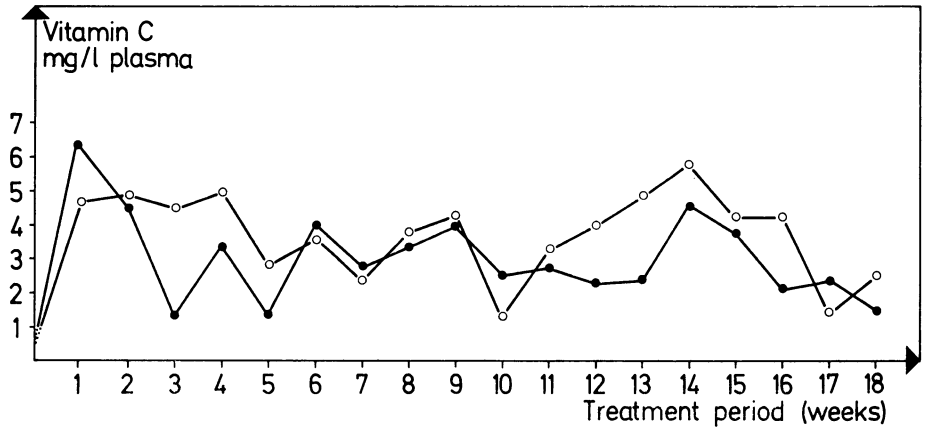

Figure 5. Blood vitamin-C levels in 2 affected pigs supplemented with ascorbic acid in the feed.

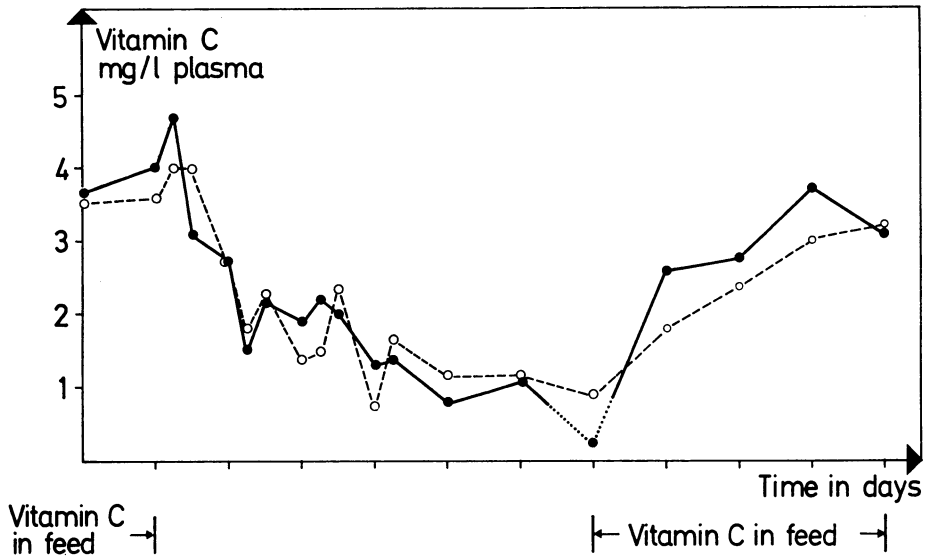

Figure 6. Blood vitamin-C levels in 2 affected pigs during and after a 6-day-period without ascorbic acid supplement to the feed.

Table 3. Ascorbic acid synthesized in test mixture with liver microsomes from different animals (μg per ml; test in triplicate).

Substrate	Affected pig	Control pigs		Rabbits		Guinea pigs	
	64-5	I	II	I	II	I	II
Saline	0.00	0.08	0.00	0.02	0.00	0.00	0.00
„	0.00	0.15	0.05	0.00	0.00	0.02	0.00
„	0.00	0.15	0.00	0.00	0.00	0.00	0.01
l-gulonolactone	0.00	0.74	0.35	1.06	1.87	0.02	0.05
„	0.00	0.61	0.28	0.87	1.61	0.00	0.08
„	0.00	0.45	0.38	0.96	1.30	0.01	0.04

affected pig and from the guinea pigs were quite unable to synthesize vitamin C from l-gulonolactone, whereas microsomes from the unrelated normal pigs and from the rabbits were producing vitamin C to the extent that could be expected.

DISCUSSION

The pathological findings: subpriostal haemorrhages, deficiency of osteoid formation, and haemorrhage and necrosis in the metaphysis, are pathognomonic of scorbutus (*Robbins & Angell* 1971) and the biochemical and radiological findings together with

the positive response to treatment confirm this daignosis in the affected pigs. The disease occurred only in some inbred litters produced by a mistake during a period when only a limited number of breeding boars were available. Genealogical and laboratory findings indicate that the primary cause of the disease is a genetic defect, probably with a simple autosomal recessive inheritance. The inability of liver microsomes from the affected pig to synthesize vitamin C with l-gulonolactone as substrate suggests that, like normally in man and guinea pigs, the enzyme concerned, i.e., l-gulonolactone oxidase, is lacking in the affected pigs. We know that the phylogenetically distant species: guinea pig and man, may live with the defect without problems if they get enough vitamin C in their food. It seems likely that the described vitamin-C-responsive scurvy in pigs has been caused by a similar mutation as must once have occurred in guinea pig and man.

