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PORCINE SALMONELLOSIS

III. PRODUCTION OF FIBRINOUS COLITIS BY INTRAVENOUS INJECTIONS OF A MIXTURE OF VIABLE CELLS OF SALMONELLA CHOLERAE-SUIS AND DISINTEGRATED CELLS OF THE SAME AGENT, OR HEMOLYTIC ESCHERICHIA COLI

By

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Fibrinous or pseudomembranous colitis is a characteristic feature in prolonged porcine infections caused by Salmonella cholerae-suis, but this enteric lesion is not present in the acute septicemic infections caused by this agent (*Jubb & Kennedy* 1963, *Nordstoga* 1970). In association with these intestinal changes extensive thrombosis commonly occurs in intestinal vessels. As disseminated intravascular coagulation is an essential feature in the generalized Shwartzman reaction (GSR), the possibility existed that this vessel damage might be part of the GSR, and that the mucosal lesions in the large intestine were secondary to the thrombosis. However, capillary thrombosis in the colonic mucosa was not observed in three pigs which were given intravenous injections of disintegrated cells of S. cholerae-suis (*Nordstoga & Fjölstad* 1970), although one of these animals developed massive bilateral renal cortical necrosis (BCN). Therefore, the hypothesis was conceived that colonic involvement might be achieved when pigs experimentally infected with living bacteria were given an additional supply of endotoxin.

MATERIALS AND METHODS

The strain of S. cholerae-suis used in this experiment was the same as that used in previous experiments (*Nordstoga* 1970, *Nordstoga & Fjölstad* 1970).

Group A

The bacterial suspension was prepared by the same procedure and diluted to the same concentration as previously described (*Nordstoga & Fjölstad*). The first administration to the pigs was performed on the day after the disintegration, at which time the suspension contained, in addition to the disintegrated cells, 48×10^6 living bacteria/ml (the number of living organisms was determined by plate counts carried out on the surface of blood agar). Four pigs (1—4) each weighing approximately 15 kg, received 3 ml of suspension intravenously. Pig 2 was killed with an intravenous injection of mebumal two days later, at which time pigs 3 and 4 got another intravenous injection of the suspension (4 ml), also containing living bacteria.

Group B

The strain of hemolytic Escherichia coli type O141 a b used in this experiment was isolated from pig intestine. The bacteria were inoculated in 37 flasks containing 100 ml solidified 2 % nutrient agar, to which 50 ml saline were added. Incubation was carried out in a rotary shaker (150 rev./min.) at 30°C for 72 hrs. The liquid contents of the flasks were centrifuged ($10{,}000 \times$ g for 20 min.), the sedimented cells washed twice in saline and resuspended in approx. 30 ml. Samples of 1 ml and 2 ml of this suspension were evaporated and dried at 120°C until constant weights were obtained. The average weight of these two samples was 0.08022 g dry substance per ml suspension. Twenty-five ml of the suspension were frozen at —20°C in an X-Press chamber (AB BIOX, Nacka 2, Sweden) and the cells disintegrated by pressing the frozen material three times through a hole having a diameter of 2.5 mm. The suspension was diluted with saline to 70 ml (thus containing 0.02865 g of dried E. coli per ml) and merthiolate and phenol added to a final concentration of 1:10,000 and 0.5 %, respectively, and then stored at 4°C. After two weeks, living bacteria could not be demonstrated in the suspension.

Four pigs (1—4) of the same age as those in group A were given 1 ml of a saline suspension containing approx. $3{,}000 \times 10^6$

living Salmonella bacteria per ml. Simultaneously, pigs 1 and 2 received 5 ml of the suspension of disintegrated cells of hemolytic E. coli, while pigs 3 and 4 got 4 and 3 ml, respectively, of the same material*.

Pieces of tissues were fixed in 10 % neutral formalin, embedded in paraffin, sections cut at about 5 µ and stained with hematoxylin and eosin (HE), methylene blue (MB), phosphotungstic acid hematoxylin (PTAH) and Lendrum's acid picro-Mallory method for demonstration of platelets and fibrin.

