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PORCINE SALMONELLOSIS

I. GROSS AND MICROSCOPIC CHANGES IN EXPERIMENTALLY INFECTED ANIMALS

By

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Salmonella cholerae-suis was once regarded as the causal agent in swine fever (*Salmon & Smith* 1886, *Salmon* 1886, 1887). When the viral origin of swine fever was elucidated some years later (*de Schweinitz & Dorset* 1903, *Dorset et al.* 1905), this view had to be abandoned, but attention is still called to the similarities these two diseases may show in their gross pathological features. Furthermore, swine fever is frequently complicated by bacterial infections, one of the more common bacteria being S. cholerae-suis, and thus the morbid picture in this condition may also be dominated by lesions produced by this organism. *Jubb & Kennedy* (1963) give a comprehensive description of the pathological lesions in porcine salmonellosis in their textbook of pathology, and recently, *Lawson & Dow* (1966) described the clinical, bacteriological and pathological findings, based on field cases. The observations of the latter authors included vascular lesions indicative of the generalized Shwartzman reaction (GSR). Similar vessel changes have also been recognized by the present author in association with acute field cases of swine salmonellosis (*Nordstoga* 1968). In the present report, the pathological changes in experimental porcine infections are briefly described. Special attention is drawn to the vascular lesions, and it is discussed to what extent these alterations are compatible with GSR.

MATERIALS AND METHODS

The strain of S. cholerae-suis used in the experiment was isolated from a septicemic porcine case* (no. 176/68), grown in

* The strain was identified by Dr. Finn Kristiansen, National Veterinary Institute, Oslo.

broth for 24 hrs. and washed in saline. A saline suspension, which according to McFarland's scale contained approx. 3,000 $\times$ 10^6 bacteria/ml, was injected intravenously into four pigs. Each of two pigs, weighing about 60 kg, was injected with 3 ml (cases 1 and 2), while two other animals, which weighed approximately 15 kg, received 2 ml each (cases 3 and 4). Two additional pigs of the same age as the latter two animals received 4 ml of the same suspension intraperitoneally (cases 5 and 6). Fourteen days later pigs 5 and 6 were killed with an intravenous injection of mebumal (phenobarbitone). Pieces of tissue 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.

RESULTS

All pigs that received the bacterial suspension intravenously developed severe dyspnea immediately after the infusion, and an intense purplish to bluish discolouration of the skin appeared in pigs 1, 2 and 4. Simultaneously, the animals went into severe shock. The cyanosis was primarily visible on the ears, which also became swollen, but it extended in a few hrs. to cover the whole body, though the intensity was somewhat unequal, the ventral abdomen being less involved. The illness developed most quickly in pig 3, which died 8 hrs. after the injection. Pig 4 died after 26 hrs., and pigs 1 and 2 after two days. From the fourth day of experimentation pig 5 revealed poor appetite and, periodically, the ears were cyanotic and thickened. This pig gradually became emaciated. Pig 6, too, partly lost its appetite, but its general condition was comparatively good, though it gradually became thinner.

Gross lesions

Pigs 1 and 2. These animals had nearly identical necropsy pictures. As already mentioned, both pigs were extremely cyanotic, and in some skin areas, especially on the ears, the tail and in the perineal region slight focal necrotic changes or diffuse incipient dry gangrene were present. The lungs were edematous, with subpleural petechial hemorrhages. A small amount of serous fluid was found in the pericardium. Scattered petechial hemor-

