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# PORCINE SALMONELLOSIS

## II. PRODUCTION OF THE GENERALIZED SHWARTZMAN REACTION BY INTRAVENOUS INJECTIONS OF DISINTEGRATED CELLS OF SALMONELLA CHOLERAE-SUIS

By

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Vascular changes compatible to the vascular lesions accompanying the generalized Shwartzman reaction (GSR) occur relatively often in pigs in association with acute septicemic infections caused by Salmonella cholerae-suis (*Lawson & Dow* 1964, 1966, *Nordstoga* 1968, *Nordstoga* 1970), and GSR, including bilateral cortical necrosis (BCN) has also been induced in rabbits by intravenous injections of endotoxin derived from this microbe (*Lawson & Dow* 1964). The present experiment shows that BCN can also be provoked in pigs in a similar way.

## MATERIAL AND METHODS

The Salmonella strain used in this experiment was the same as that used in a previous experiment (*Nordstoga* 1970). The bacteria were inoculated in 12 flasks containing 100 ml 3 % nutrient agar and 50 ml saline. Incubation was carried out in a shaker (150 rev./min.) at 30°C for 24 hrs. The contents of the flasks were centrifuged (10,000 $\times$ g for 20 min.), the sedimented bacteria washed twice in saline and resuspended in saline to a volume of 31 ml. By the plate count method carried out on the surface of blood agar the suspension was found to contain $24 \times 10^8$ living bacteria/ml. Thirty ml of this suspension were frozen at —20°C in an X-Press (AB BIOX, Nacka 2, Sweden) and the cells disintegrated by pressing them three times through a hole having a diameter of 2.5 mm. The suspension was diluted

in saline to 50 ml, merthiolate added to a concentration of 1:10,000 and stored at 4°C. Ten days later samples taken from the suspension were inoculated on nutrient agar and nutrient broth and incubated for 48 hrs. at 37°C. No growth occurred.

Three pigs, each weighing approximately 15 kg, received 4 ml of the suspension intravenously. Twenty-four hrs. later another dose (5 ml) was intravenously administered to all the animals, and on the following day (48 hrs. after the first infusion) pigs 1 and 2 received 10 ml of the same material intravenously. Twenty-four hrs. later the pigs were killed with intravenous injections of mebumal and necropsied immediately. 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), phosphotungstic acid hematoxylin (PTAH) and Lendrum's acid picro-Mallory method.

## RESULTS

Half an hour after the first injection all animals showed moderate dyspnea and a few hours later a slight diarrhea occurred. After the second injection pig 1 became cyanotic on the tail and on the skin adjacent to the base of the tail. All animals were in good general condition at this time. After the third infusion the cyanosis on the tail of pig 1 increased in intensity, and the animal revealed signs of posterior weakness.

### *Gross lesions*

*Pig 1.* This animal had considerably enlarged kidneys with hemorrhagic and completely necrotic cortices (Figs. 1—2). The cortical tissue was extremely friable and the capsule was almost entirely loosened from the external surface. Great blood extravasations occurred around the kidneys, and the renal lymph nodes were considerably enlarged and hemorrhagic. The bronchial lymph nodes, too, were large and hemorrhagic, whereas no macroscopic lesions were visible in the pulmonary tissue.

*Pig 2.* This animal had pale and somewhat enlarged kidneys and the inner part of the cortical layer appeared severely degenerated, or almost necrotic on the cut surface. This pig had also circumscribed hyperemic spots on the lungs, otherwise no macroscopic lesions occurred in any organ.

*Pig 3.* Necropsy did not reveal any gross changes in this pig.