RESULTS

Group A

All animals developed severe dyspnea and vomiting within half an hour. Pig 1 went into shock after one hour, had strongly forced respiration and the ears gradually became thickened and cyanotic. The cyanosis extended soon to the whole body; the pig had frequent, thin and bloody rectal discharge and died approximately 24 hrs. after inoculation. Two days after inoculation the remaining two pigs developed thickened and cyanotic ears, but their general condition was comparatively good. After the second infusion both animals gradually became weaker and some hours later they went into shock while the cyanosis progressed to the whole body. The animals died two days later.

Group B

All animals showed slight transient cyanosis, dyspnea and vomiting immediately after the infusions. Successively all animals went into severe shock. Pig 1 showed an intense cyanosis in the perineal region and on the distal part of the extremities, while the ears were only slightly cyanotic. The remaining pigs had widespread cyanosis. The pigs died after approx. 10 hrs. (pig 1), 80 hrs. (pig 2), 40 hrs. (pig 3) and 60 hrs. (pig 4).

Gross Lesions

External changes are already mentioned.

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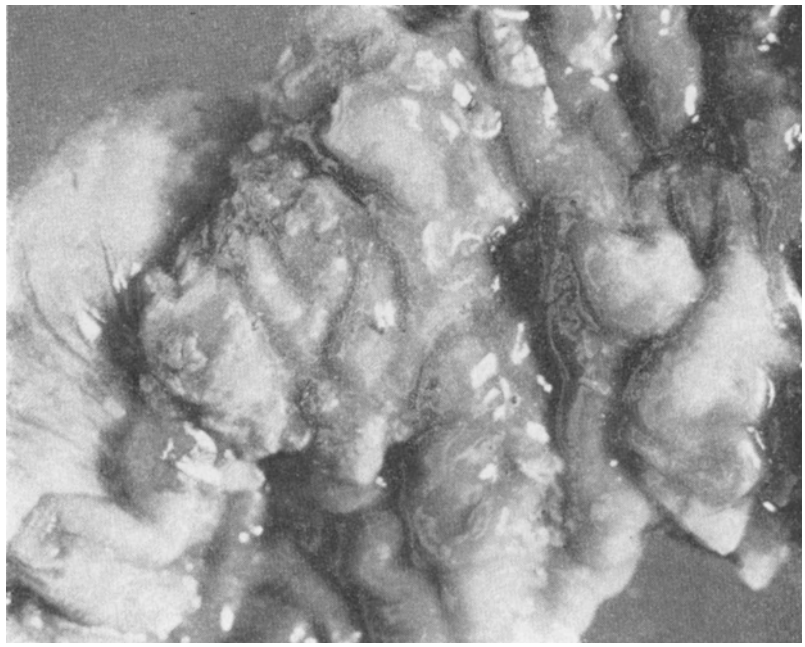
* Three additional pigs were given the same intravenous dose of living Salmonella bacteria, but the intravenous challenge of crushed E. coli was delayed until severe cyanosis appeared. These animals were highly susceptible to endotoxin, and died without showing any macroscopic evidence of gastrointestinal lesions. These animals were therefore excluded from the experiment.

Group A

Pigs 1, 3 and 4. In the fundic area of the stomach the mucosa was hyperemic and covered with a film of tenacious and blood-stained mucus. The colonic mucosa was hyperemic and partly hemorrhagic, and fibrinous membranes occurred in scattered areas in pigs 1 and 4 (Fig. 1). The mesenteric lymph nodes were



F i g u r e 1. Fibrinous lesions in the colonic mucosa. Case 1, group A.

moderately enlarged and hyperemic; the liver and spleen were also congested, the spleen being distinctly enlarged. Pigs 3 and 4 had congested and slightly enlarged kidneys. All animals had congested and edematous lungs and somewhat dilated hearts.

Pig 2. This animal did not show any macroscopic visceral lesions.

Group B

Pig 1. In the fundic region of the stomach the mucosa was hemorrhagic and covered with pseudomembranes. The serosal surface of the caudal part of colon was somewhat hemorrhagic and was partly covered with wisps of fibrin; the descending

colon had a bloody content and an intensely hyperemic or almost hemorrhagic mucosa. In minor scattered areas of the colonic mucosa unattached bloody fibrinous membranes were found. The spleen was moderately enlarged. The body cavities contained large amounts of serous fluid, and in the mesentery of the spiral colon a distinct and partly hemorrhagic edema was present. Scattered bloody areas appeared in the pulmonary tissue.