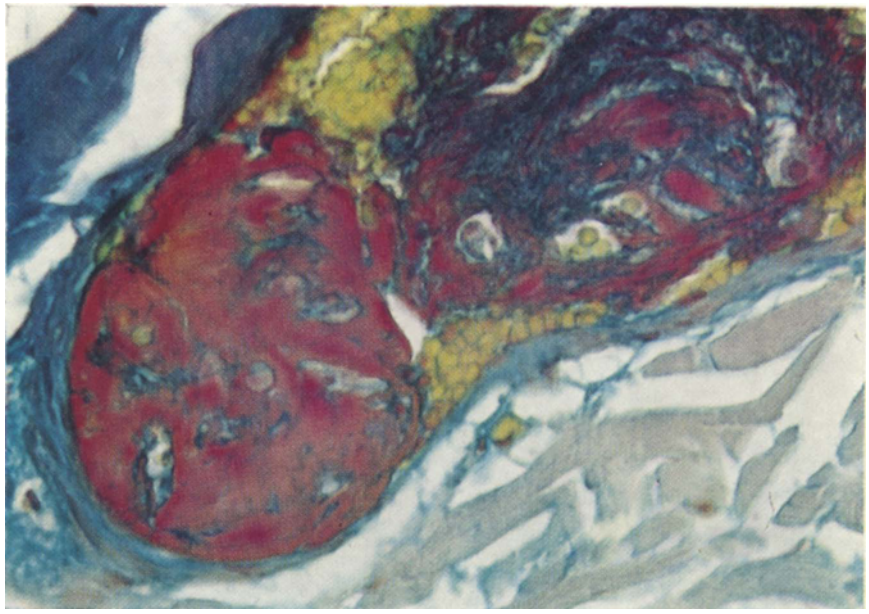

Figure 1. Mixed thrombus in skin vessel containing fibrin (red) and platelets (blue). Erythrocytes are stained orange. Case 2. Lendrum's acid picro-Mallory method, × c. 250.

rhages were noticed on the serosal surface of the jejunum, elsewhere the digestive tract appeared unchanged. The kidneys were considerably enlarged and congested, especially the papilla were intensely hyperemic. The lymph nodes were congested, the renal lymph nodes also considerably enlarged, and the spleen, too, was congested and enlarged.

Pig 3. This animal showed a slight cyanosis on the distal part of the extremities. The lungs were edematous and congested, while the other organs appeared unaffected.

Pig 4. This animal was cyanotic on the ears, the extremities and the croup. The pig was dehydrated; the jejunum was congested, with a distinct vascular pattern and had some dilated areas. Its contents were loose, almost watery. The kidneys appeared unchanged.

Pig 5. This animal was emaciated. The abdominal cavity contained a yellow, serous fluid. In scattered areas of the serosal surface of colon, firmly attached fibrinous flakes were found and the mucosa was in some areas covered with loosely fibrinous membranes. The mesenteric lymph nodes were considerably enlarged.

Pig 6. This animal had numerous circumscribed colonic lesions which were prominating on both the serosal and the mucosal surface. Ulcerations occurred in some of these nodules. Outside these focal lesions the colonic mucosa was rough and thickened, and the lymph nodes of the colon were considerably enlarged.

Microscopic lesions

Pigs 1 and 2. There was great resemblance between cases 1 and 2. Sections from various skin areas revealed extensive thrombosis. The thrombi in the capillaries and venules were acellular and consisted, apparently, merely of fibrin, whereas the thrombi in the greater vessels also contained platelets, leucocytes, erythrocytes and sometimes also rods (Fig. 1).

Hepatic sections revealed acute congestion and distinct leucostasis, minor hemorrhages, dissociation of the hepatic cells and scattered cellular necrosis. In the kidneys, there was severe parenchymal degeneration in the cortical layer, and in some areas, regular necrosis of the tubular epithelium. Widespread cast formation occurred in both the cortical zone and in the

medulla. The casts were predominantly homogenous and eosinophilic and had partly tinctorial properties of fibrin. Hemorrhages and edema occurred in the interstitium. Severe vascular lesions with fibrinoid necrosis of the vessel walls and fibrin thrombi within the lumina, were present in the interlobular arteries, the precapillary arterioles and the glomerular capillary loops (Fig. 2). The pulmonary septa appeared edematous and thickened with infiltrations of mononuclear cells. In some areas the alveoli

