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Extensive Hepatic Cell Necrosis Produced by the Shwartzman Mechanism

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Summary. Acute, severe, and extensive necrosis of the liver was produced in pregnant and non-pregnant female adult rabbits by the Shwartzman mechanism. Shwartzman reagent (*E. coli* endotoxin) was administered in various combinations by three routes of injection, the portal vein (mesenteric vein), the bile duct, and the ear vein. Morphologic changes of the extrahepatic organs were minimal. The similarity to massive necrosis in human liver and the effect of pregnancy on hepatic necrosis in rabbit and man were discussed. The lesion is presented as a new animal model for acute massive hepatic necrosis and is proposed as a third category of Shwartzman reaction, designated the univisceral type.

Key words: Hepatic necrosis – Fulminant hepatitis – Shwartzman mechanism – Endotoxin – Pregnancy.

Introduction

One of the remaining problems in hepatic pathology is the pathogenesis of massive necrosis of the liver, which is often fatal. The lack of success following various treatments is due to lack of knowledge of the pathogenesis of the disease which can, in part, be attributed to the lack of a good experimental model in animals.

In a study of human autopsy material, some morphological similarities in hepatic necrosis and in tissue changes induced by the Shwartzman reaction was noted by one of us (W.M.) after modified forms of the Shwartzman reaction in rabbits had caused massive hepatic necrosis in preliminary experiments (Mori,

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1972). For further clarification of the lesion including its organ specificity, the investigation was extended to increase the variation in administration routes of the Shwartzman reagent, in both pregnant and non-pregnant rabbits.

The present experiment was designed in view of the facts that massive hepatic necrosis in man has a relatively high incidence in pregnancy and that the role of endotoxins in liver disease is now clear (Nolan, 1975).

Materials and Methods

Adult female albino rabbits weighing approximately 3 kg were used. Out of 64 rabbits, 23 were pregnant. *E. coli* endotoxin (DIFCO Lab., 011:B4) dissolved in saline (5 mg/ml) served as the original solution of the Shwartzman reagent. It was diluted to adjust the amount of injected solution (5 ml) to contain an appropriate dose of endotoxin for three routes of administration; 0.005–0.05 mg for the bile duct, 0.3 mg for the portal vein (the mesenteric vein), and 0.05 mg for the marginal vein of the ear.

Laparotomy under ether anesthesia was performed to permit local administration to the liver; the veins on the mesentery served as the site of injection. For the bile duct, a catheter was inserted into the duct and fixed, the distal segment of the common bile duct and the cystic duct were ligated, and injection of 5 ml of the contrast medium, Urographin (sodium diatrizoate, Shering Chemicals) through the catheter showed uniform filling of the intrahepatic biliary system (Fig. 1). After injection of 5 ml of the solution, the catheter was kept clamped for 30 min and then released. The external end of the catheter was fixed on the closed abdominal wall for subsequent injections. The interval between preparative and provocative injections was 24 h.

