Acta vet. scand. 1964, *5*, 305—310.

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THE INTESTINAL FLORA IN PIGS WITH PARAKERATOSIS

IV. DETERMINATION OF ZINC LEVELS IN BLOOD AND URINE

By

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In 1955, *Tucker & Salmon* demonstrated the good effect of zinc on parakeratosis in pigs. Their results have been verified by others but it still remains to find out how zinc prevents the disease. In part, this uncertainty arises from methodological difficulties in studying zinc in the body.

The blood zinc values obtained by *Hoekstra et al.,* 1956, with the dithizone test did not give a definite answer i. a. because only a few pigs with parakeratosis were examined. In the organs the zinc content was normal, a conclusion also reached by others (*Grünberg,* 1961, among others). As yet there are no systematic studies of the blood zinc content in pigs with parakeratosis. For further references on zinc and analytical methods, see *Månsson et al.,* 1964.

Details of an experiment in which three groups of pigs were fed a high protein diet have already been described (*Månsson,* 1964). Pigs given supplementary zinc did not develop signs of parakeratosis. In one group of pigs (group III), also clinically healthy, there was no increase in the anaerobic component of the intestinal flora. Blood zinc levels were followed for all animals in the experiment and for some of them, the urine zinc content as well. The purpose was to find out whether or not the experimental groups differed in this respect and if so, whether there was an interplay between zinc levels, feed, composition of the intestinal flora, and state of the skin.

MATERIAL AND METHODS

Group I (6 animals) was fed the basal diet. Group II (6 animals) was also fed the basal diet but three animals in the group, pigs 1, 2 and 4, were given a zinc supplement. Group III (6 animals), was fed the basal diet but with the fishmeal (Icelandic codmeal) replaced by Peruvian sardine meal (*Månsson*, 1964).

Zinc in blood was determined by spectrography of mineralized whole blood in a high tension arc, with Cd 2288 as reference (*Månsson et al.*, 1964).

Zinc in urine was determined by means of the dithizone test described by *Huff* (1948) in samples collected during urination.

RESULTS

Blood zinc levels

The blood zinc values obtained, expressed as ppm, are listed in table 1.

In *group I* there was a pronounced tendency towards lower zinc values during the first weeks of the experiment. The initial value for pig 293 was 4.3 ppm, and by day 9, the zinc content had dropped to 3.8 ppm. Pig 299 had an initial value of 5.0 ppm and a value of 2.7 ppm on day 20. The values for pig no 303 were low from the beginning of the experiment. The rise in the zinc values for pigs 292 and 303 on day 27 reflects the intravenous injections of zinc sulphate during the preceding week. After day 27, zinc was added to the feed and the blood zinc values began to increase. There was a degree of individual variation, the values for pig 303 were invariably lower than the values for pig 292 for example.

On day 11 the blood levels in *group II* still exceeded 5.5 ppm. In pigs 6, 7 and 9, which did not receive supplementary zinc, the blood levels dropped still more but later on began to rise again.

In *group III* the zinc values dropped during the first part of the experiment and then levelled off at about 3 ppm for the remainder of the experimental period.

Presence of zinc in the urine

The values obtained are listed in table 2; only groups I and III were examined. On day 5, urine from four of the six pigs in *group I* contained no zinc and urine from the other two contained traces of zinc. On days 9 and 20 samples from all the animals were negative. From day 27 onwards the feed was supplemented

Table 1.
Zinc levels in blood (ppm).

Pig no.	No of days from the beginning of the exp.							
	1.	9.	20.	27.	34.	49.	63.	77.
Group I.								
292			6.1	9.2	6.3	7.4	7.1	9.0
293	4.3	3.8						
299	5.0		2.7	3.1	5.9	5.0	5.8	5.2
300	5.2		5.5	5.0	6.3	7.0	5.5	4.6
303	3.1	2.3	3.2	8.5	4.3	4.9	6.9	4.3

	No of days from the beginning of the exp.			
	11.	25.	34.	42.
Group II.				
1	9.0	8.4	6.2	6.5
2	5.8	5.6	5.4	8.2
4	7.0	6.1	5.8	6.2
6	5.5	4.2	4.5	4.6
7	7.0	5.0	4.5	8.5
9	6.5	4.5	5.6	9.5

	No of days from the beginning of the exp.			
	1.	14.	28.	42.
Group III.				
42	5.7	3.6	3.3	3.5
50	4.4	3.3	3.7	3.4
51	3.8	3.2	3.0	2.8
52	3.8	2.1	2.6	4.5
53	5.4	3.0	2.7	3.4
55	7.5	3.2	3.9	3.8

with zinc. Of the samples taken after this point, two were positive for zinc on day 41 and all four on day 69. All the samples taken from the pigs in *group III* on the day before the experiment began contained zinc. On day 8 all samples were negative and the urine seems to have remained free from zinc throughout the experiment.

DISCUSSION

In confirmation of earlier observations (*Månsson et al.*, 1964), the amount of zinc in the whole blood decreased during the first part of the experiment. The composition of the experimental diets differed radically from the feed given the pigs in the interval between weaning and the beginning of the experiment; the drop in blood zinc levels may illustrate the importance of the diet for

Table 2.
Dithizone test of urine.