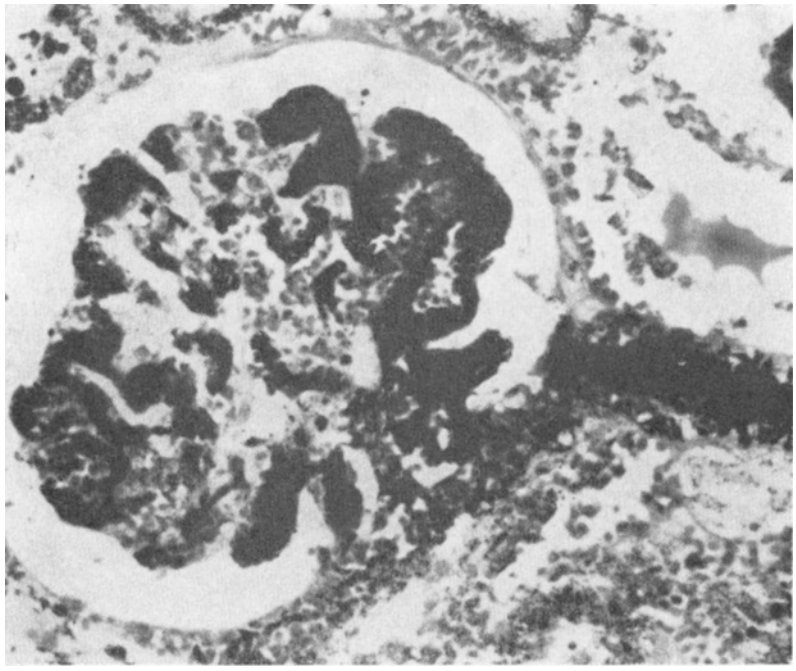

Figure 2. Fibrin deposits in glomerular capillary loops and afferent arteriole. Case 2. PTAH, × 114.

contained edema fluid and red blood cells. Septal capillaries and some greater vessels contained thrombi, the capillary thrombi were acellular and homogenous and stained as fibrin (Fig. 3), while the thrombi in the greater vessels also constantly contained leucocytes, platelets and erythrocytes, sometimes also rods (Fig. 4). In cerebral and leptomeningeal vessels, too, numerous thrombi were seen, with the same characteristics as those in the skin and lungs. Endothelial necrosis was noticed in arteries as well as in veins. In the lymph nodes hyperemia and frequently

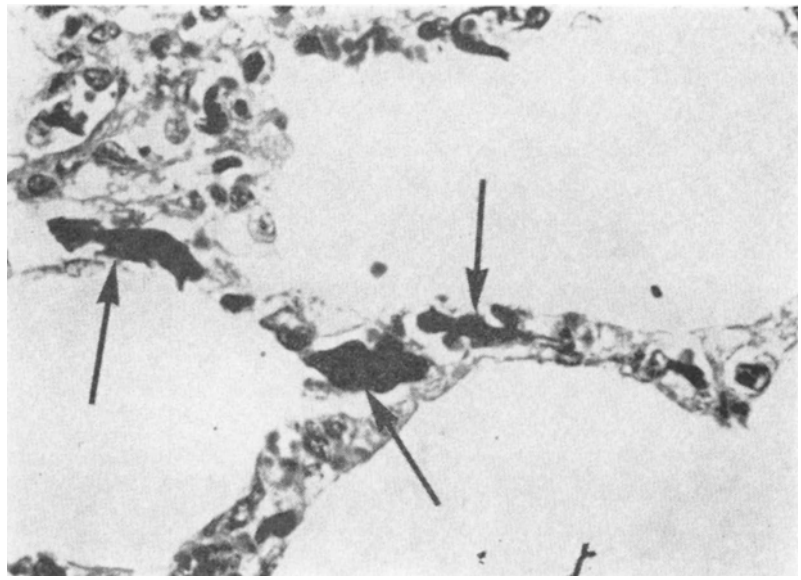

Figure 3. Lung. Fibrin thrombi within septal capillaries (arrows). Case 1. PTAH, × 114.

