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IRON POISONING IN PIGLETS AUTOPSY FINDINGS IN EXPERIMENTAL AND SPONTANEOUS CASES

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As piglets are born with very small stores of iron and the iron-content in sow's milk does not at all satisfy their iron requirement, it is necessary to give them iron in sufficient amounts to prevent anaemia. The current doses are usually tolerated without any complications. Occasionally, however, it will be found that some or most of the pigs in a litter show signs and symptoms of disease and die within a few hours or days of the iron treatment. This holds true for both oral and parenteral iron.

Waxy degeneration of the muscles in pigs which died after oral or parenteral administration of iron has been observed by, for instance, *Brag* (1957, 1958), *Lannek & Tollerz* (1962), *Tollerz* (1962), *Henriksson* (1962), *Ludvigsen* (1962), and *Guarda* (1963).

At autopsy of pigs which died after having eaten soil besprinkled with ferrous-sulphate solution or been treated orally with a paste containing ferrum reductum and copper, *Brag* found waxy degeneration of skeletal and heart muscle.

Lannek & Tollerz induced experimentally hypersensitivity to iron in piglets whose sows had been fed a vitamin-E-deficient diet. The post-mortem picture in pigs treated with current doses

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of iron dextran was characterized by hydropericardium, hydrothorax, and extensive waxy degeneration of the body muscles, but not in the heart.

Even low doses of ferrofumarate as tablets were lethal to such pigs (*Tollerz* 1962). The autopsy picture will be described under Results.

At examination of 10 piglets which had died after conventional prophylactic treatment with intramuscular iron dextrin against anaemia, *Nilsson* (1960) did not find any waxy degeneration of skeletal muscle but, instead, focal hydropic degeneration of the heart muscle, hydropericardium, and hydrothorax.

Hydropericardium, in particular, seems to be common in these deaths from iron poisoning (*Lannek & Tollerz, Henriksson, Ludvigsen,* and *Guarda*).

MATERIAL AND METHODS

The autopsy material comprises all the pigs that died after iron treatment, except 6, in a large experimental series used for the purpose of studying why sporadic deaths occur after conventional treatment to prevent anaemia in piglets.

The whole experimental series includes 42 litters produced by 21 sows which were used in 49 experiments, as well as mice and chicks. Material, methods, and results for these experiments will be reported in full (*Tollerz,* in press) but have in part been reported preliminary by *Lannek & Tollerz* (1962), *Tollerz* (1962), *Lannek* et al. (1962), and *Tollerz & Lannek* (1964). The results of autopsy of the above-mentioned 6 pigs which are not included in this paper were reported by *Lannek & Tollerz* in 1962.

The autopsy findings in 78 piglets and 8 mice will be reported here. Data on the piglets and the iron treatment of the piglets are set out in Table 1.

Diet A is the experimental vitamin-E-deficient diet, consisting mainly of grain (76 %) heated at 80—100°C under continuous flow of air, cottonseed oil (4.6 %) similarly heated, and skim-milk powder (17 %). Diet D is "Forss Suggfoder" (food for pregnant sows) without the ordinary addition of alfalfa-leaf meal, vitamin E, and ethoxyquin. "Stensta" is a food containing 80 % oats obtained from the farm with the same name, plus 18 % skim-milk powder. On Stensta Farm oats together with skim milk had caused an outbreak of waxy muscular degeneration in por-

Table 1. Data on the autopsy cases.

