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THE INTESTINAL FLORA IN PIGS WITH PARAKERATOSIS

II. ELECTROPHORETIC STUDIES OF BLOOD SERUM, THE ERYTHROCYTE SEDIMENTATION RATE AND HAEMOGLOBIN DETERMINATIONS

By

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In an experiment comprising three groups of pigs it could be demonstrated (*Månsson,* 1964) that a feed containing 20 per cent fishmeal (Icelandic codmeal), when given dry and ad libitum, caused a great increase in the number of atypical *Clostridium perfringens* in the intestinal contents and also induced parakeratosis. When zinc was added to the feed, the pigs developed no signs of parakeratosis but the high counts of atypical Clostridium perfringens in the intestinal contents were not affected. If the Icelandic codmeal was replaced by an equivalent amount of Peruvian sardine meal, there was no increase in the clostridial flora and no parakeratosis. The animals in this experiment were utilised for a number of special studies intended to explore whether or not an aetiological relationship exsists between the skin lesions and the changes known to occur in the composition of the intestinal flora.

This paper covers electrophoretic studies of blood serum, the erythrocyte sedimentation rate (ESR), and haemoglobin determinations.

Several reports have been published on the electrophoresis of pig blood serum including the amounts of the different fractions

and the changes occurring with age. For references see the paper by *Larsen et al.* (1962) among others. *Smith et al.* (1960) could not demonstrate any significant differences in the γ-globulin fractions between pigs with parakeratosis and zinc-treated pigs without parakeratosis. The γ-globulin levels were high from the beginning of the experiment. The changes in the electrophoretic pattern which were observed "were definitely not specific for parakeratosis" but could have reflected an "increased susceptibility to invasion by infectious organisms".

Hoefer et al. (1960) note an increase in the amount of γ-globulin in samples taken on the 53rd day from pigs with experimentally induced parakeratosis. There is no way of knowing when this increase first occurred; it is possible that it resulted from secondary infection of the skin.

When evaluating the amounts of some serum fractions the protein content of the diet has to be taken into account. From studies by *Cartwright et al.* (1948) it appears that the dietary protein level influenced the amount of the albumin fraction.

MATERIAL AND METHODS

Three groups of pigs on different diets have been followed. The experimental conditions have been described in an earlier paper (*Månsson,* 1964). Group I (6 animals) was fed the basal diet. Group II (6 animals) was also fed the basal diet but three of the pigs, nos. 1, 2 and 4, were given a zinc supplement. For Group III (6 animals), the fish meal in the basal diet — Icelandic codmeal — was replaced by Peruvian sardine meal.

Electrophoresis of serum protein

Total serum protein was determined colorimetrically by using Weichsel and Baum's reagent and is expressed in g/100 ml.

Paper electrophoresis of serum was carried out on an LKB apparatus, type LKB No 3276, with Schleicher & Schüll paper No 2043B and TRIS-buffer pH 8.9. The samples were run for 18 hours at 150 V and 6 mA. The papers were stained with Amido-Schwartz 10B and the relative intensities of the stained fractions were measured in a Beckman Spinco Analytrol.

Erythrocyte sedimentation rate (ESR)

The ESR was determined according to Westergren and read after 60 minutes. Heparinised blood samples were used.

Haemoglobin determinations

The extinction point for a mixture of 0.1 ml blood with 25 ml 0.4 per cent ammonia solution was measured in a Bechman model C colorimeter. The haemoglobin level is expressed in g/100 ml. Standardization as pyridine hemochromogen.

RESULTS

Total protein levels and different protein fractions

Total protein levels and amounts of the different protein fractions are listed in table 1. Among the globulin fractions the most obvious changes were in the γ-globulin. This fraction is the only one to be dealt with in any detail; for information concerning the other serum fractions, see table 1.

Group I. During the first three weeks of the experiment the total serum protein increased from about 6 to about 8 g/100 ml, and then levelled off at this value for the rest of the experiment. Changes in the electrophoretic patterns became obvious from the end of the third week. There was a great absolute and relative increase in pigs 292 and 299 and to some extent in pig 300. The decrease in the albumin fraction was mainly relative. The A/G ratio was reduced by about half.

Group II. There was a distinct increase in the γ-globulin in pigs 7 and 9 in samples on day 33. There was a slighter increase in pig 6 and no change for pigs 1, 2 and 4.

Group III. None of the changes noted in the serum electrophoretic patterns for the pigs in Group I or for pigs 6, 7 and 9 in Group II could be noticed.

Erythrocyte sedimentation rate (ESR)

The values obtained for the different groups can be found in table 2.