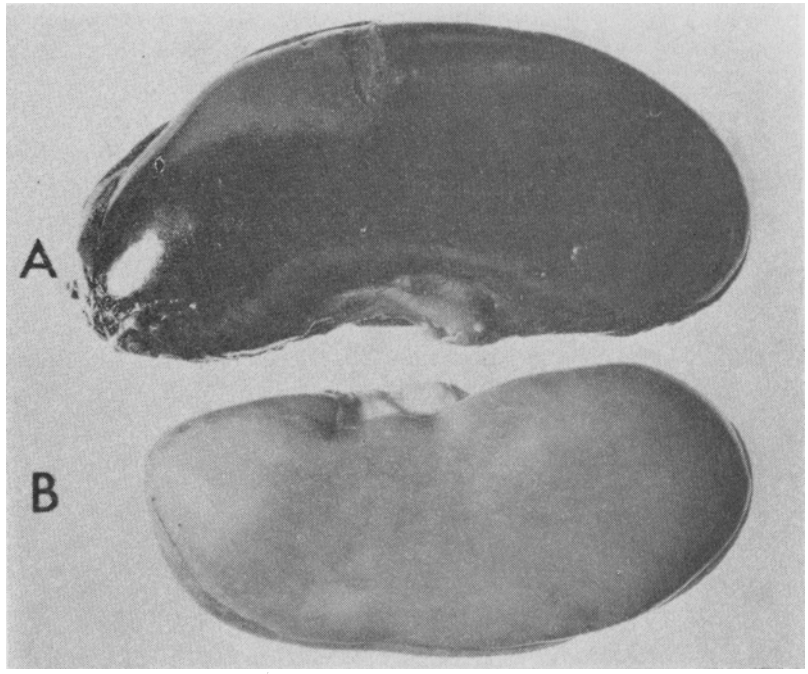


Figure 1. A: Renal cortical necrosis. Case 1. B: Kidney of normal pig of the same size.

*Microscopic lesions*

*Pig 1.* Renal sections revealed extentive parenchymal destruction with complete cortical necrosis, also partly including the juxtamedullary portion, and widespread extravasations of erythrocytes. In HE-stained sections the glomeruli were scarcely visible as pale, necrotic structures. Sections stained for fibrin exhibited fibrin thrombi in glomerular capillaries, afferent arterioles and interlobular arteries (Fig. 3). In the greater vessels the vascular damage also included fibrinoid necrosis of the vascular walls (Fig. 4). Occasionally, fibrin thrombi were also noted in intertubular capillaries. Within some areas the necrotic renal parenchyma was penetrated by a network of fibrin threads. In the juxtamedullary zone the glomeruli were somewhat less damaged, but in this location, too, fibrin thrombi occurred in the capillary loops. In relatively isolated areas considerable calcification was observed in glomeruli as well as in necrotic tubules, and cast formation was noted throughout the necrotic cortex. The casts were composed of desquamated epithelial cells, partly

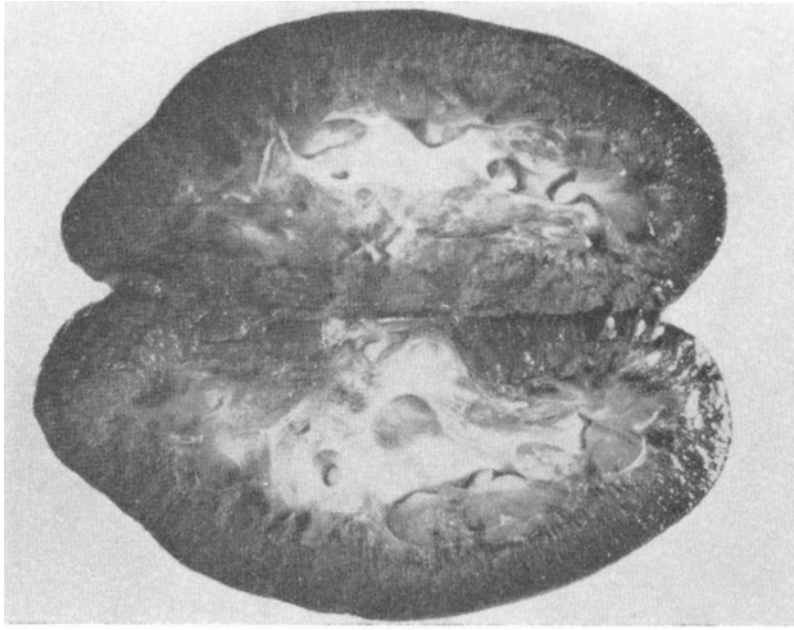

Figure 2. Cut surface of the same kidney as shown in Fig. 1 A; completely necrotic and hemorrhagic cortical layer.