Diet	Iron compounds	Route of administration	Number of litters	Number of pigs	Age (days) at iron treatment					Iron dose (mg/piglet) at time 0					Mortality at time in days				
					3	4	5	6	7-13	80	150	200-400	600-1000	1500	1	2	3	4	5
A	iron dextran	i. m.	18	47	5	9		9	24		7	5	32	3	38	4	2	1	2
				6[1]	2	1		3				1	5		4	1		1	
	iron dextrin	i. m.	1	3					3				3		1	2			
	ferrous fumarate	orally	2	6		3			3	6					6				
	ferrous sulphate	orally	1	1					1			1			1				
D	ferrous sulphate	orally	1	6				6				3[2]	3[2]		4		1	1	
Stensta	iron dextran	i. m.	1	1	1						1				1				
A farmer's diet (Gunsta)	iron dextran	i. m.	2	8				8			8				4	4			
Total			26 (11 litters after gilts)	78											59	11	3	3	2

[1]) 3 pigs were given dl-α-tocopherylacetate at the same time as or before the iron treatment. The other 3 received ethoxyquin between 6 and 12 hours after iron treatment.

[2]) 3 pigs were given 400 mg and 3 pigs 800 mg of iron per kg body weight. One pig at each dose level was given an injection of dl-α-tocopherylacetate on the day before the iron treatment.

kers. The Stensta diet was fed for 6 weeks before farrowing to a gilt whose piglets were then tested for iron sensitivity.

In a field outbreak at Gunsta Farm, 12 out of 18 piglets died after conventional prophylactic doses (100—150 mg per kg) of iron dextran. Eight of the piglets were subjected to autopsy and the findings will be reported below.

Eight mice which were fed a modified diet A containing 70 % grain, 23 % skim-milk powder, and 4.5 % heated cottonseed oil, were examined post mortem. They died within 6—12 hours of experimental treatment with iron dextran.

Histological methods

In all the cases histological examination was made of specimens from the body musculature (the rectus femoris, triceps brachii, and psoas), myocardium, lung, liver, kidney, spleen, a body lymph-node, and, in some cases, of other organs as well. The material was fixed in 10 % aqueous solution of formaldehyde. The frozen sections were stained for fat with Scharlach R and the paraffin-embedded sections were stained with Ehrlich's haematoxylin and eosin. Hueck's method was used for iron-staining, and Hotchkiss-Mc.Maren's periodic acid-Schiff technique for demonstration of glycogen.

RESULTS

As there was good agreement between the autopsy findings in 71 of the 78 examined pigs, the results will be reported together for the 71 pigs (group A), special observations being mentioned in the course of the account. In this group, 62 pigs were given intramuscular iron dextran, 3 intramuscular iron dextrin, and 6 oral ferrofumarate. For the other 7, which received ferrous sulphate orally, the findings will be reported individually (group B).

Group A. The skin and muscles were markedly pale. The muscles and subcutis around the sites of injection on the neck and the thigh were oedematous, exhibiting a brownish-black discoloration.

Macroscopical changes in the muscles in the form of rice-grain to oat-grain-sized streaky yellowish white or hyperaemic foci were seen in the muscles of the thigh, back, shoulder, or diaphragm in 6 cases only. The foci were distinctly visible but could easily be overlooked, unless the muscle was cross-sectioned and examined systematically. These pigs had died on the third to fifth day after the iron treatment. They represent all the

deaths in the series (Table 1) which occurred later than 2 days after the injection of iron in piglets whose sows received diet A.

The colon and the rectum, and to some extent the small bowel, showed varying degrees of greyish-black discoloration, irrespective of the mode of administration of iron. The intestinal contents in the piglets that had been given the higher intramuscular doses were of a greyish colour.

Ten pigs showed evidence of mild acute catarrhal enteritis. Bacteriological examinations of the liver, spleen, and lymph-nodes in these enteritis cases were negative. Three of these 10 pigs belonged to the group that received oral ferrofumarate.

No or insignificant hydrothorax or hydropericardium was noted in the diet-A-fed pigs or the "Stensta"-fed pig.

Examination of the 7 or 8 day old pigs from Gunsta Farm showed slight hydrothorax with about 5 ml of yellow fluid in the thoracic cavity but no hydropericardium.

The predominant microscopical finding in the group-A pigs was waxy muscular degeneration at different stages, depending upon the age of the morbid process, in most cases involving the greater part of the muscle fibres in the specimens taken from three different muscles. It should be noted that only 1 pig showed slight waxy myocardial degeneration.