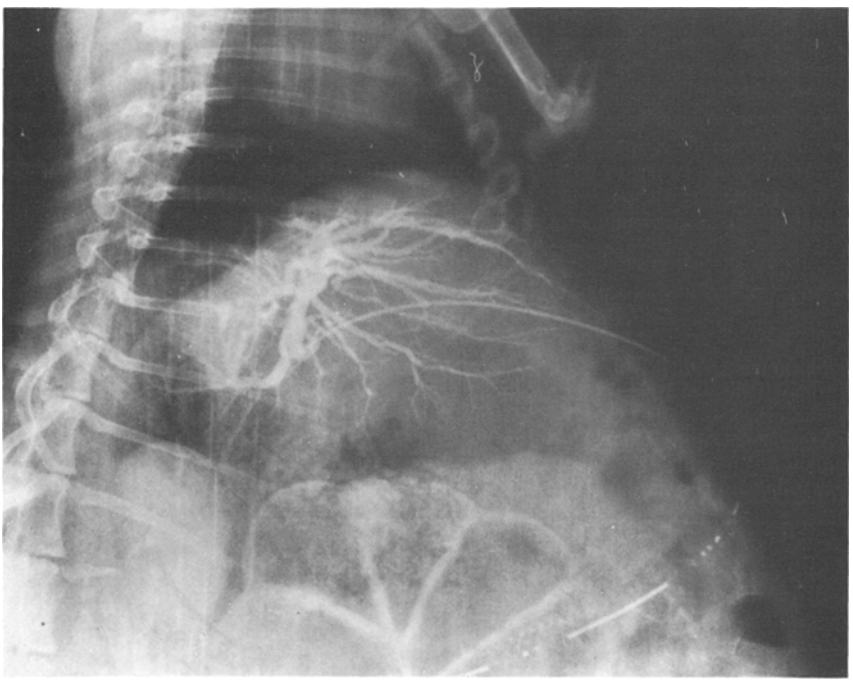


Fig.1. An X-ray film taken to confirm the result of our method of injection via the bile duct. Note uniform filling of the contrast medium throughout the liver

Table 1. Hepatic necrosis caused by Shwartzman reaction in non-pregnant animals (figures indicate the number of animals)

Group	A (Control group)	B (Standard group)	C (Modified standard group)	D (Repetition group)
Total number of animals	14	13	9	5
	Injection routes of endotoxin[a]			
Preparative	Portal route (2) ductal route (12)	Ductal route (13)	Portal route (6) ductal route (3)	More than two injections (5)[b]
Provocative	none	ear vein (13)	ear vein (5) portal route (3) ductal route (1)	
Grade of hepatic necrosis (gross)[c]				
I (none)	13	0	2	0
II (mild)	1	1	1	0
III (moderate)	0	6	4	0
IV (severe)	0	4	1	1
V (extremely severe)	0	2	1	4

[a] Figures in parentheses indicate the number of animals in each group.
[b] Endotoxin was injected more than twice as follows:
via bile duct – via ear vein – via bile duct – via ear vein (for 1 animal)
via bile duct – via portal vein – via bile duct – via portal vein (for 2 animals)
via bile duct × 6 times (for 1 animal)
via bile duct – via ear vein – via bile duct (for 1 animal)
[c] Including fibrosis

Combinations of experimental procedures used in the animals of the respective groups are summarized in Tables 1 and 2.

Among four groups (A–D) of non-pregnant animals, the rabbits in group A (control group) received a single injection of the endotoxin (preparative dose), either via the portal vein or the bile duct, without subsequent provocative injection. The rabbits in group B (standard group) were exposed to the conventional procedure for the Shwartzman reaction, preparative injection via the bile duct and provocative via the ear vein. In group C (modified standard group), the rabbits received both preparative and provocative injections in different combinations of injection routes. In group D (repetition group), more than two injections of the endotoxin were given to repeat the Shwartzman reaction by various routes, as indicated in Table 1.

The pregnant rabbits were divided into four groups (E to H) dependant on the period between the injections and delivery (Table 2). All the pregnant animals received only a single injection of the endotoxin, because it caused such severe reactions that it prevented another injection.

All rabbits were autopsied as soon as possible after natural death or sacrifice. Time interval between the last injection of the endotoxin and death ranged from 2 h to 6 days. Gross changes of hepatic necrosis were classified into five grades as follows: Grade I (none). No hepatic necrosis; Grade II (mild). Small scattered foci of hepatic necrosis; Grade III (moderate). Necrosis up to one-half of a lobe; Grade IV (severe). Necrosis larger than one-half of a lobe; Grade V (extremely severe). Necrosis covering a whole lobe.

The liver, kidneys, lungs, heart, spleen, and placenta were examined histologically. Chemical determinations of SGOT, SGPT, and serum bilirubin before and after injections of endotoxin were performed in selected cases.