Pig 2. The mucosal and serosal surfaces of the small intestine were hyperemic, and the jejunal contents were scanty and mucous. The kidneys were congested, but of normal size; the spleen was slightly enlarged. A small amount of serous fluid was found in the body cavities.

Pig 3. In the fundic area the gastric mucosa was hemorrhagic and partly covered by pseudomembranes. Colon descendens had hyperemic mucosal membrane, and its contents were bloody and mucous. There was general hyperemia and slight enlargement of the lymph nodes of the colon. The kidneys were distinctly enlarged and congested, with a dark bluish discolouration, they were extremely friable and appeared heavily degenerated on their cut surface, although total cortical necrosis was not present. The spleen was distinctly enlarged. Serous effusions occurred in the body cavities.

Pig 4. This animal showed similar macroscopic alterations to pig 3 except for the gastric and colonic lesions which were not as pronounced as in case 3.

Microscopic Lesions

Group A

Pigs 1 and 3 had hemorrhagic necrosis of the gastric and colonic mucosa, and fibrin thrombi occurred in the capillaries of the lamina propria (Fig. 2). In pig 1 fibrinous membranes covered the necrotic epithelium of the large intestine (Fig. 3) and, in some areas, an incipient inflammatory response that tended to demarcate the pseudomembranes was observed. Pig 3 also revealed minor fibrinous membranes covering the colonic mucosa, and fibrin capillary thrombi. All animals had thrombosed vessels in the skin of the ear. In the smallest vessels the thrombi consisted merely of fibrin, while the greater thrombi also contained platelets, polymorphonuclear leucocytes and bacteria. In pigs 1, 3 and 4 similar thrombi were also observed in

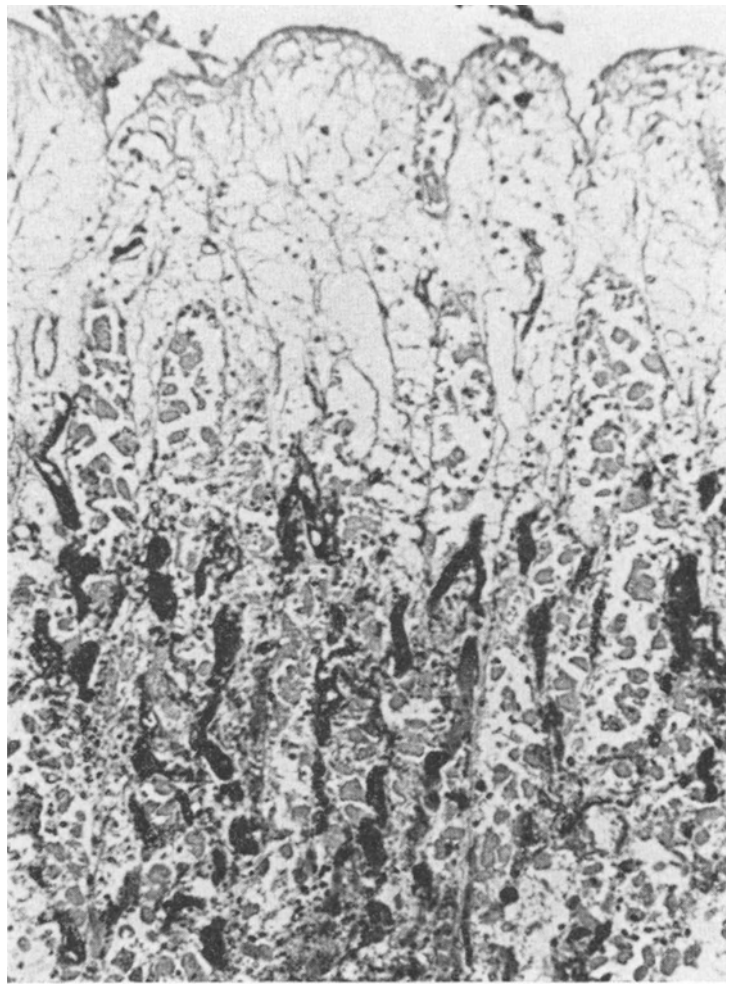


Figure 2. Necrotic gastric mucosa with numerous fibrin thrombi in the capillaries. Case 1, group A. PTAH, × 50.