destroyed red blood cells, polymorphonuclear leucocytes and fibrin. Excessive hemorrhages and incipient necrotic changes with the presence of a few inflammatory cells, including some eosinophils, were demonstrated in the renal lymph nodes. Dissociation of the parenchymal cells and slight hyperplasia of the reticulo-endothelial cells were found in hepatic sections together with minor cellular infiltrations in the periportal spaces (mononuclear cells and some eosinophils). In the lungs, the interalveolar septa appeared thickened, and, to a less extent, they were also infiltrated by large, mononuclear cells. Inside the areas where these changes were most evident hemorrhages into alveoli occurred; in some alveoli a fibrinous material and macrophages desquamated from the lining epithelium could also be demonstrated. Sections from the bronchial lymph nodes revealed changes similar to those in the renal nodes, though less pronounced. Fibrin thrombi were found in vessels from the skin of the tail (Fig. 5), in meninges of the posterior part of the spinal cord, and in the caudal spinal nerve roots.

*Pig 2.* Renal specimens revealed extensive tubular damage

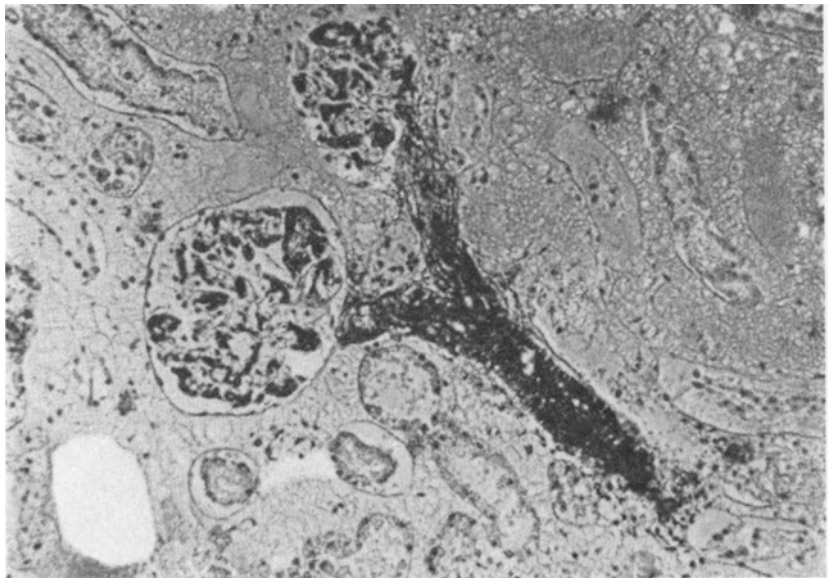

Figure 3. Kidney. Fibrin thrombi in glomerular capillary loops and afferent vessels; necrotic tubular epithelium. Case 1. PTAH, × 114.