In the present case the genetic change is considered to be a deletion mutation. *Ginter* (1976) has described the opposite phenomenon: guinea pigs being able to synthesize ascorbic acid, and thus presumably having an active l-gulonolactone oxidase enzyme.

The very rapid occurrence of the first weak clinical signs in the vitamin C-deprived affected pigs, together with the fall in the blood vitamin C level, is consistent with the appearance of clinical symptoms about two weeks after weaning, since piglets normally get plenty of vitamin C from the sow's milk (*Lund et al.* 1980). The rapid occurrence of symptoms and decrease in plasma vitamin C levels suggest that vitamin C is metabolized in the same way in pigs as in guinea pigs (*Burns* 1960). Also in guinea pigs obvious signs of vitamin C deficiency will normally appear between the second and third week after they are put on a vitamin C-free diet. In man the situation is very different, and it will normally take months without vitamin C in the food before the first deficiency symptoms appear.

Plasma and tissue samples from litter mates and other normal control pigs (Table 1 and 2) had the vitamin C level as previously found by *Lund* (1980) in young Danish Landrace pigs.

If it is possible successfully to raise a strain of pigs devoid of the gene responsible for synthesizing the l-gulonolactone oxidase, new possibilities will be opened for studying the effect of vitamin C in pigs, which is an animal species often used as a model in experimental medicine.

REFERENCES

Bessey, O. A.: A method for the determination of small quantities of ascorbic acid dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem. 1938, *126*, 771—784.

Burns, J. J.: Ascorbic acid. *In:* Metabolic Pathways Vol. I (ed. D. M. Greenberg) Acad. Press 1960, pp. 341—356.

Burns, J. J., P. Peyser & A. Moltz: Missing step in guinea pigs required for the biosynthesis of L-ascorbic acid. Science 1956, *124*, 1148—1149.

Chatterjee, I. B., J. J. Ghosh, N. C. Ghosh & B. C. Guha: Effect of cyanid on the biosynthesis of ascorbic acid by an enzyme preparation from goat-liver tissue. Biochem. J. 1958, *70*, 509—515.

Ginter, E.: Ascorbic acid synthesis in certain guinea pigs. Internat. J. Vit. Nutr. Res. 1976, *46*, 173—179.

Grollman, A. P. & A. L. Lehninger: Enzymatic synthesis of L-ascorbic acid in different animal species. Arch. Biochem. Biophys. 1957, *69*, 458—467.

Lund, C.: Studier over askorbinsyres betydning for svin. Variationer i askorbinsyrekoncentrationen i plasma og væv. (Studies on the importance of ascorbic acid in pigs). Licentiatafhandling. Royal Vet. and Agric. Univ., Copenhagen 1980.

Lund, C., I. D. Christensen & I. Vegger: Askorbinsyreomsætningen hos svin. Undersøgelser over ascorbinsyreindhold i blod, mælk og væv. (Ascorbic acid metabolism in swine. Studies on ascorbic acid content in blood, milk and tissues). Ann. Rep. Steril. Res. Inst., Royal Vet. and Agric. Univ., Copenhagen 1980, *23*, 48—60.

Nielsen, N. C. & K. Vinther: Influence of dietary vitamin C supplement on leg-weakness in pigs. Proc. Int. Pig. Vet. Soc. Congress, Mexico 1982, 269.

Robbins, S. L. & M. Angell: Basic Pathology, W. B. Saunders Company 1971, pp. 311—312.

Roe, J. H. & C. A. Knether: The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. 1943, *147*, 399—407.

Sandholm, M., T. Honkanen-Buzalski & V. Rasi: Prevention of navel bleeding in piglets by preparturient administration of ascorbic acid. Vet. Rec. 1979, *104*, 337—338.

SAMMENDRAG

Medfødt ascorbinsyremangel hos svin.

I en svineproduktionsbesætning blev der diagnosticeret skørbug hos nogle smågrise 2—3 uger efter fravænningen. Alle afficerede grise havde den samme orne til såvel morfar som farfar. Hos afficerede grise fandtes kun spor af ascorbinsyre i blod og organer sammenlignet med kuldsøskende og andre normale grise. Forholdet mellem det samlede antal normale og afficerede grise i de relevante kuld var i overensstemmelse med den forventede 3:1 udspaltning, som er karakteristisk for simpel autosomal recessiv arvegang ved parring mellem ikke afficerede anlægsbærere.

To afficerede grise blev rekonstituerede ved ascorbinsyretilsætning til foderet. Afficerede grise, der ikke fik vitamin C tilskud, døde af skørbug eller måtte aflives for at undgå unødig lidelse.

I modsætning til normale kontrolgrise kunne levermicrosomer fra en afficeret gris ikke danne ascorbinsyre in vitro med l-gulonolacton som substrat.

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