skin sections from other areas. Pigs 3 and 4 exhibited degenerative changes in the tubular epithelium of the renal cortex, and in the interstitium edema and hemorrhages were noted. Thrombosis, with fibrin thrombi, occurred in interlobular arteries, afferent arterioles and glomerular capillaries. In cases 3 and 4 fibrin thrombi were noted in the adrenal capsule. In pigs 3 and 4 pulmonary interalveolar septa were thickened and had increased cellularity, and in some areas there were blood and edematous fluid in the alveoli; some alveoli contained also fibrin.

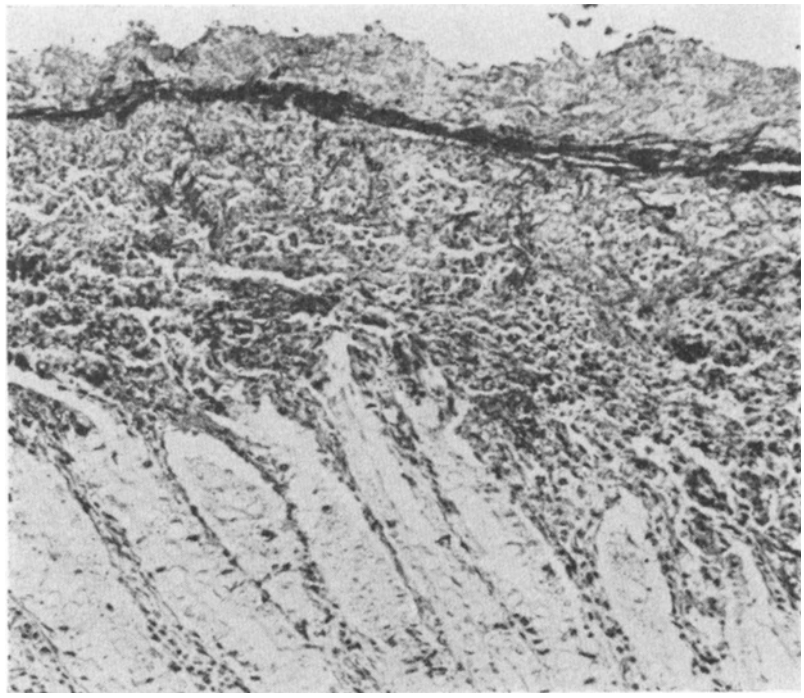


Figure 3. Necrotic colonic mucosa covered by pseudomembrane; the membrane is partly stained as fibrin. Case 1, group A. PTAH, × 50.

Fibrin thrombi were seen in septal capillaries, and in pig 4 mixed thrombi were demonstrated in some larger lung vessels. Sections from the brain of pigs 1, 3 and 4 showed congestion, and occasionally, also thrombosis.

Group B

In cases 1 and 3, hemorrhages and necroses together with extensive capillary thrombosis were found in the gastric mucosa; in case 1 similar changes were also present in the colonic mucosa. Incipient thrombosis was noticed in case 1 in capillary loops of renal glomeruli, while renal sections from pigs 2, 3 and 4 revealed severe glomerular thrombosis and lesions also in afferent arterioles and interlobular arteries, including fibrinoid necrosis of the vascular walls and the presence of fibrin thrombi. In pigs 3 and 4 the cortical parenchyma was severely degenerated with extensive interstitial hemorrhages. In pig 1 minor fibrin thrombi were recognized in skin preparations from the perineal area,

whereas the other cases showed widespread thrombosis in skin vessels, with the presence of mixed thrombi in greater vessels. In pigs 3 and 4 extensive thrombosis also occurred in the lungs.