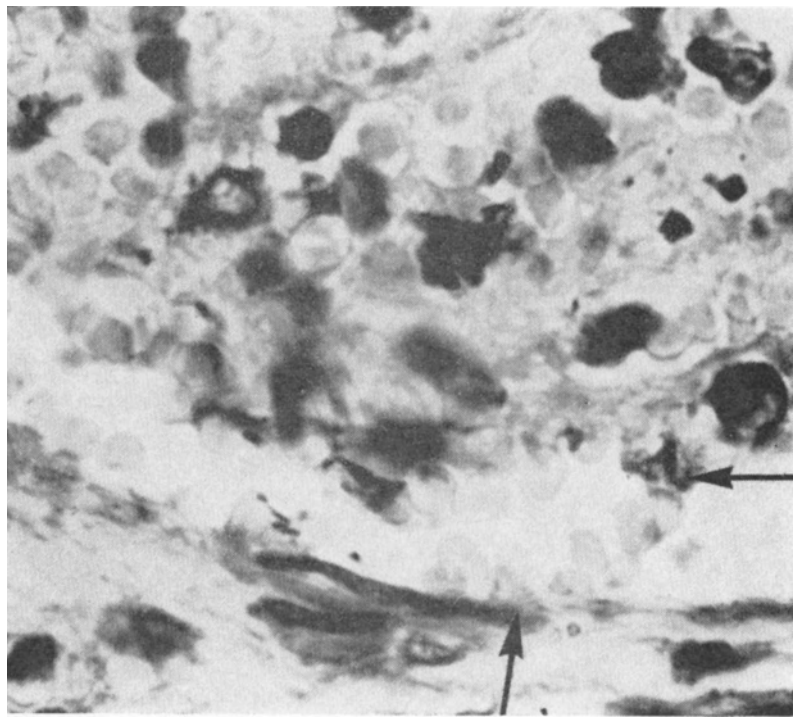

Figure 4. Lung. Mixed thrombus. Leucocytes and clusters of rods (upper arrow) are included in the thrombus. Lower arrow indicates endothelium. Case 1. MB, × 470.

hemorrhages were found, the changes being always most pronounced in the marginal zone where edema and incipient necrosis were also occasionally recognized. These latter changes were most pronounced in the renal lymph nodes. In the heart muscle and the jejunal submucosa fibrin thrombi were observed.

Pig 3. This pig had acute congestion of the viscera and hepatic leucostasis. Vascular damage was not observed in the kidneys, while pulmonary sections showed thrombi similar to cases 1 and 2. Severe bleeding into the alveoli occurred, in some areas fibrin could also be demonstrated in the alveolar content. Thrombi were also present in the jejunum. A distinct leucostasis was noted in the brain, but no vascular changes occurred in this location.

Pig 4. In this animal, extensive thrombosis was found in the skin, the thrombi having characteristics similar to those in cases 1 and 2. Thrombi were also present in the brain, the submucosa of the jejunum and colon, and in the pituitary gland, while vascular modifications were not demonstrable in the kidneys.

Pig 5. Dissociation of the parenchymal cells and a moderate hyperplasia of the reticulo-endothelial elements occurred in the liver. Focal infiltrations of histiocytes were also demonstrated, together with a few minor accumulations of eosinophilic cells. Focal proliferations of histiocytes were also recognized in the renal cortex. The lymphoid tissue in the submucosa of colon showed proliferative modifications where central necrosis and infiltrations of polymorphonuclear leucocytes were prominent; some eosinophilic cells were also present, and peripherally, comparatively many histiocytes were observed. Some of the processes were ulcerated and opened into the intestinal lumen. In the lamina propria an almost diffuse infiltration of mononuclear inflammatory cells was observed.

Pig 6. This animal showed microscopic changes quite similar to pig 5, but the cellular response in the renal lesions was more evident, especially in the medulla where cellular casts occurred, predominantly consisting of polymorphonuclear cells.