Group I. There was an increase in ESR for all animals in this group and particularly during the first 20 days. The ESR for pig 303, however, did not rise before day 27. The increase in ESR for pigs 293 and 302 was noticeable on day 9. The values for pigs 292 and 299 rose to 25 and 49 mm from the initial value of 6 mm. From day 27 and onwards the ESR gradually returned to normal after oral zinc supplementation was begun.

Group II. There was a slight increase in ESR for pigs 6, 7 and 9, and no change for pigs 1, 2 and 4.

Table 1.
Total serum protein levels and amounts of different serum protein fractions.

Pig no.	No of days from the beginning of the exp.	Total protein g/100 ml	Albumin		Globulin						A/G
					α		β		γ		
			rel. %	g/100 ml	rel. %	g/100 ml	rel. %	g/100 ml	rel. %	g/100 ml	
Group I.											
292	8	5.8	50.6	2.93	21.3	1.24	15.7	0.91	12.4	0.72	1.0
	20	7.7	31.8	2.44	28.5	2.19	18.4	1.42	21.2	1.63	0.4
	49	8.2	45.2	3.71	14.5	1.18	22.7	1.86	17.7	1.45	0.8
	63	8.7	42.7	3.71	17.6	1.53	19.9	1.73	19.9	1.73	0.7
	77	8.1	48.3	3.91	17.1	1.39	17.6	1.43	17.1	1.39	0.9
299	8	5.9	48.6	2.87	22.4	1.32	16.4	0.97	12.6	0.74	0.9
	20	7.6	28.7	2.18	28.6	2.14	18.1	1.38	24.6	1.87	0.4
	26	7.8	24.8	1.93	24.8	1.93	17.7	1.38	32.7	2.55	0.3
	49	8.3	39.3	3.26	14.5	1.20	19.5	1.62	26.6	2.21	0.6
	63	8.6	37.7	3.24	14.0	1.20	19.6	1.69	28.6	2.46	0.6
	77	8.1	46.2	3.74	14.7	1.19	18.9	1.53	20.2	1.64	0.8
300	8	6.6	46.0	3.04	25.2	1.66	15.3	1.01	13.5	0.89	0.8
	20	7.5	35.1	2.63	20.0	1.50	18.7	1.40	26.2	1.97	0.5
	49	7.9	38.0	3.00	17.2	1.36	19.7	1.67	25.1	1.98	0.6
	63	8.5	32.9	2.80	17.4	1.48	19.9	1.69	29.8	2.53	0.4
	77	7.8	30.3	2.36	21.4	1.67	20.2	1.58	28.1	2.19	0.4
303	8	4.6	42.0	1.93	25.9	1.19	17.6	0.81	14.5	0.67	0.7
	20	7.1	47.5	3.37	20.8	1.48	14.2	1.01	17.5	1.24	0.9
	49	7.6	44.5	3.38	18.1	1.38	21.0	1.60	16.4	1.25	0.8
	63	8.2	45.2	3.71	16.1	1.32	19.1	1.57	19.5	1.60	0.8
	77	7.5	47.0	3.53	15.9	1.19	18.1	1.36	18.9	1.42	0.8
Group II.											
1	5	7.4	56.2	4.16	21.2	1.57	14.9	1.10	7.7	0.57	1.2
	18	6.9	59.2	4.08	15.3	1.19	15.3	1.76	10.2	0.70	1.4
	47	7.1	54.3	3.86	19.2	1.36	16.9	1.20	9.6	0.68	1.1
2	5	6.7	58.6	3.93	21.2	1.42	13.6	0.91	6.5	0.44	1.4
	18	6.2	52.6	3.26	19.7	1.22	17.5	1.09	10.2	0.63	1.1
	33	6.7	54.3	3.64	16.8	1.13	17.9	1.20	10.9	0.73	1.1
	47	7.5	51.7	3.88	16.2	1.22	19.2	1.44	12.8	0.96	1.0
4	5	6.9	64.6	4.46	17.2	1.19	13.7	0.95	4.4	0.30	1.8
	18	6.2	49.5	3.07	21.5	1.46	15.9	0.99	13.1	0.81	0.9
	47	7.1	53.8	3.82	18.7	1.33	16.1	1.14	11.4	0.81	1.1
6	5	6.7	58.8	3.94	17.3	1.16	17.7	1.19	6.2	0.42	1.4
	18	6.5	53.5	3.48	18.8	1.22	14.9	0.97	12.9	0.84	1.1
	25	7.1	53.0	3.76	19.5	1.38	14.8	1.05	12.8	0.91	1.1
	33	7.8	57.6	4.49	18.7	1.46	11.2	0.87	13.1	1.02	1.3
	47	7.5	52.9	3.97	18.7	1.40	12.3	0.92	16.0	1.20	1.1