DISCUSSION

The present experiment shows that acute hemorrhagic lesions, including necrosis of the gastric and colonic mucosa and pseudomembranes in the large intestine, may be produced in pigs by intravenous injections of disintegrated cells of S. cholerae-suis or hemolytic E. coli when viable Salmonella bacteria also are present. This is contrary to our observations in a previous experiment where only disintegrated cells of Salmonella were employed (*Nordstoga & Fjölstad* 1970). The experimental model of our investigation is the finding of *Sanarelli* (1924, 1936), who, during a study on experimental cholera, observed that guinea-pigs primarily inoculated intravenously with non-lethal doses of "cholera vibrio" and secondly with a small normally well tolerated dose of culture filtrate of "colon bacilli" died with a necropsy picture similar to that of acute cholera in humans, i.e. epithalaxia of the intestinal mucosa. According to *McKay* (1965) *Sanarelli*'s observation appears to be the first recognition of the phenomenon which is presently known as the GSR (or Sanarelli-Shwartzman reaction).

The findings in the present experiments suggest that endotoxins derived from Salmonella and hemolytic E. coli have a related effect in provoking gastrointestinal changes although the lesions in the colonic mucosa were considerably more severe in group A than in group B.

Outside the alimentary canal the findings in this experiment were nearly identical with the picture described in acute experimental Salmonella infections (*Nordstoga* 1970). It should be pointed out, however, that these cases were not accompanied by colonic lesions, nor were such changes observed in a limited material when sterile suspensions of disintegrated bacteria were used (*Nordstoga & Fjölstad*). On the other hand, intraperitoneal injection of living bacilli, which established an infection running a more prolonged course, was followed by colonic lesions characteristic of subacute or chronic spontaneous Salmonella infections ("button ulcers", *Nordstoga* 1970). The reasons for these dif-

ferent findings may possibly be that the presence of endotoxins during several days is necessary for the development of the intestinal lesions. Acute infections have probably too rapid a course, while in prolonged cases, endotoxins are gradually released from dying bacterial cells and may exert their toxic effect on the intestinal vessels during a period sufficient for the development of the typical Salmonella changes in the large intestine. In experimental situations, such as in this instance, similar, although more acute, intestinal lesions may be produced by replacing the successive release of endotoxin by application of large amounts of a corresponding exogenous material.

Under the present experimental conditions, one might probably have expected development of BCN (pigs 3 and 4 in group A). However, the two subsequent dosages of Salmonella suspension were given with a 48 hrs. interval, while the optimal interval is about 24 hrs., when GSR is brought about by endotoxins of Gram-negative bacteria. The injected quantities were also considerably smaller than in the previous experiment where GSR, including BCN, was provoked (*Nordstoga & Fjölstad*). Furthermore, great individual variations probably exist in the susceptibility to the GSR. Therefore, in limited investigations such as in these instances, one cannot expect quite corresponding results in two or more experiments.

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SUMMARY

Hemorrhagic necrosis of the gastric and colonic mucosa with capillary thrombosis were induced in pigs experimentally infected with Salmonella cholerae-suis when an additional intravenous supply of disintegrated cells of Salmonella cholerae suis or hemolytic Escherichia coli was administered. Except for the gastrointestinal lesions, the necropsy findings were almost identical with the picture found in acute septicemic salmonellosis. The gastrointestinal changes were interpreted as a special gastroenteric effect of endotoxins of Gram-negative bacteria during the development of septicemic salmonellosis.

SAMMENDRAG

Salmonellose hos gris.

III. Fibrinøs kolitt framkalt ved intravenøs injeksjon av en blanding av levende Salmonella cholerae-suis bakterier og knuste celler av samme mikrobe eller hemolytisk Escherichia coli.

Hemorrhagisk nekrose med trombosering i slimhinnen i ventrikkelen og i tykktarmen ble fremkalt hos gris eksperimentelt infisert med Salmonella cholerae-suis når det i tillegg ble gitt knuste Salmonella cholerae-suis eller hemolytiske Escherichia coli bakterier intravenøst. Utenfor fordøyelseskanalen var forandringene nesten identiske med seksjonsbildet ved akutte septikemiske tilfeller av salmonellose. Forandringene i fordøyelseskanalen ble oppfattet som en spesiell gastro-enterogen effekt av endotoksiner fra Gram-negative bakterier, under utviklingen av septikemisk salmonellose.

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