Table 2. Hepatic necrosis caused by Shwartzman reaction in pregnant animals (figures indicate the number of animals)

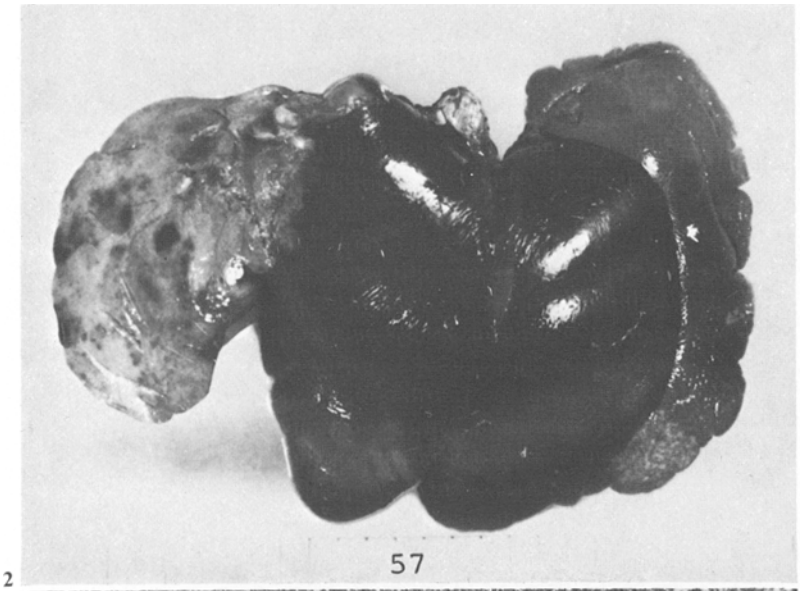
Group	E (Between 1 to 2 weeks before expected delivery date)	F (Within 1 week before expected delivery date)	G (Within 1 week after delivery date)	H (Between 1 to 2 weeks after delivery date)
Total number of animals	2	9	10	2
	Single injection[a]			
	Via portal vein (1) Via bile duct (1)	Via portal vein (2) Via bile duct (7)	Via portal vein (2) Via bile duct (8)	Via bile duct (2)
Grade of hepatic necrosis (gross)				
I (none)	1	0	0	1
II (mild)	1	0	0	1
III (moderate)	0	3	2	0
IIV (severe)	0	1	4	0
V (extemely severe)	0	5	4	0

Figures in parentheses indicate the number of animals

Results

Gross Hepatic Changes. Grossly, the areas of hepatic necrosis were grayish to yellowish red, irregular in shape, and of varying sizes. In milder cases, there were many, miliary-sized spots of necrosis scattered on the surface of the liver and in the parenchyma. These were larger and confluent forming massive areas of necrosis of irregular shape in more severe cases, sometimes involving an entire lobe in the severest instances (Fig. 2).

Histological Hepatic Changes. The changes observed in the liver were acute, severe, and extensive necrosis, including both lytic and coagulative forms. Cloudy swelling and vacuolar change of the hepatocellular cytoplasm with ballooning and nuclear pyknosis occurred in lytic necrosis while eosinophilic, hyalin degeneration of hepatocytes was seen with coagulative change. The hepatic necrosis occurred in the centrilobular or midzonal areas, with higher frequency of the former; but sometimes an entire lobule was necrotic in more severe cases (Figs. 3, 4, and 5). Segmented leukocytes frequently accumulated in and around the area of necrosis, and hemorrhage was also common. In the liver of these animals, small thrombi were seen scattered in the sinusoids or small portal vein branches, which were not recognized grossly. Densely packed accumulations of red blood cells (hemagglutination) was quite often seen in sinusoids and blood vessels. In the hepatic tissue remaining rather intact, there found so-called "dunkle Leberzellen" (Helmke, 1939) abundantly, especially in the area bordering the necrosis.