If severe muscle degeneration is taken to denote cases with only a few normal muscle fibres and moderate degeneration to denote cases with less than half the muscle fibres intact in at least 2 out of 3 muscle specimens, 61 cases (86 %) were severe, 9 were moderate, and 1 case was mild (Figs. 1—4). The severe acute form was characterized partly by swelling and disappearance of striation of the muscle fibres, partly by complete destruction of large parts of the muscle fibres.

In areas of incomplete destruction there was usually slight interstitial oedema. At the subacute stage (the 6 cases in which the degeneration was macroscopically observable) reparatory processes had begun, and very short and thin regenerating muscle fibres were seen, with one or several cell nuclei and a sparsity of histocytes and a few leucocytes in the interstitium.

In most cases there was fatty degeneration of otherwise normal muscle fibres and occasionally also dystrophic calcification of the hyaline discs. The fatty degeneration was in most cases diffuse and finely globular. In the few cases in which there was focal fatty degeneration, degenerated muscle fibres were

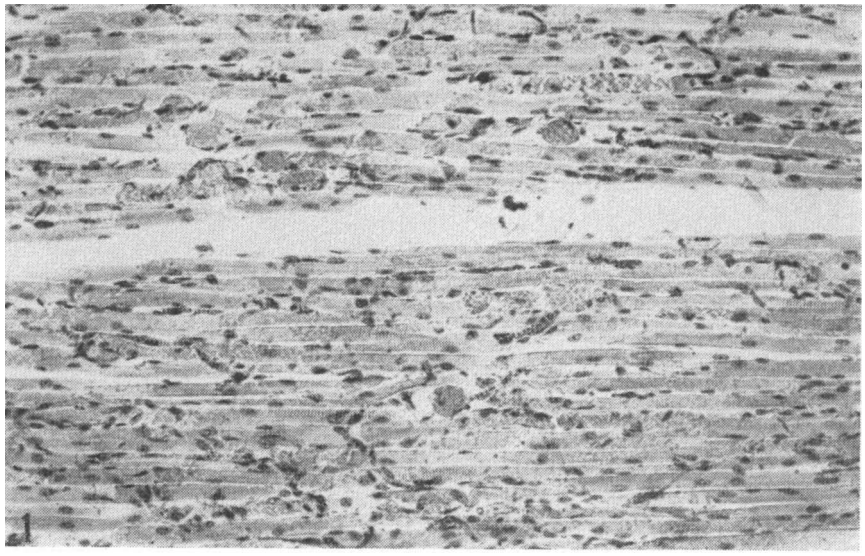

Fig. 1. O 2334/63. Muscle specimen from piglet, which died about 12 hours after injection of iron (375 mg per kg).
Slight acute waxy degeneration. A few muscle fibres are swollen or show discoid destruction. Heamatoxylin and eosin. 150 ×.