Pig no.	No of days from the beginning of the exp.				
	5.	9.	20.	41.	69.
Group I.					
292	neg		neg	trace	trace
293	neg	neg			
299	neg		neg	neg	trace
300	neg		neg	neg	strongly pos
302	trace	neg			
303	trace		neg	trace	strongly pos
Group II.	not investigated				

	No of days from the beginning of the exp.			
	1.	8.	27.	40.
Group III.				
42	pos	neg	neg	trace
50	trace	neg	neg	neg
51	pos	neg	neg	neg
52	pos	neg	neg	neg
53	strongly pos	neg	trace	neg
55	strongly pos	neg	neg	neg

the absorption of zinc. The degree of reduction, however, varied widely. In groups I and II, the drop was greatest for the animals which developed skin lesions. The time of onset for the skin lesions (*Månsson,* 1964) accorded with the lowest zinc values obtained. Low blood zinc levels were accompanied by the absence of detectable zinc in the urine. This is well illustrated by the results for group III. The results for this group also imply that low zinc values are not in themselves sufficient to induce parakeratosis since these animals remained clinically healthy. Nor was the intestinal flora dominated by anaerobes. This *can* be a hint that interplay between several factors is a prerequisite for the induction of the disease. Of these factors, the amount of zinc — reflected by the low levels in the blood and urine — and the composition of the intestinal flora with a dominating clostridial component appear to be basic.

It is difficult to compare the results obtained here and those reported by *Hoekstra et al.* (1956) among others because of the methods used.

Examination of the urine for the presence of zinc was sug-

gested by previous observations that animals on the basal diet often did not excrete detectable amounts of zinc in the urine and that animals on a protein-poor diet — the control diet (*Månsson & Olsson,* 1961) — did excrete zinc. The results for groups I and III in this experiment confirmed the influence of the basal diet on the zinc content of the urine even although practical difficulties limited the number of samples obtained. Samples have to be collected while the animals are urinating, zinc-free utensils have to be used, and all contamination has to be avoided in order to be able to rely on the results of the sensitive dithizone test. The presence of zinc in dithizone-positive urine samples has been confirmed by using a spectrographical method similar to the one used for whole blood.

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SUMMARY

Zinc in the blood was determined spectrographically and in the urine by the dithizone test. There was a tendency towards a drop in the zinc content of the blood during the first days of the experiment and also a tendency towards low values at the skin changes appeared.

The blood zinc content subsequently rose. The lowest values were encountered among the pigs in group III which remained free from clinical signs and which had been fed a different type of fish meal. The results obtained are listed in tables 1 and 2. The presence of demonstrable zinc in the urine appears to be closely related to the blood zinc content. No zinc could be detected in urine samples obtained at the time of onset of the skin changes or from the animals in group III after the first sampling (table 2).

The interplay between diet, composition of the intestinal flora, and the state of the skin is discussed in view of the results obtained.

ZUSAMMENFASSUNG

Die Darmflora bei Schweinen mit Parakeratose.

IV. Bestimmung vom Zinkgehalt im Blut and Urin.

Die Bestimmung des Zinkgehaltes im Blut wurde spektrografisch und im Urin mit der Methode mit Dithizon ausgeführt. Der Zinkgehalt im Blut zeigte eine Tendenz zu sinken während der ersten Tage und auch eine Tendenz niedrig zu sein bei dem Zeitpunkt wo Hautveränderungen auftreten um danach wieder zu steigen. Die niedrigsten Werte wurden bei Schweinen der Gruppe III nachgewiesen, die symptomfrei waren und die anderes Fischmehl als die Tiere der Gruppen I und II erhielten. Siehe Tabelle 1 und 2. Das Vorkommen von nachweisbaren Mengen Zink im Urin scheint recht gut mit dem Blutzinkgehalt zu korrelieren. So konnte in der Regel kein Zink bei Tieren zum Zeitpunkt des Auftreten von Hautsymptomen nachgewiesen werden und auch nicht bei Tieren der Gruppe III nach der ersten Probenabnahme. Tabelle 2.

Der Zusammenhang zwischen der Diet, der Zusammensetzung der Darmflora und der Hautstatus wird gegen den Hintergrund der erhaltenen Zinkwerte diskutiert.

SAMMANFATTNING

Tarmfloran hos grisar med parakeratos.

IV. Bestämning av zinkhalten i blod och urin.

Bestämning av zinkhalten i blod utfördes spektrografiskt och i urin med dithizontest. Zinkhalten i blod visade en tendens att sjunka under försökets första dagar och också en tendens att vara låg vid tidpunkten för hudförändringarnas uppträdande för att därefter åter stiga. Lägst värden påvisades emellertid hos grisar i grupp III, som voro symtomfria och som erhållit annat fiskmjöl än djuren i grupperna I och II. Se tabell 1 och 2. Förekomsten av påvisbar mängd zink i urinen synes tämligen väl korrelerad till blodzinkhalten. Sålunda kunde i regel ej zink påvisas hos djur vid tidpunkten för hudsymtomens uppträdande och ej heller hos djur i grupp III efter första provtagningen. Tabell 2.

Sambandet mellan foderstat, tarmflorans sammansättning och hudstatus diskuteras mot bakgrund av de erhållna zinkvärdena.

(Received December 31. 1963).