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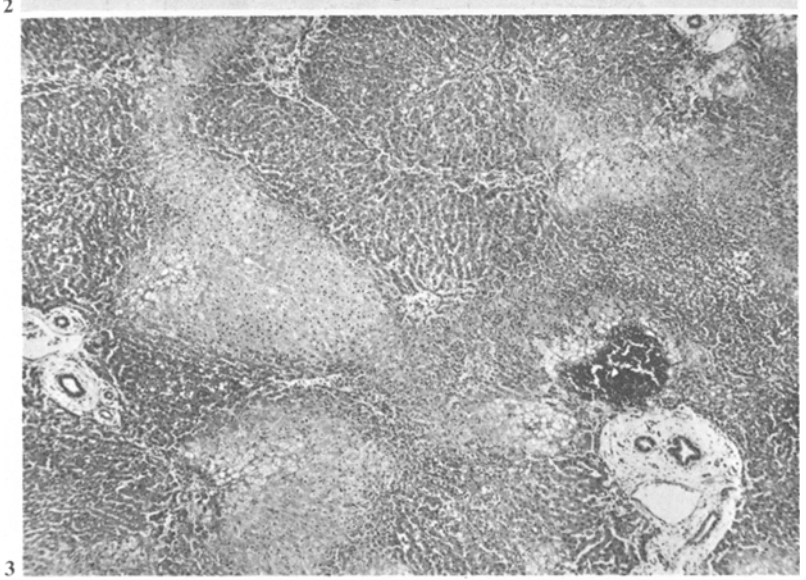

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Fig. 2. A liver fallen into extensive and severe necrosis. An entire lobe on the left is necrotic, and one at the center is hemorrhagic and atrophied (note wrinkles on the surface). (Grade V)
Fig. 3. A liver showing acute necrosis of a moderate degree. Necrosis is fundamentally centrilobular, and associated by occasional hemorrhages. (H-E. × 50)

Liver Function. Laboratory tests were done on three animals. For example, SGOT activity rose from 29 to over 800, SGPT from 34 to over 800 in one rabbit in group F with grade V necrosis.

Dose and Injection Routes. No definite dose-response relationship existed between the amount of endotoxin used and the severity of the hepatic changes. In

