STUDIES OF THE GENERALIZED SHWARTZMAN REACTION PRODUCED BY DIET*

I. Pathology

By DONALD G. McKAY, M.D., and TING-CHAO WONG, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City)

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In the early studies of the generalized Shwartzman reaction it was thought that the phenomenon could be elicited only in rabbits after two properly spaced intravenous injections of bacterial endotoxin. In recent years it has become apparent that under certain conditions only one injection is necessary and that animal species other than the rabbit are susceptible to the phenomenon (1, 2). Pregnancy is one of the most effective means of "preparing" for the reaction. In the pregnant rabbit only one intravenous injection of endotoxin is required and the reaction is found in higher incidence in pregnant than in non-pregnant rabbits (3). It has recently been demonstrated that rats, which in the non-pregnant state resist the development of the generalized Shwartzman reaction, become susceptible during pregnancy (4). The mechanism by which pregnancy increases this sensitivity to bacterial endotoxin remains to be elicited.

In 1956 and 1959 Stamler (5, 6) reported a fatal disease of rats which appeared under the influence of pregnancy and an "anti-vitamin E stress diet." Upon repeating Stamler's experiments, it became apparent that the pathologic changes in these rats were similar to those of the generalized Shwartzman reaction. It is the purpose of this report to call attention to the fact that the generalized Shwartzman reaction may be produced by dietary means in the absence of any injection of exogenous bacterial endotoxin.

Materials and Methods

Rats of the Sprague-Dawley and Columbia-Sherman strains were fed a basic diet of Rockland pellets for a period of 8 to 16 weeks. The Rockland diet contains 3 mg of vitamin E per 100 gm and the rats ate an average of 10 gm of food per day. When they reached sexual maturity at a weight of approximately 150 to 200 gm they were mated. The females were exposed to the males for a period of 5 days. At the end of this time they were fed the oxidized

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cod liver oil diet used by Stamler (6) which consisted of the following ingredients: corn starch, 61 per cent; purified casein, 20 per cent; brewer's yeast, 10 per cent; HMW salt mixture, 4 per cent; oxidized cod liver oil, 5 per cent. The cod liver oil was oxidized by bubbling air through the oil for 60 hours at 100°C. The peroxide number at the end of this time was 100 to 160. The diet was mixed and stored at −20°C until ready for use. It was fed to the animals as a powder, which they ate freely. They were given water *ad libitum*. The majority of the animals were allowed to go to term at which time they either delivered normally or died and were autopsied. A few were sacrificed near term as they developed clinically obvious disease or when they were to be used as controls for histologic examination.

Four groups of animals were studied.

Group I, Controls.—Twenty rats were mated and fed Rockland or Purina diets before and during gestation and these served as the control series for the purpose of observing the normal histology of the pregnant rat at term.

Group II, First Generation.—80 rats were placed on the Rockland diet for 2 months. They were then mated and fed the oxidized cod liver oil diet throughout gestation.

TABLE I

	Number of animals	Number pregnant	Number died	Sacrificed	With disease (pregnant)
					per cent
Group I, control	20	20	0	0	0
Group II, 1st generation	80	61	5	0	8
Group III, 2nd generation	171	112	35	9	31.2
Group IV, 3rd generation	32	19	7	1	36.8

Group III, Second Generation.—171 surviving infant rats who were delivered of the mothers of the second group were placed on the Rockland diet until they reached sexual maturity. They were then mated and fed the oxidized cod liver oil diet during gestation.

Group IV, Third Generation.—32 surviving infant rats delivered of mothers of group III were placed on the Rockland diet until sexual maturity. They were then mated and fed the oxidized cod liver oil diet during gestation.

RESULTS

Clinical Observations.—

No functional abnormalities were noted during the first 19 to 20 days of gestation in any of the rats on the oxidized cod liver oil diet. Near term many of the rats died unobserved, often at night. A few of the animals were observed to have a variety of alterations. The most notable change was a lethargic appearance and the rat lay quietly in a corner of the cage. The hair was ruffled and respirations became rapid and difficult. Some animals developed a frothy exudate from the nose. In some, convulsions developed and were rapidly followed by death, and a few of the animals died quietly without convulsions. Approximately one-third of the animals that died had antepartum vaginal bleeding which was of 6 to 48 hours' duration. A few animals died postpartum.