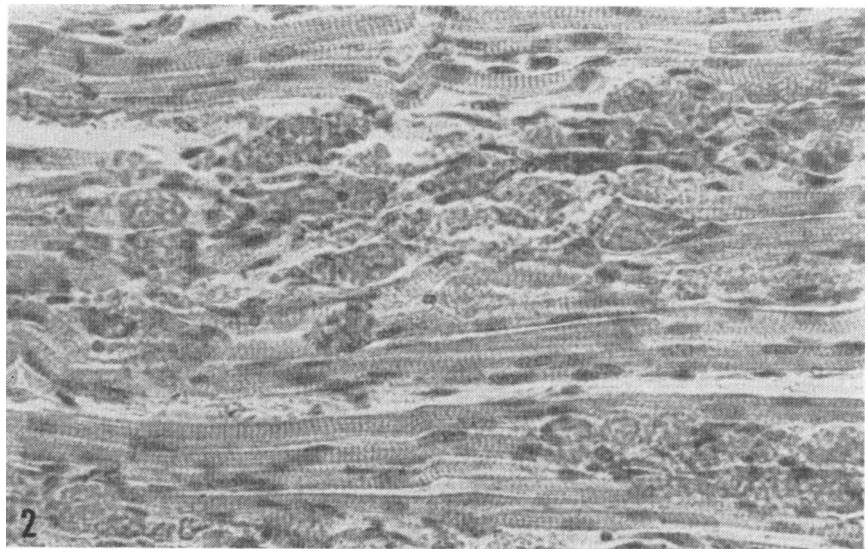

Fig. 2. O 2678/63. Muscle specimen from piglet, which died about 15 hours after injection of iron (375 mg per kg).
Moderate acute waxy degeneration. Haematoxylin and eosin. 300 ×.

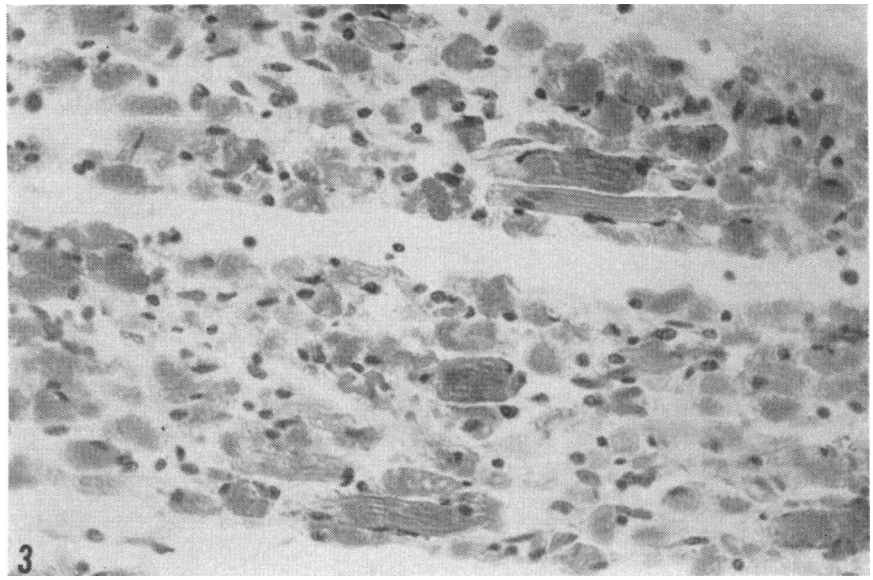

Fig. 3. O 465/62. Specimen of muscle from piglet, which died about 12 hours after oral administration of iron (80 mg).
Severe acute waxy degeneration. A few muscle fibres are intact.
Haematoxylin and eosin. 300 ×.