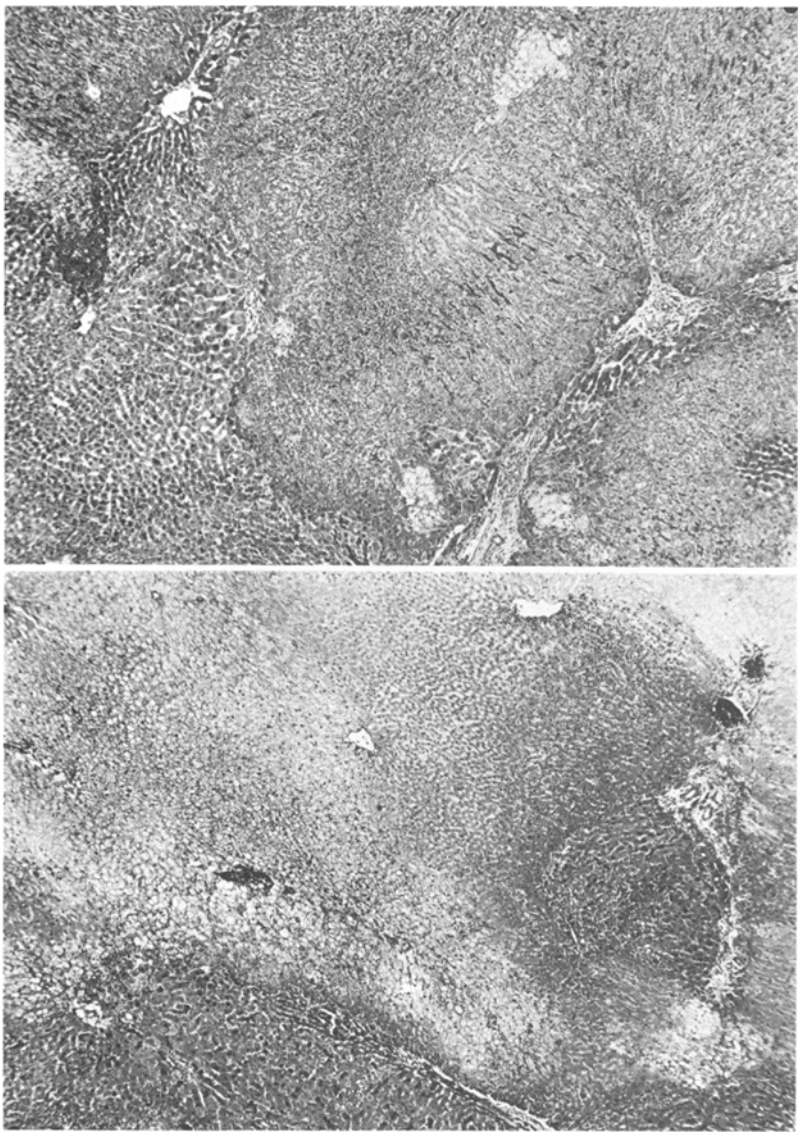

Fig. 4. Severe and extensive necrosis of the liver. Uninvolved area is seen on the left. Note small viable areas in periportal region on the right. (H-E. × 50)

Fig. 5. Severe and extensive necrosis of the liver. All hepatic cells in this field have fallen into necrosis, partly coagulative and partly lytic. (H-E. × 50)

general, animals receiving a bile duct injection as the preparative dose showed more severe necrosis, while those given the portal vein injection showed a tendency to more marked hemorrhage in the non-pregnant groups. The pregnant groups showed no significant differences in results between the two procedures.