Table 1 (continued).

ig no.	No of days from the beginning of the exp.	Total protein g/100 ml	Albumin rel. %	Albumin g/100 ml	Globulin α rel. %	Globulin α g/100 ml	Globulin β rel. %	Globulin β g/100 ml	Globulin γ rel. %	Globulin γ g/100 ml	A/G
7	5	6.7	62.4	4.17	16.2	1.09	15.8	1.06	5.8	0.39	1.65
	18	6.5	50.6	3.29	23.6	1.52	14.6	0.95	11.2	0.73	1.02
	25	7.1	61.7	4.38	15.2	1.08	12.6	0.89	10.4	0.74	1.61
	33	7.9	52.4	4.14	20.5	1.60	11.8	0.92	15.3	1.21	1.10
	47	8.7	43.8	3.81	21.9	1.91	16.3	1.42	18.1	1.57	0.78
9	5	6.9	54.2	3.74	23.6	1.63	14.3	0.99	7.4	0.51	1.18
	18	6.7	60.7	4.07	16.7	1.12	12.7	0.85	9.9	0.66	1.55
	25	6.9	63.3	4.37	15.9	1.10	11.6	0.80	9.2	0.63	1.72
	33	7.1	46.8	3.32	20.9	1.48	14.4	1.02	18.0	1.27	0.88
	47	8.2	57.8	4.74	14.7	1.21	12.8	1.05	14.7	1.21	1.37
roup III.											
42	10	5.9	47.7	2.81	19.6	1.16	17.8	1.05	15.0	0.89	0.91
	28	7.3	39.1	2.85	23.9	1.74	16.6	1.21	20.3	1.48	0.64
	42	7.6	58.7	4.31	18.0	1.37	12.4	0.92	11.1	0.84	1.42
50	10	6.5	40.9	2.66	24.4	1.59	18.1	1.18	16.5	1.07	0.69
	28	6.6	44.7	2.95	22.0	1.45	20.0	1.32	13.5	0.88	0.81
	42	7.1	55.4	3.93	19.1	1.36	14.2	1.01	11.3	0.80	1.24
51	10	6.5	53.8	3.50	18.8	1.22	15.3	0.99	12.2	0.79	1.16
	28	7.3	50.8	3.71	16.7	1.22	19.1	1.39	13.3	0.97	1.04
	42	7.9	60.5	4.80	15.8	1.25	13.3	1.04	10.5	0.83	1.53
52	10	6.6	46.0	3.04	20.7	1.37	19.5	1.29	13.8	0.91	0.85
	28	7.2	38.1	2.74	28.7	2.07	16.6	1.21	16.6	0.88	0.62
	42	7.6	52.1	3.96	19.9	1.51	16.1	1.22	11.8	0.90	1.09
53	10	6.5	44.0	2.86	20.7	1.35	16.4	1.07	19.0	1.23	0.78
	28	7.1	42.6	3.02	23.5	1.67	16.5	1.17	17.4	1.24	0.74
	42	7.4	55.9	4.14	18.6	1.38	13.1	0.82	12.4	0.92	1.27
55	10	6.5	51.8	3.37	17.3	1.12	18.2	1.18	12.7	0.83	1.08
	28	6.8	42.9	2.92	27.0	1.84	15.1	1.03	15.1	1.03	0.75
	42	7.1	55.6	3.95	14.1	1.00	18.7	1.33	11.6	0.82	1.25

Group III. The initial values for ESR, 2—3 mm, remained more or less unchanged throughout the experiment.

Haemoglobin levels

Values were recorded every tenth day during the experiment, and lay between 8 and 12 g/100 ml for all pigs regardless of group and the state of the skin.

Table 2.
Erythrocyte sedimentation rate (ESR) values
(mm after 60 minutes).

Group I.								
No of exp. days	1	9	20	27	34	49	63	77
Pig no.								
292	6		25	8	11	5	8	10
293	6	9						
299	6		49	33	42	11	28	3
300	8		11	7	6	2	3	5
302	6	19						
303	6		4	10	12	7	9	6
Group II.								
No of exp. days	4	11	18	25	34	42		
Pig no.								
1	2	3	3	2	4	2		
2	2	2	2	3	3	2		
4	2	3	3	2	4	3		
6	2	2	2	3	4	5		
7	2	3	2	6	5	12		
9	2	2	2	7	5	4		
Group III.								
No of exp. days	3	14	28	42				
Pig no.								
42	2	3	2	2				
50	2	4	5	2				
51	2	3	3	3				
52	3	5	5	6				
53	3	5	4	5				
55	2	2	2	2				

DISCUSSION

The results listed in tables 1 and 2 demonstrate the changes in the electrophoretic patterns and the increase in ESR which occurred in all pigs in Group I and pigs 6, 7 and 9 in group II but not in the other animals in the experiment. The most obvious change in the serum protein fractions was the increase in the γ-globulin fraction. At the same time the albumin fraction decreased slightly. The A/G ratio was reduced.