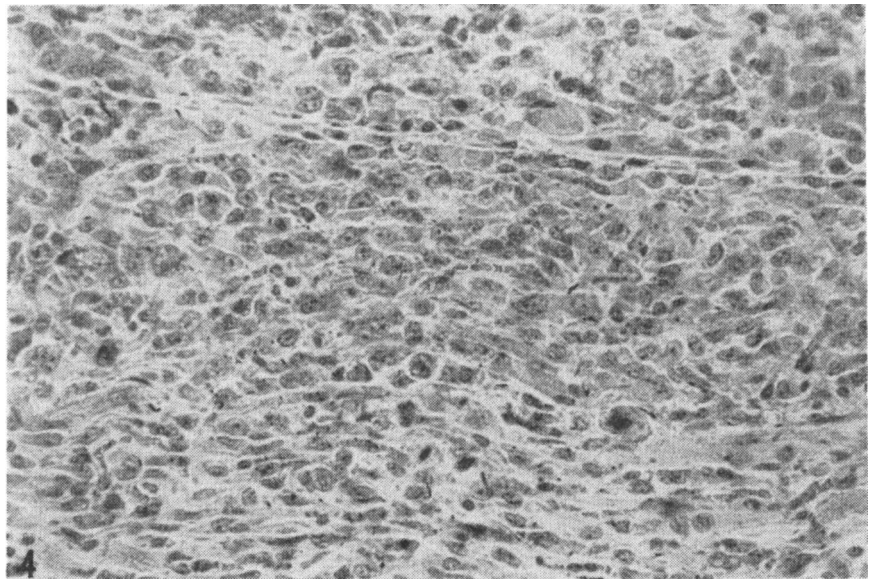

Fig. 4. O 3588/64. Muscle specimen from piglet, which died 5 days after injection of iron (375 mg per kg).
Severe subacute waxy degeneration. Most muscle discs are absorbed. Notable new formation of cell nuclei and beginning regeneration of muscle fibres. Haematoxylin and eosin. 300 ×.

seen in the foci. There seemed to be no relation between the degree of fatty degeneration and the degree of waxy muscular degeneration.

PAS-staining of the skeletal muscles showed in most cases sparse occurrence of glycogen.

No deviations from the above-described appearance of the muscles were noted in the Gunsta material.

Neither macroscopical nor microscopical changes of importance were seen in the liver. Slight to moderate, diffuse, finely and coarsely globular fatty degeneration of the liver cells was seen in 10 pigs. There was no correlation between this finding and the rest of the autopsy findings.

Slight vacuolisation in the liver cells with a few lytic nuclei was noted in 5 cases, seemingly occurring at random among the pigs.

Other organs showed no morphological changes of significance.

Staining for iron in 16 cases, 10 iron-dextran-treated and 6 ferrofumarate-treated pigs, showed iron in the blood-plasma, endothelial cells in the glomeruli of the kidneys, the intestinal mucosa, and the reticuloendothelial system.

Group B. The post-mortem picture of the 7 ferrous-sulphate-treated pigs deviated from that of group A in two respects. There was no waxy muscular degeneration in 4 cases but marked to moderate degeneration of the skeletal muscles in 3 cases. In all the 7 cases there was severe acute catarrhal to necrotizing gastro-enteritis with necrosis and haemorrhages in the mucous membrane. Bacteriological examination was negative.

The predominant autopsy finding in the 8 mice was moderate to marked acute waxy degeneration of the skeletal muscles, with the same microscopical picture as in the pigs.

The post-mortem picture of the mice deviated from that of the pigs in one respect, namely in that it showed slight acute waxy myocardial degeneration with small foci of discoid destruction in the muscle fibres in 4 cases.

There was moderate fatty degeneration of the skeletal muscles in 4 mice and slight fatty degeneration of the liver in 1 mouse.

DISCUSSION

The most important autopsy findings were waxy degeneration of the skeletal muscles, acute catarrhal to necrotizing gastroenteritis in the 7 pigs given ferrous sulphate orally, and anaemia. Anaemia in itself is a normal finding in pigs that have not been given iron, and the degree of anaemia is depending mainly upon the age of the pigs at the time of iron treatment. As the haemoglobin determinations, made on the day of the iron treatment of the pigs at the age of 3—13 days, did not suggest that the pigs were abnormally anaemic, and as uncomplicated anaemia does not usually cause death of pigs until they are 3—5 weeks old, the anaemia was judged as being of secondary importance.

The waxy muscular degeneration, on the other hand, was of severe nature and, together with the afore-mentioned anaemia, it was in 71 pigs the only significant finding. In view of the degree and extent of the muscle degeneration — about 25 % of the young body consists of muscles — it is obvious that the muscle damage was the central manifestation of iron poisoning in these pigs, even though the absence of other changes does not, of course, exclude non-observable important metabolic effects of the iron.

An interesting observation is that the microscopical waxy muscular degeneration, which in most cases was severe, could not be seen macroscopically until the dystrophic process was 3 days old or older. In the 6 cases of longer duration (Table 1), the macroscopical findings were distinctly observable but not very extensive. The presence of macroscopical changes thus shows only that the muscles are damaged but not the extent of the damage. The results of these studies also show that there is no correlation between the finding of local pale muscle areas and acute waxy muscular degeneration.