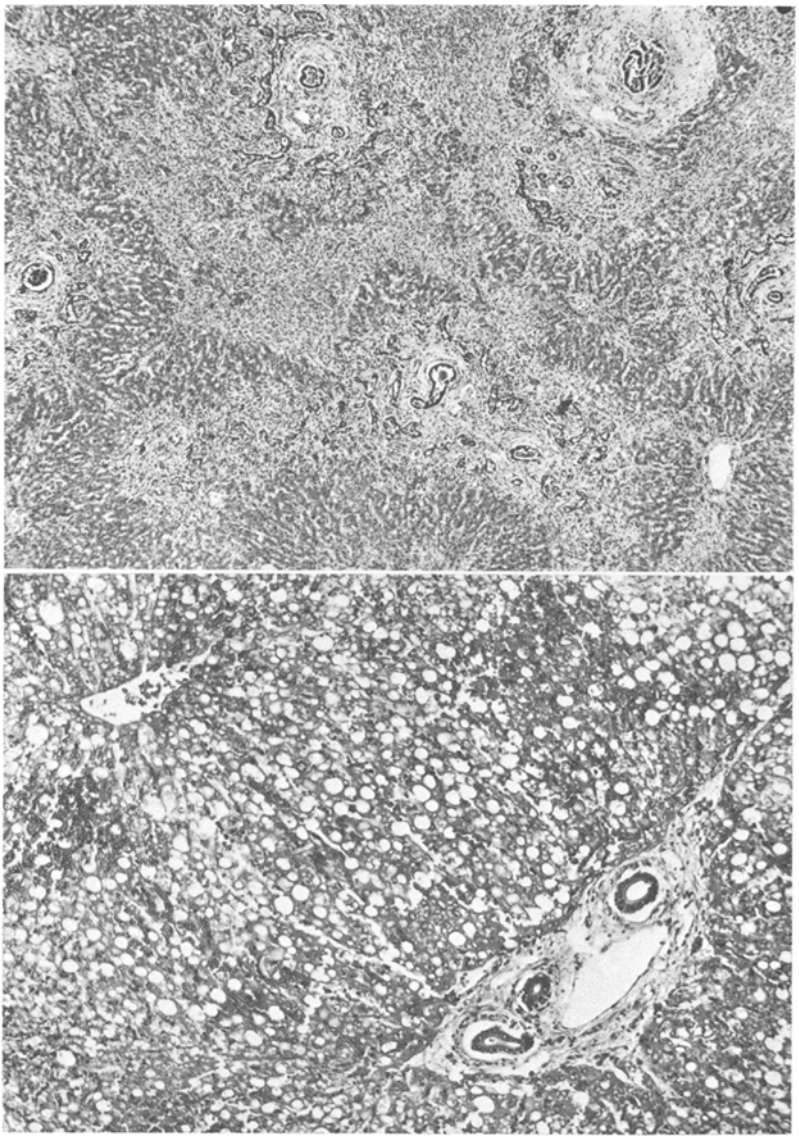

Fig. 6. Result of repeated endotoxin injection to the liver. Note marked fibrosis, bile duct proliferation, and inflammatory cell infiltration. (H-E. × 50)

Fig. 7. Severe fatty metamorphosis and hemorrhagic necrosis of the liver. A rabbit in pregnancy. (H-E. × 120) (H-E. = Hematoxylin-Eosin stain)

Comparison of Hepatic Changes Between Groups. The hepatic changes were compared in the groups (Tables 1 and 2). In group A, frequent vacuolar degeneration or spotty necrosis of the hepatic cells to a mild degree was seen. In group B, extremely severe hepatic necrosis was seen in two cases, severe necrosis in four, and moderate in six. In group C, changes were similar to those observed

in group B, but slightly milder. In group D, fibrosis was frequent in addition to acute necrosis and in 3 cases was so conspicuous that it suggested early cirrhosis. Lymphoplasmacytic infiltration was often found. Proliferation of the bile ducts was also noted (Fig. 6).

In the pregant groups (E–H), gross and histological changes in groups F and G were severe. The degree of hepatic necrosis was the same as, or more severe than that of the non-pregnant, twice-injected rabbits. The differences in hepatic changes were similar in mature pregnant and in non-pregnant groups. Severe changes in pregnancy were seen only when the injection was given within one week before or after delivery (Groups F and G). Besides necrosis, steatosis of various degrees occasionally occurred in the pregnant groups (Fig. 7).

Sequential Changes. Sequential changes of the liver were also studied with cases developing severe necrosis. Two hours after preparatory injection infiltration of leucocytes as well as degeneration of Kupffer cells were observed, however, these changes were mild and could not be considered as "Seröse Hepatitis" (Rössle, 1943). Massive hepatic necrosis occurred only after the second, provocative injection. Namely, 2 h after provocative injection, numerous leucocytes were seen accumulating in the sinusoid with fibrin or fibrin like materials; the change is quite compatible with the findings of the kidneys by generalized Shwartzman reaction (Watanabe and Tanaka, 1977). Three hours after the provocative injection massive hepatic necrosis appeared rather suddenly. Fibrosis was noted 3 days later, and this fibrosis was basically same as that found in the rabbit which survived for 11 days. No newly formed elastic fibre was seen.

Extrahepatic Changes. Changes in organs other than the liver were minimal. Occasionally, some thrombus formation was noted in kidneys and lungs and oedema was present in the latter. In 3 animals from the pregnant groups, foci of hemorrhage were found in the placenta.

Discussion

The most important results of this study is the successful production of acute, severe, and extensive necrosis of the liver by the Shwartzman mechanism with minimal changes in extrahepatic organs. The histopathological picture of this lesion resembles some human cases and the results of laboratory tests support this similarity.

Since Shwartzman (1928) produced severe hemorrhagic necrosis of the skin in rabbits after preparative and provocative injections of the culture filtrate of typhus bacterium, numerous modifications of the original experiment have been performed. The Shwartzman reaction has been classified into two major categories, localized and generalized, and the latter is considered to have a closer relation to human pathologic features in various conditions, especially in pregnancy. However, no modification producing necrosis in a single organ has been reported. Our preliminary experiment (Mori, 1972) revealed that one