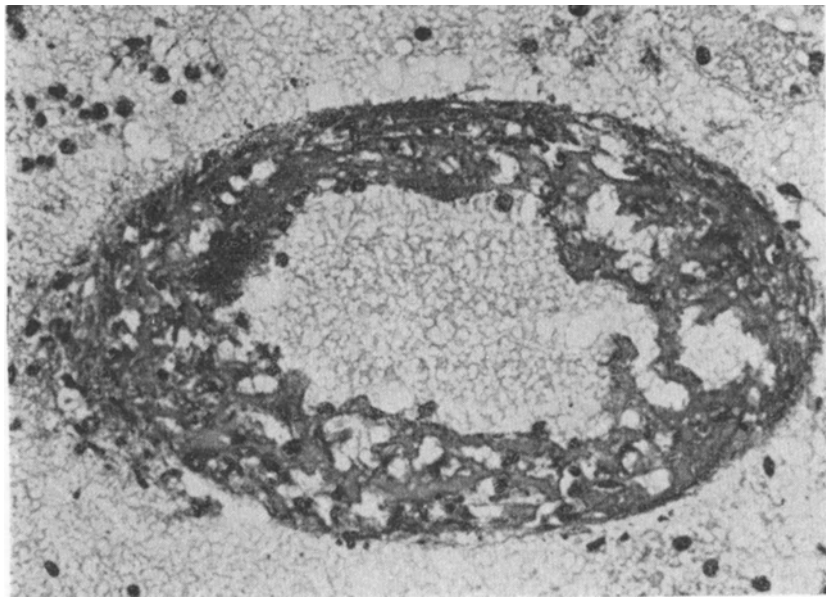

Figure 4. Interlobular renal artery with fibrinoid necrosis of the wall. Case 1. Lendrum's acid picro-Mallory method, × 114.

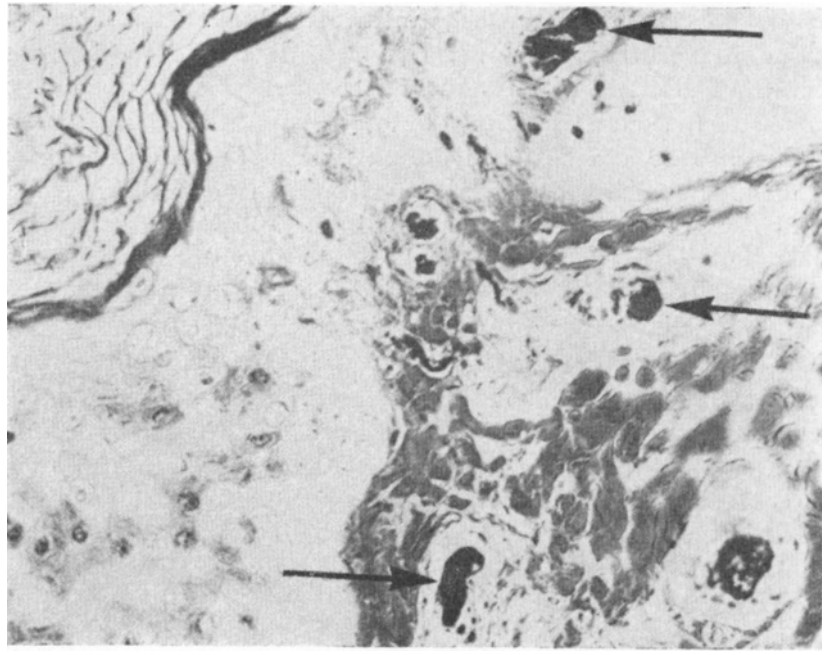

Figure 5. Skin (tail). Fibrin thrombi in vessels in corium (arrows). Case 1. PTAH, × 114.

in the inner portion of cortex where the epithelium in circumscribed areas was completely necrotic. In the same areas minor casts occurred, the casts consisting of partly decomposed red blood cells as well as of other cells with a marked preponderance of polymorphonuclear leucocytes. In the interstitium as well, there were minor cellular accumulations, consisting almost exclusively of mononuclear cells. In some degenerated tubules precipitation of calcium occurred. Glomeruli appeared normal, and thrombosed vessels were not observed in the kidneys. In the lungs, a picture was found somewhat similar to case 1, whereas preparations from other organs did not reveal any changes.

*Pig 3.* This animal did not show microscopic lesions in any organ.

## DISCUSSION

The classic changes of GSR, including BCN, which is regarded as the hallmark of GSR (*Thomas* 1959), may be produced in