Among the 7 pigs with severe gastroenteritis, 3 had coexistent marked or moderate degeneration of the skeletal muscles. The latter was so advanced that it can be regarded as the cause of death. As for the toxic gastroenteritis caused by ferrous sulphate, it is difficult to assess the degree of its severity. The negative bacteriological examinations together with the absence of evidence of septicaemia would probably indicate that septicaemia was not the cause of death. The role of gastroenteritis caused by orally administered iron-compounds has been studied in comprehensive experiments by *Reissmann et al.* (1955 a, b). They report that deaths occurred in adult dogs and rabbits after about the same

doses of ferrous sulphate, whether the iron caused gastrointestinal disorder or not, that is, whether they gave it per rectum instead of per os. They conclude that, compared with the toxic effect of the absorbed iron, the gastrointestinal affection is of secondary importance. On the presumption that this result would also apply to the piglets in our experiments, it would mean that the toxicity mechanism for the absorbed iron in the 4 piglets with intact muscles differs from the mechanism that causes the severe muscle lesions. On the other hand, it is possible that severe affections of the stomach and intestine play a more important role in sucking pigs than in adult dogs and rabbits and, therefore, these affections cannot be excluded as a possible cause of death (see further *Tollerz*, in press).

Slight acute catarrhal enteritis also occurred in 10 piglets, 3 of which had received ferrofumarate orally. On some occasions diarrhoea occurred among the pigs, independently of the experiments. It was of mild and transient nature and required no treatment. As far as possible, experiments were avoided during the periods of intercurrent enteritis but, inevitably, occasional diarrhoeas occurred when the experiments started. It is probable that most of these 10 pigs already had diarrhoea when the iron was given, although the ferrofumarate or the parenteral iron, as will be seen in the following, could have played some part in causing this symptom.

From the degree of blackish-grey discoloration of the intestinal wall even after parenteral administration of iron it may be reasonably assumed that the iron is present at a fairly high concentration in the intestinal wall. Investigations by *Braude et al.* (1962) support this assumption. They found that 3 % of a single injection of 180 mg of iron as iron dextran were present in the gastrointestinal wall 12 hours after the injection. They also found that a small part of the injected amount of iron appeared in the gastrointestinal contents. Four hours after the injection the iron concentration was 8.2 µg per g of gastrointestinal content, and at 12 hours it was 2.8 µg per g, an amount that would equal 1 mg of iron if the gastrointestinal contents are assumed to be 100 mg. In the present investigation much higher doses of iron dextran were usually given and, therefore, it seems probable that the greyish colour of the intestinal content is due to excreted iron.

No noteworthy transudations to the body cavities were noted in this material of animals which, besides experimental pigs, included pigs that had died after iron treatment at Gunsta Farm. A remarkable fact is that *Lannek & Tollerz*, in the preliminary experiments published separately, in which the same experimental diet was used, found marked transudation to the pericardium and pleural cavity.

Nilsson, in his material of fatal iron-treated cases comprising 10 newborn piglets, found no waxy muscle degeneration but noted focal hydropic degeneration of the heart muscle. This change in the heart muscle is difficult to see at routine staining with Ehrlich's haematoxylin and eosin, unless attention is directed especially towards detecting it. In the present material, this type of heart affection did not occur, which also agrees with the absence of hydropericardium. Only in one pig slight waxy degeneration was seen in the heart muscle. It seems reasonable to assume that the heart-muscle change demonstrated by *Nilsson* can very well have been present in the pigs with hydropericardium as a manifestation of iron poisoning in the other papers mentioned here, but that it escaped notice.

In the material of 78 pigs presented here, slight pathological changes in the heart muscle of the waxy-degeneration type were seen in only one pig. Slight acute waxy myocardial degeneration, on the other hand, was found in 4 out of the 8 mice. No valid explanation of the different manifestations of iron poisoning can be given here.