of the preparative and provocative injections of the endotoxin must be administered directly to the liver through the portal vein or bile duct to produce necrosis exclusively in the target organ. The present study not only confirmed the organ specificity of the experimental model, but also revealed that necrosis is generalized, not localized, within the target organ, despite some differences in extent. Diffuse but exclusive distribution of necrosis in the target organ strongly suggests that the current model belongs to neither the localized nor generalized types of classical Shwartzman reactions. It is therefore tentatively designated the univisceral type as third category of the Shwartzman reaction. Repetition of the Shwartzman reaction (group D) led to the most extensive necrosis and fibrosis providing further evidence for this contention. The pancreatic necrosis (Thal and Brackney, 1954) and enterocolitis (Berry and Fraser, 1968) both produced by Shwartzman mechanism might be some models of univisceral type for other target organs. Bilateral cortical necrosis of the kidney, a manifestation of the generalized Shwartzman reaction (Thomas and Good, 1952) was not observed in the present series. A small number of fibrin thrombi were seen in the liver, contrary to our expectations, however rapid clearance of fibrin in the liver by reticuloendothelial cells may have occurred (Lee, 1962).

Toxic chemicals, viruses, immunological disorders, etc., are usually considered the cause of the massive hepatic necrosis seen in man which can also be produced experimentally to some extent. The morphological similarity of the present model, at least at the light-microscopic level, to the hepatic necrosis produced by Paronetto and Popper (1965) using immune complexes, indicate that the alterations might not be specific.

Among clinical entities leading to massive hepatic necrosis, the lesion produced resembles both eclampsia and herpes simplex. The non-involved hepatocytes appear intact, rouleaux formation of the red blood cells is often present, segmented leucocytes are sometimes prominent, and fibrin thrombi are not rare. Eclampsia is of particular interest since pregnancy is frequently considered to be an equivalent of the preparative step of the Shwartzman reaction (Apitz, 1935; Wong, 1962) and is the commonest basis for the generalized Shwartzman reaction in human cases (Mckay et al., 1953; Hjort, 1965; Clarkson, 1969). In our model, in pregnant animals a single injection resulted in extremely severe necrosis of the liver. Some histologic similarity in the hepatic change in the present experiment and that seen in the so-called fulminant hepatitis in man is suggested. Viral hepatitis may be aggravated in pregnancy (WHO, 1964; Koff and Isselbacher, 1970), especially in the last trimester (Borhanmanesh et al. 1973), and the obstetric hepatic necrosis described by Sheehan (1940) and later confirmed by Ober and LeCompte (1955) might also be related to the Shwartzman reaction.

Similarly, some consequences of gram-negative infections in biliary tract and portal veins might be regarded to be clinical counterparts of injection of endotoxin into the bile duct and mesenteric veins. The effectiveness of heparin in both the Shwartzman reaction (Good and Thomas, 1953) and clinical hepatic injuries also suggests a common. Moreover, both clinical and pathological features of viral hepatitis might be complicated by the shwartzman reaction, although the basic pathogenesis of human viral hepatitis appears to be immunological (Gudat and his associates, 1975; Edgington and Chisari, 1975).

In conclusion, this experimental model of acute massive necrosis of the liver produced by the Shwartzman reaction (univisceral Shwartzman reaction of the liver) seems to have similarities with herpes and eclampsia in man. It also suggests that the Shwartzman mechanism may be a complicting factor in the histologic appearances of massive necrotic viral hepatitis, pernicious steatosis of pregnancy, and in particular, bacterial infections of the bile duct system of the liver.