Table I shows the incidence of death in pregnant animals on the Rockland diet for varying periods of time followed by the oxidized cod liver oil diet during gestation. These results indicate that the incidence of disease increases

with prolonged exposure to the Rockland diet and with an increase in the number of previous exposures of the mothers' antecedents to the oxidized cod liver oil diet.

Pathologic Alterations.—

Examination in the gross revealed congestion and hyperemia of the abdominal and thoracic viscera. The lungs were atelectatic, and occasionally had petechial hemorrhages on the pleural surfaces. The liver was congested and occasionally showed petechiae on the capsular and cut surfaces. The gastrointestinal tract was usually within normal limits but in two instances showed focal, segmental, hemorrhagic necrosis of the mucosa of the ileum. Two animals had hemorrhage into the vitreous of the eyes.

Major changes were found in the adrenals, kidneys, and uterus. The adrenals were enlarged and often showed hemorrhages in the cortex. The kidneys were enlarged and some had a mottled red and yellow discoloration of the surface. This change extended through the thickness of the cortex but was not seen in the medulla. There was often hemorrhage n the uterus. Unclotted blood was found in the uterine lumen and around many placental

TABLE II

Incidence of Fibrin Thrombi in Autopsied Animals

	Cases	Kidney	Liver	Adrenal	Spleen	Lung
Animals dying spontaneously	35	31	4	14	4	33
Animals sacrificed antepartum	13*	4	3	2	1	3

* Contains two animals from a more recent study.

sites. Some of the placentas were separated from the uterine wall and these were often enlarged and congested. Occasional dead, macerated fetuses were found but for the most part the fetuses were well preserved and healthy in appearance.

Microscopic examination revealed the proximate cause of death in these animals. In all the rats that died spontaneously there was evidence of disseminated intravascular coagulation with thrombi in capillaries, arterioles, and venules of the lungs, spleen, kidneys, and rarely the adrenals, liver, and gastrointestinal tract. The incidence of fibrin thrombi in the autopsied cases is presented in Table II.

Lungs.—Numerous thrombi occluded the small arterioles of the lung (Fig. 1). These thrombi were rarely associated with extravasation of blood into the alveolar spaces. The lungs were usually focally atelectatic. Although intra-alveolar edema was rare and focal, interstitial edema was frequent. The connective tissue spaces around the large vessels and bronchi were widened and contained precipitated protein in the edema fluid (Fig. 2). Occasionally, blood was found in these interstitial spaces.

Kidneys.—The most constant finding was fibrin thrombi in the glomerular capillary loops (Fig. 3). 50 to 100 per cent of the glomeruli were obstructed in this manner. Precapillary arterioles were sometimes filled with fibrin. Patches of tubular tissue were necrotic in about one-third of the cases. In two cases there were hyaline droplets in the cells of the renal tubules.

Fat droplets near the basement membrane of the cells of the convoluted tubules were a constant feature.

Adrenals.—Fibrin thrombi were present in an irregular distribution in the small afferent vessels of the capsule and outer cortex. These were accompanied by focal hemorrhagic necrosis of the underlying cortex (Fig. 4).

Liver.—The most constant alteration of the liver was the presence of fat droplets in the liver cells. This change was not extensive and was usually central-lobular in distribution although in some instances involved all cells. In a few animals there was an intense periportal congestion and extravasation of blood from the hepatic sinusoids. This was often accompanied by fibrin thrombi in the sinuses of the periportal region (Fig. 5).

Spleen.—The sinuses of the red pulp were congested and in a few instances were lined by fibrin. Macrophages were prominent in the red pulp and many contained a yellow-brown pigment that did not react to Prussian blue (Fig. 6).

Uterus and Placenta.—In a few cases thrombi were found in the uterine veins and in the decidual veins. Degeneration of the trophoblast of the labyrinth and the giant cell layer was a constant feature. Congestion of the maternal blood spaces of the labyrinth and deposits of fibrin in these spaces were frequently observed. In some cases one or two of the placentas were separated from the uterus. In 10 per cent of the animals there was an intense chorioamnionitis with a thick layer of leukocytes over the placental margins and extending over the fetal surface.