DISCUSSION

The pathological changes reported in this communication are, for the major part, in accordance with the descriptions of acute Salmonella infections given by *Jubb & Kennedy* (1963) and

Lawson & Dow (1966). This experiment suggests that the widespread renal thrombosis may be developed within 48 hrs. when bacteriemia is severe. In the present experiment, the renal vascular injury was confined to glomerular thrombosis and fibrinoid necrosis with fibrin thrombi in the afferent arterioles and interlobular arteries, whereas extrarenal lesions in greater vessels included mixed thrombi. As the described vascular alterations in the kidneys are an essential part of GSR, the renal vascular damage observed in this experiment (cases 1 and 2) may be explained entirely as a result of GSR. However, other authors (*Jubb & Kennedy, Lawson & Dow*) have also reported an inflammatory response in renal lesions in association with acute porcine salmonellosis. This discrepancy obviously depends on the duration of the septicemia; if the course is somewhat prolonged, one would expect bacillary propagation and cellular response in various organs.

The extrarenal vascular changes observed in this investigation included the presence of mixed thrombi in larger vessels, indicating that these lesions may be regarded rather as septic emboli, than as part of GSR. However, enterobacteriaceae are not known to produce exotoxins, and the pathogenicity of these organisms is to a great extent dependent on their ability to elaborate endotoxins (*Jubb & Kennedy*). It seems likely, therefore, that an enhanced tendency to disseminated intravascular coagulation, which is a basic feature in GSR, may also have contributed to the extensive extrarenal thrombosis. As a possible relationship between septic emboli, which also occur in Gram-positive infections, and GSR has not been elucidated, this question will not be further discussed in this paper.

Species of the genus Salmonella are considered to have a special affinity for the intestinal tract in prolonged cases, although acute infections, without any obvious enteric involvement also occur in various species. Therefore, as the main purpose of this work was to study the extraintestinal vascular lesions accompanying acute porcine salmonellosis, the intravenous route of transmission was chosen (cases 1—4). The purpose of intraperitoneal challenge (cases 5—6) was to induce a subacute or chronic infection, with the presence of numerous Salmonella organisms in the abdominal cavity, a condition which conceivably would permit an intestinal localization of bacteria without rapid development of fatal septicemia. This aim was attained, as pro-

longed infections, with the presence of colonic changes corresponding to the typical "button ulcers" in porcine salmonellosis, and hepatic and renal lesions equivalent to the "paratyphoid nodules" seen in various organs of animals infected with Salmonella bacteria, were present (cases 5 and 6). As a conclusion it must be emphasized that the renal vascular alterations following acute experimental porcine salmonellosis are exclusively of Shwartzman type, while the extrarenal vessel lesions may be explained, only in part, as induced by the Shwartzman mechanism, and that, as expected, no evidence of GSR occurred in prolonged infections.

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SUMMARY

Renal vascular lesions associated with experimentally induced septicemic porcine salmonellosis consisted of fibrinoid necrosis of the vessel walls and fibrin thrombi within the lumina of interlobular arteries, afferent arterioles and glomerular capillary loops. Extrarenal vascular alterations were predominantly localized to the skin and the lungs. In these sites, too, mere fibrin thrombi were found in the capillaries, whereas mixed thrombi, consisting of fibrin, platelets, leucocytes and erythrocytes were present in larger vessels. The conclusion is drawn that the renal vascular injury is completely compatible to the generalized Shwartzman reaction, while the extrarenal vascular changes may only in part depend on this mechanism.

SAMMENDRAG

Salmonellose hos gris.

I. Makroskopiske og mikroskopiske forandringer hos eksperimentelt infiserte dyr.

Karskadene i nyrene i forbindelse med eksperimentell septikemisk salmonellose hos gris besto av fibrinoid nekrose av karveggene og fibrintromber i lumina i interlobular-arteriene, i de afferente arterioler og kapillarslyngene i glomeruli. Utenom nyrene ble det i første rekke funnet karforandringer i huden og i lungene. Også i disse lokalisasjonene ble det funnet rene fibrintromber i kapillærene, mens det fantes blandede tromber, som besto av fibrin, blodplater, leukocytter og erythrocytter i de større kar. Det blir konkludert med at karforandringene i nyrene i sin helhet er forenlig med en generalisert Shwartzmans reaksjon, mens de ekstrarenale karforandringene bare delvis kan bero på denne mekanisme.

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