rabbits by two properly spaced intravenous injections of endotoxins derived from Gram-negative organisms. The optimal interval is about 24 hrs. However, approximately 40 % of rabbits submitted to such a treatment fail to develop GSR (*McKay* 1965). It has also been demonstrated that two subsequent injections of disintegrated cells of Haemophilus parainfluenzae may elicit GSR in pigs (*Nordstoga & Fjölstad* 1967a). The present experiment shows that intravenous injections of disintegrated cells of S. cholerae-suis, too, are capable of provoking GSR in pig. Certainly, our material is scanty, and three subsequent infusions were used, but there is no reason for assuming that mechanisms other than GSR were involved. Pigs 1 and 2 tolerated remarkably great amounts of endotoxin in the third injection, without lethal effect, but it must be remembered that a small dose of endotoxin may render experimental animals resistant to lethal dosages of endotoxin in 24 hrs. (*Bertók & Berczi,* cited by *Bertók* 1968). Thus, their susceptibility may have been reduced by the previous injections. The eruption of GSR is apparently dependent on an added effect of the first ("preparatory") and the second ("provoking") dose of endotoxin, during increasing impairment of the reticulo-endothelial system, and if insufficient amounts are given, it is not surprising that a third injection can elicit GSR, provided that complete resistance has not already been acquired. It is clear from experimental studies that considerable individual differences exist as to the susceptibility to GSR, and that young rabbits are more sensitive to GSR than adult animals. We have, therefore, not made any attempt to determine the incidence of GSR in pigs, since this, in our opinion, would be almost purposeless without application of varying doses of endotoxin to a great number of experimental pigs of various ages. Furthermore, in many experimental situations, one cannot quite neglect a possible participation of "endogenous" endotoxin. The present authors have considered a similar possibility in a few porcine cases of spontaneous hemophilic infections which were accompanied by GSR, but where also hemolytic strains of Escherichia coli were recovered from the jejunum (*Nordstoga & Fjölstad* 1967b).

The severe acute renal injury observed in case 2 cannot definitively be interpreted as caused by endotoxin, but it seems possible that these changes, too, were related to endotoxin action since such lesions are otherwise rare in pigs, and since endo-

toxins are known to produce vasomotoric alterations (*Gilbert* 1960, *Alican* 1962, *Wachtel & Lyhs* 1968) which in turn, may possibly lead to ischemic lesions in various organs, in the absence of GSR.

Interstitial pneumonia is a characteristic feature in porcine salmonellosis, a lesion which is attributed to bacillar damage on the alveolar vessels (*Jubb & Kennedy* 1963). Our findings in cases 1 and 2 may possibly indicate that endotoxin released from S. cholerae-suis may also exert a toxic effect upon pulmonary tissue. We have, however, too few observations to permit any conclusions at this point as similar lesions may also be provoked by viral agents widely distributed in swine herds.

Although species differences in the response to endotoxins obviously exist, endotoxins released from Gram-negative agents are, regardless of their origin, considered to have a nearly uniform, nonspecific effect in experimental situations (*Bennett* 1964). The results obtained in case 1 in the present experiment are similar to the findings in porcine cases of GSR, induced by intravenous injections of disintegrated cells of Haemophilus parainfluenzae (*Nordstoga & Fjölstad* 1967a), and in both experiments fibrin thrombi were demonstrated in cyanotic skin areas. However, the distribution of the cyanotic areas was somewhat different in these two experiments, since no cyanosis appeared on the ears in the present case, whereas the most severe cyanosis was present in this localization when endotoxin from hemophilic organisms was used. Although the material is scanty, there seem to be reasons for assuming that a difference exists in the mechanism involved as to the localization of fibrin thrombi in skin vessels when endotoxins from those two bacteria are implicated. Furthermore, it is obvious that different mechanisms are involved in the development of vascular lesions in animals infected with these two agents, as the vascular changes occurring in porcine hemophilic infections were entirely reproducible by the Shwartzman mechanism, while the lesions in skin vessels in the present experiment were minimal as compared with those accompanying Salmonella infections where the thrombi not only consist of fibrin, but also of platelets and polymorphonuclear leucocytes (*Jubb & Kennedy, Nordstoga* 1970). For these mixed thrombi to be formed, the presence of viable bacteria is most probably necessary. However, in the early phase of experimental Salmonella infection (*Nordstoga* 1970),

and prior to the development of the generalized cyanosis, the ears of the experimental pigs were cyanotic and thickened, and in this phase of experimentation there was great resemblance between these pigs and those which developed GSR after intravenous administration of disintegrated hemophilic cells (*Nordstoga & Fjölstad* 1967a). At present, however, sufficient evidence is not available to explain these phenomena.