DISCUSSION

A. Identification of the Generalized Shwartzman Reaction.—These observations confirm and add to those originally made by Stamler. Perhaps the single most important consequence of these experiments is the recognition of this tissue response as the generalized Shwartzman reaction since at no time did the rats receive any intravenous injections of exogenous bacterial endotoxin or any other substance, and since the only experimental alteration in their treatment resided in an altered diet.

In attempting to define the generalized Shwartzman reaction certain difficulties are encountered. It has been shown that this reaction is readily induced by the intravenous injection of bacterial endotoxin in small amounts in nonpregnant rabbits in two properly spaced injections, and that this results in two episodes of disseminated intravascular coagulation (7). The first injection deposits thrombi in the liver, lungs, and spleen, and the second injection causes similar fibrin deposits in the renal glomeruli with the ultimate development of bilateral renal cortical necrosis. From these observations several possible definitions of the generalized Shwartzman reaction may be proposed.

1. It is the effect of an intravenous injection of bacterial endotoxin: This is an inadequate definition because the factors of time and dosage are not taken into account. One large dose kills the animals rapidly with no pathologic change detectable. One small dose produces thrombi in the non-pregnant animals in the lungs, liver, and spleen, but not the kidney.

2. It is the effect of two, properly spaced, intravenous injections of bacterial endotoxin: This is closer but still inadequate because, (*a*) 40 per cent of the

animals treated in this manner show no tissue reaction whatever and survive unscathed, and (*b*) only one injection is necessary to produce all the pathologic changes including renal glomerular thrombosis in pregnant or cortisone-treated rabbits.

3. It is disseminated intravascular coagulation: This is true but an inadequate definition because intravascular coagulation is caused by a wide variety of agents such as thrombin, thromboplastin, and particulate matter, which, when injected intravenously, are not capable of producing the renal involvement characteristic of the Shwartzman reaction.

4. It is a series of alterations in the hemostatic mechanism, i.e., a shortening of the coagulation time, a lowering of circulating platelets, leukocytes, and fibrinogen, with a subsequent abnormal elevation of all elements except platelets: Since these are merely the hemostatic changes attendant upon disseminated intravascular coagulation, the criticism of *3* applies here.

5. It is shock due to endotoxemia: This is inadequate because it does not take into account the wide variety of histologic and hematologic changes occurring in the reaction.

6. It is bilateral symmetrical renal cortical necrosis: This is close but not acceptable for two reasons, (*a*) bilateral renal cortical necrosis may be produced by staphylococcal alpha toxin by a mechanism of vasospasm and vascular damage which is quite different from the pathogenetic mechanism in the Shwartzman reaction (8), and (*b*) some animals with the etiologic factor of endotoxin-induced bilateral cortical necrosis, *i.e.*, organic obstruction of the renal capillaries by disseminated capillary glomerular thrombosis, do not survive long enough for ischemic infarction to appear.

All the above definitions have their element of truth, but would have to be lumped together as a description rather than a definition. The single feature that makes this reaction different from all others is embodied in the last proposed definition.

7. It is disseminated renal glomerular capillary thrombosis: This is the one element of the reaction which at present distinguishes it clearly from all others, and gives it identity.

Using this definition, it is clear that the pregnant rats dying after exposure to the Rockland and oxidized cod liver oil diets exhibit the generalized Shwartzman reaction.

B. Comparison with the Shwartzman Reaction in Rabbits.—The location and incidence of the disseminated thrombi in the Shwartzman reaction induced by diet in pregnant rats differ somewhat from that in the rabbit injected with endotoxin.

In the non-pregnant rabbit after the *second injection* of endotoxin, thrombi were found in the renal glomeruli (53 per cent), sinusoids of the red pulp of the spleen (64 per cent), pulmonary vessels (76 per cent), and in the central

veins of the liver (69 per cent). After *one injection* in pregnant rabbits, thrombi were found in the renal glomeruli (82 per cent), malpighian corpuscles of the spleen (64 per cent), pulmonary vessels (88 per cent), peripheral sinusoids of the liver (76 per cent), and adrenal sinusoids (41 per cent). With the injection of endotoxin, the "preparation" of pregnancy shifts the localization of thrombi from the central vein to the periportal sinusoids of the liver, from the sinuses of the red pulp to the malpighian corpuscles of the spleen, and causes thrombi to appear in the adrenal (3).