In the preliminary reports it has been shown that the vitamin-E-deficient diet A fed to the sows is attended with a potential risk for iron poisoning in the piglets. This risk can be reduced by the administration of vitamin E.

Henriksson (1962), at autopsy of 3 piglets in the same litter which, probably, had died of iron poisoning, made the interesting observation that 2 of them had profuse serofibrinous transudation into the pericardium and the pleural sac, 1 had advanced degeneration of the skeletal muscles, 1 had hepatosis dietetica, and 1 had alterative myocarditis of the type seen in cases of mulberry-heart disease, that is, a sample of different vitamin-E-deficiency manifestations. Whether all the autopsy findings are due to the administered iron is uncertain, however. Our knowledge of what factors determine the way in which vitamin-E deficiency mani-

fests itself is still incomplete. It may be mentioned that selenium also causes a significant increase of the iron tolerance of mice (*Tollerz*, in press).

REFERENCES

Brag, S.: Profylax och terapi vid järnbristanemi hos spädgrisar. Medlemsblad för Sveriges Veterinärförbund 1957, *9*, 321—324.

Brag, S.: Iron poisoning in piglets. Proc. VIIIth Nordic Veterinary Congress, Helsinki 1958, 98—102.

Braude, R., A. G. Chamberlain, M. Kotarbinska & K. G. Mitchel: The metabolism of iron in piglets given labelled iron either orally or by injection. Brit. J. Nutr. 1962, *16*, 427—449.

Guarda, F.: Incidenti mortali nella terapia della anemia dei suinetti con composti organici di ferro. Ann. Fac. Med. vet. Torino 1963, *13*, 3—39.

Henriksson, K.: Poisonings of piglets during iron therapy. Finsk Veterinärtidskr. 1962, *68*, 293—297.

Lannek, N., P. Lindberg & G. Tollerz: Lowered resistance to iron in vitamin E deficient piglets and mice. Nature (Lond.) 1962, *195*, 1006—1007.

Lannek, N. & G. Tollerz: Överkänslighet hos vitamin E-fattiga smågrisar mot järnbehandling. Medlemsblad för Sveriges Veterinärförbund 1962, *14*, 29—33.

Ludvigsen, J. B.: Indledende undersøgelser over jernforgiftning hos pattegrise. Landøkonomisk Forsøgslab. efteraarsmøde 1962, 429—434.

Nilsson, P. O.: Några fall av akut järnförgiftning med myokardskada hos smågrisar. Nord. Vet.-Med. 1960, *12*, 113—119.

Reissmann, K. R., T. J. Coleman, B. S. Budai & L. R. Moriarty: Acute intestinal iron intoxication. I. Iron absorption, serum iron and autopsy findings. Blood 1955 a, *10*, 35—45.

Reissmann, K. R. & T. J. Coleman: Acute intestinal iron intoxication. II. Metabolic, respiratory, and circulatory effects of absorbed iron. Blood 1955 b, *10*, 46—51.

Tollerz, G.: Överkänslighet hos E-vitaminfattiga smågrisar vid peroral järnbehandling. Medlemsblad för Sveriges Veterinärförbund 1962, *14*, 510.

Tollerz, G.: Studies on the tolerance to iron in piglets and mice, with special reference to vitamin E, synthetic antioxidants and sodium selenite (in press).

Tollerz, G. & N. Lannek: Protection against iron toxicity in vitamin E-deficient piglets and mice by vitamin E and synthetic antioxidants. Nature (Lond.) 1964, *201*, 846—847.

SUMMARY

An account is given of the autopsy findings in 78 piglets which died from iron poisoning. They belonged to an experimental series which included a further 6 piglets that also died but will not be accounted for here. The experiment was designed to study why iron treatment causes occasional deaths among piglets. The results will be reported in full elsewhere (*Tollerz,* in press).