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References

Apitz, K.: A study of the generalized Shwartzman phenomenon. J. Immunol. **29**, 255–266 (1935)

Berry, C.L., Fraser, G.C.: The experimental productin of colitis in the rabbit with particular reference to Hirschsprung's disease. J. Ped. Surg. **3**, 36–42 (1968)

Borhanmanesh, F., Haghighi, P., Hekmat, K., Rezaizadeh, K., Ghavami, A.G.: Viral hepatitis during pregnancy Severity and effect on gestation. Gastroenterology **64**, 304–312 (1973)

Clarkson, A.R.: Consumption coagulopathy and acute renal failure due to gram-negative septicemia after abortion. Complete recovery with heparin therapy. Ann. Int. Med. **70**, 1191–1200 (1969)

Edgington, T.S., Chisari, F.V.: Immunological aspects of hepatitis B virus injection. Am. J. Med. Sci. **270**, 213–227 (1975)

Good, R.A., Thomas, L.: Studies on the generalized Shwartzman reaction. IV. Prevention of the local and generalized Shwartzman reactions with heparin. J. Exp. Med. **97**, 871–888 (1953)

Gudat, F., Bianchi, L., Sonnabend, W., Thiel, G., Aenishaenslin, W., Stalder, G.A.: Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. Lab. Invest. **32**, 1–9 (1975)

Helmke, K.: Über den Zellkollaps. Virchows Arch. Path. Anat. and Histol. **304**, 255–270 (1939)

Hjort, P.F.: The Shwartzman reaction: Pathogenetic mechanisms and clinical manifestations. Ann. Rev. Med. **16**, 135–168 (1965)

Koff, R.S., Isselbacher, K.J.: Acute hepatitis. In: Harrison's principles of internal medicine, M.M. Wintrobe, G.W. Thorn, R.D. Adams, E. Braunwald, K.J. Isselbacher, R.G. Petersdorf, I.L. Bennett (eds.), 6th edition, pp. 1535–1541. New York: McGraw-Hill Co. 1970

Lee, L.: Reticuloendothelial clearance of circulating fibrin in the pathogenesis of the generalized Shwartzman reaction. J. Exp. Med. **115**, 1065–1082 (1962)

McKay, D.G., Merrill, S.J., Weiner, A.E., Hertig, A.T., Reid, D.E.: The pathologic anatomy of eclampsia, bilateral renal cortical necrosis, pituitary necrosis, and other acute fatal complications of pregnancy, and its possible relationship to the generalized Shwartzman phenomenon. Am. J. Obst. Gynec. **66**, 507–539 (1953)

Mori, W.: Fulminant hepatitis – some considerations on its pathology. Gastroenterol. Jap. **7**, 46–47 (1972)

Nolan, J.P.: The role of endotoxin in liver injury. Gastroenteroloy **69**, 1346–1356 (1975)

Ober, W.B., LeCompte, P.M.: Acute fatty metamorphosis of the liver associated with pregnancy. A distinctive lesion. Am. J. Med. **19**, 743–758 (1955)

Paronetto, F., Popper, H.: Aggravation of hepatic lesions in mice by in vivo localization of immune complexes (auer hepatitis). Am. J. Path. **47**, 549–563 (1965)

Rössle, R.: Über die serösen Entzündungen der Organe. Virchows Arch. Path. Anat. **311**, 252–284 (1943)

Sheehan, H.L.: The pathology of acute yellow atrophy and delayed chloroform poisoning. J. Obst. Gynec. Brit. Emp. **47**, 49–62 (1940)

Shwartzman, G.: A new phenomenon of local skin reactivity to B, typhosus culture filtrate. Proc. Soc. Biol. Med. **25**, 560–561 (1928)

Thal, A., Brackney, E.: Acute hemorrhagic pancreatic necrosis producted by local Shwartzman reaction. JAMA **155**, 569–574 (1954)
Thomas, L., Good, R.A.: Studies of the generalized Shwartzman reaction. J. Exp. Med. **96**, 605–623 (1952)
Watanabe, T., Tanaka, K.: Electronmicroscopic observation of the kidney in the generalized Shwartzman reaction. Virchows Arch. A Path. Anat. and Histol. **374**, 183–196 (1977)
Wong, T.-C.: A study on the generalized Shwartzman reaction in pregnant rats induced by bacterial endotoxin. Am. J. Obst. Gynec. **84**, 786–797 (1962)
World Health Organization Technical Report Series No. 285, WHO Expert Committee on Hepatitis. Second report. WHO, Geneva, 1964

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