As can be seen from the descriptions of the composition of the intestinal flora and of the skin changes (*Månsson*, 1964) it was only for animals with parakeratosis that changes in the

serum fractions and ESR could be demonstrated. Pigs in which the composition of the intestinal flora was the same but which received a zinc supplement, i. e. pigs 1, 2 and 4 in group II, did not develop these changes. Nor did the pigs in group III, the group in which there was no increase in the atypical *Clostridium perfringens* in the intestinal contents.

The changes in the serum protein fractions could be demonstrated in samples taken about 10 days after the onset of the skin changes. The magnitude of these changes, particularly in the γ-globulin fraction, tallied fairly well with the severity of the disease. Pigs 292, 299 and 303 in group I were severely affected and the increase in γ-globulin was very distinct. The skin changes were mild for pigs 6, 7 and 9 in group II and the increase in γ-globulin was smaller. A similar correlation could be discerned between the ESR and the severity of the skin changes. The time when the changes in the ESR were first manifest was followed more closely than was the case for the changes in the serum protein fractions. The ESR for pigs 293 and 303 was increased by day 9 of the experiment, when these animals first developed clinical signs. From subsequent experiments it appears that the ESR can be increased 24 to 72 hours before the onset of the skin changes. The increase in the ESR, then, does not seem to be a consequence of the skin changes and the secondary infection which can occur.

It remains to find out whether the changes in the ESR and the electrophoretic patterns are the result of antigen stimulation by one or more components of the intestinal flora. This aspect will be dealt with in another report.

The good clinical effect of zinc is accompanied by normalisation of the ESR (group I) and a reduction in the increased γ-globulin fraction. It is not known whether the blood zinc levels are influenced by this treatment or whether the blood levels are depressed in pigs with parakeratosis. These points will be taken up in a separate publication.

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SUMMARY

Parakeratosis in pigs (groups I and II) is accompanied by an increase in the γ-globulin content and a reduction in the albumin content of the blood serum (table 1). The ESR also rises (table 2). These changes did not occur in pigs which received an oral zinc supplement (group II) or in pigs (group III) fed Peruvian sardine meal instead of Icelandic codmeal.

Haemoglobin levels were unaffected by diet or the presence of skin changes.

ZUSAMMENFASSUNG

Die Darmflora bei Schweinen mit Parakeratose.

II. Elektroforetische Untersuchungen über Blutserum, Bestimmung der Blutsenkungsreaktion (ESR) und dem Hämoglobinhalt.

Bei Schweinen mit Parakeratose (Gruppe I und II) lag eine Steigerung von dem γ-Globulingehalt und eine Abnahme von dem Albumingehalt im Blutserum vor, Tabelle 1. Ausserdem wurden erhöhte ESR-Werte nachgewiesen, Tabelle 2. Derartige Veränderungen konnten nicht bei Schweinen festgestellt werden, die einen extra Zuschuss von Zink im Futter erhalten haben (Gruppe II) oder bei Schweinen wo isländisches Dorschmehl durch peruanisches Sardinenmehl ersetzt wurde (Gruppe III). Der Hämoglobingehalt war bei sämtlichen Tieren gleich, abgesehen von der Diet und evt. vorhandenen Hautveränderungen.

SAMMANFATTNING

Tarmfloran hos grisar med parakeratos.

II. Elektroforetiska undersökningar över blodserum, bestämning av sänkningsreaktionen (SR) och hemoglobinhalten.

Hos grisar med parakeratos (grupp I och II) förelåg en ökning av γ-globulinhalten och en viss minskning av albuminhalten i blodserum, tabell 1. Dessutom påvisades förhöjda SR-värden, tabell 2. Dylika förändringar kunde ej iakttagas hos grisar, som erhållit extra tillskott av zink till fodret (grupp II) eller hos grisar där isländskt torskmjöl ersatts av peruanskt sardinmjöl (grupp III). Hemoglobinhalten var lika hos samtliga djur oavsett foderstat och ev. förefintliga hudförändringar.

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