In 63 piglets, the sows had been fed special experimental diets, and in 8 piglets from a field case where deaths occurred after conventional iron treatment to prevent anaemia, the predominant autopsy finding was waxy degeneration of the skeletal muscles, which appeared irrespective of whether iron dextran or iron dextrin was injected intramuscularly, or whether ferrofumarate or ferous sulphate was given orally. Only one piglet had slight heart-muscle degeneration, and hydropericardium was not present.

The remaining 7 piglets received ferrous sulphate orally, which resulted in catarrhal to necrotizing gastroenteritis. Three of these piglets had waxy degeneration of the skeletal muscles, but in the other 4 the musculature was intact, which indicates another mechanism of toxicity than that in the cases of muscle degeneration.

ZUSAMMENFASSUNG

Eisenvergiftung bei Ferkeln. Obduktionsbefunde in experimentellen und spontanen Fällen.

Es wird über die Obduktionsbefunde an 78 Ferkeln, die an Eisenvergiftung starben, berichtet. Die Tiere gehören zu einer experimentellen Gruppe, zu der noch 6 weitere Ferkel gehörten, die ebenfalls starben, über die aber hier nicht berichtet wird. Die Experimente waren geplant um zu studieren, weshalb Eisenbehandlung bei Ferkeln gelegentlich zu Todesfällen führt. Über die Resultate wird in extenso anderweits berichtet werden (Tollerz, im Drucke).

Bei 63 Ferkeln waren den Säuen spezielle experimentelle Diäten verabfolgt worden, und 8 Ferkel stammten von einem Falle, bei dem Todesfälle nach der gebräuchlichen Eisenbehandlung zur Anämiprophylaxe auftraten. Bei allen diesen Ferkeln war der auffälligste Obduktionsbefund eine wachsartige Degeneration der Skelettmuskulatur, unabhängig davon, ob Eisendextran oder Eisendextrin intramuskulär injiziert worden war, oder ob Ferrofumarat oder Ferrosulphat oral verabreicht wurde.

Nur ein Ferkel hatte eine leichte Herzmuskeldegeneration, ohne Auftreten von Hydroperikard. Die übrigen 7 Ferkel hatten Ferrosulphat per os erhalten, welches zu einer katarrhalischen bis nekrotisierenden Gastroenteritis führte. Drei von diesen Ferkeln hatten wachsartige Degeneration der Skelettmuskeln, während bei den restlichen 4 die Muskulatur intakt war. Letzterer Befund deutet auf einen anderen Toxicitätsmechanismus, als denjenigen, der bei den Fällen mit Muskeldegeneration auftrat.

SAMMANFATTNING

Järnförgiftning hos grisar. En patolog-anatomisk undersökning av experimentella fall och fältfall.

Obduktionsresultatet från 78 smågrisar, som dött av järnförgiftning, redovisas. Grisarna ingår i en serie experiment, i vilka ytterligare 6 grisar dog. Dessa redovisas ej här. Avsikten med undersökningen var att utröna varför järnbehandling orsakar sporadiska dödsfall bland grisar. Resultaten kommer att fullständigt redovisas (Tollerz, under tryckning).

Hos 63 grisar, vars suggor erhållit särskilda experimentella koster, och hos 8 grisar från ett fältfall, där gängse anemiprofylax orsakade dödsfall, var det väsentligaste obduktionsfyndet vaxartad degeneration av skelettmuskulaturen, som förekom oberoende av om järndextran eller järndextrin injicerades intramuskulärt eller om ferrofumarat eller ferrosulfat gavs oralt. Endast en gris visade lindrig hjärtmuskeldegeneration och hydroperikard förekom ej.

De återstående 7 grisarna erhöllo ferrosulfat oralt, vilket orsakade katarral till nekrotiserande gastroenterit. Tre av dessa grisar hade vaxartad skelettmuskeldegeneration, men hos de 4 övriga var muskulaturen normal, vilket torde bero på en annan toxicitetsmekanism än vid fallen med muskeldegeneration.

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