Intestinal changes are characteristic findings in prolonged cases of S. cholerae-suis infection (*Jubb & Kennedy, Lawson & Dow* 1966), but such lesions were not found in the present experiment. This question will be discussed in more detail in a subsequent paper (*Nordstoga & Fjölstad* 1970).

## REFERENCES

*Alican, I.:* Pathophysiology of endotoxin shock. Clinical observations and experimental studies. Amer. J. med. Sci. 1962, *244*, 237—257.

*Bennett jr., I. L.:* Approaches to the mechanisms of endotoxin action. *In* Bacterial Endotoxin. Proceedings of a symposium, Rutgers University. Quinn & Boden Co., Rahway, New Jersey 1964.

*Bertók, L.:* Effect of endotoxin tolerance on the lead acetate-induced endotoxin hypersensivity of rats. J. Bact. 1968, *96*, 569.

*Gilbert, R. P.:* Mechanisms of the hemodynamic effect of endotoxin. Physiol. Rev. 1960, *40*, 245—279.

*Jubb, K. V. F. & P. C. Kennedy:* Pathology of Domestic Animals. Academic Press, New York 1963, vol. 2, 109—113.

*Lawson, G. K. H. & C. Dow:* Production of the generalized Shwartzman reaction with Salmonella cholerae-suis. J. comp. Path. 1964, *74*, 482—486.

*Lawson, G. K. H. & C. Dow:* Porcine salmonellosis. A study of the field disease. J. comp. Path. 1966, *76*, 363—371.

*McKay, D. G.:* Disseminated Intravascular Coagulation. An Intermediary Mechanism of Disease. Harper Row, New York, Evanston & London 1965.

*Nordstoga, K.:* Unpublished observation 1968.

*Nordstoga, K.:* Porcine salmonellosis. I. Gross and microscopic changes in experimentally infected animals. Acta vet. scand. 1970, *11*, 361—369.

*Nordstoga, K. & M. Fjölstad:* The generalized Shwartzman reaction and Haemophilus infections in pigs. Path. vet. 1967a, *4*, 245—253.

*Nordstoga, K. & M. Fjölstad:* Unpublished observation 1967b.

*Nordstoga, K. & M. Fjölstad:* 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. Acta vet. scand. 1970, *11*, 380—389.

*Thomas, L.:* The Shwartzman phenomenon and other reactions produced by the endotoxins of Gram negative bacteria. Immunopathology. 1st International Symposium. ed. P. Grabar & P. Miescher. Benno Schwabe, Basel-Stuttgart 1959, 325—338.

*Wachtel, W. & L. Lyhs:* Die Wirkung hoher Endotoxindosen von E. coli auf den Blutkreislauf von Läuferschweinen. (The effect of high doses of E. coli endotoxin on the blood circulation of store pigs). Arch. exp. Vet.-Med. 1968, *22*, 639—646.

SUMMARY

Three pigs were given intravenous injections of disintegrated cells of Salmonella cholerae-suis. One of these pigs developed macroscopic bilateral renal cortical necrosis. Histologic examination revealed, besides total cortical parenchymal necrosis, also severe vascular injury, characterized with fibrinoid necrosis of the vessel walls and fibrin thrombi within their lumina. These findings were interpreted as a result of a generalized Shwartzman reaction, provoked by the action of bacterial endotoxin.

SAMMENDRAG

*Salmonellose hos gris.*

*II. Framkallelse av generalisert Shwartzmans reaksjon ved intravenøse injeksjoner av knuste celler av Salmonella cholerae-suis.*

Tre griser ble gitt knuste Salmonella cholerae-suis bakterier intravenøst. En av disse grisene utviklet makroskopisk bilateral nyrebarknekrose. Histologisk undersøkelse viste, foruten total nekrose av nyrebarken, også utbredt karskade karakterisert ved fibrinoid nekrose av karveggene og fibrintromber i lumina. Disse funn ble tolket som resultat av en generalisert Shwartzmans reaksjon, utløst gjennom virkningen av bakterielle endotoksiner.

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