In comparing the incidence of involvement of the various organs with the dietary Shwartzman reaction (Table III) several facts are apparent. The kidneys and lungs are more frequently involved in the diet-induced Shwartzman reaction, and the liver and spleen are less frequently involved than by any

TABLE III

Incidence of Thrombi in Various Organs by 4 Different Methods of Producing the Generalized Shwartzman Reaction

	Kidney	Lungs	Liver	Spleen	Adrenal
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Non-pregnant rabbits (endotoxin, 2 injections)	53	76	69	64	0
Pregnant rabbits (endotoxin, 1 injection)	82	88	76	64	41
Pregnant rats (endotoxin, 1 injection)	47	23	26	—	18
Pregnant rats (dietary Shwartzman reaction)	88.5	94.2	11.4	11.4	40

other means of inducing the reaction. Adrenal thrombi are usually seen in the pregnant animals but not in the non-pregnant animals.

There are differences in the location of the thrombi within organs. Fibrin is infrequently deposited in the spleen in the diet-induced Shwartzman reaction and is located in the sinusoids of the red pulp whereas endotoxin injection into a pregnant rabbit causes the deposition in the malpighian corpuscles of the spleen, and in high incidence. The localization in the periportal sinusoids of the liver is the same but the incidence is much lower in the diet-induced Shwartzman reaction.

It is clear that the basic tissue reaction is the same in each of these 4 experiments but that there are minor quantitative and qualitative differences in the distribution of the thrombi. It is likely that differences in the blood flow and/or reactivity of the blood vessels under these conditions may account for the varying minor differences (9).

C. Miscellaneous Pathologic Changes.—The accumulation of fat in the kidney and liver is confined to the diet-induced Shwartzman reaction. This is probably a result of the altered lipid diet.

The interstitial edema in the lungs is of interest because it is somewhat

reminiscent of the effect of vitamin E deficiency in the chick. The disease process in the chick is apparently mediated by an increased vascular permeability which allows the leakage of large amounts of protein and fluid through the endothelium into the interstitial connective tissue. It is referred to as an "exudative diathesis" (10).

The observation of dyspnea terminally, coupled with the microscopic finding of pulmonary vessels obstructed and dilated by fibrin thrombi makes it quite likely that these animals die of acute cor pulmonale and the rapid development of shock.

D. General Features of the Diet-Induced Shwartzman Reaction.—1. The generalized Shwartzman reaction is produced by a diet low in tocopherol and containing oxidized fats.

2. Supplements of tocopherol prevent the appearance of the disease.

3. No non-pregnant animals developed the generalized Shwartzman reaction under the conditions of these experiments; therefore, pregnancy is required for the appearance of the disease.

4. The reaction takes place shortly before or after delivery. Therefore, full term pregnancy is required for the reaction.

5. There are alterations in the chorionic placenta in this reaction which consist of premature separation, deposition of fibrin in the maternal blood spaces of the labyrinth, congestion, infarcts, degeneration of the trophoblast, decidual hemorrhage, and thrombosis of the uterine veins and placentitis in 10 per cent of the cases.

6. This reaction in the pregnant rat bears many resemblances to the toxemias of pregnancy in human patients.

7. Since the generalized Shwartzman reaction is identified by the appearance of fibrin thrombi in the renal glomeruli, the ultimate effect of this diet is to produce an episode of disseminated intravascular coagulation. The steps involved between the feeding of the oxidized lipid diet, the presence of pregnancy and inadequate tocopherol diet, and the *in vivo* coagulation of blood remain to be demonstrated.

One of the possible explanations for the appearance of the generalized Shwartzman reaction in animals that have not been injected with bacterial endotoxin is that the animals might have been rendered more susceptible to a bacterial infection which could discharge endotoxin into the circulation from an endogenous source. No infections were found in any of the organs at autopsy in the majority of the cases. However, in 10 per cent of the autopsied animals there was a severe suppurative placentitis which in these cases may have provided the focus from which bacterial endotoxins entered the maternal circulation. This possible explanation, however, does not apply to the remaining 90 per cent of the cases.

Since bacterial infection cannot account for the majority of the cases, other

explanations must be sought. One possibility that cannot be ruled out is that bacterial endotoxin from the gastrointestinal tract is being absorbed into the blood stream across the intestinal mucosa. A mechanism of this type has been postulated by Fine (11) in certain experiments dealing with hemorrhagic shock in the rabbit. This must be considered along with the possibility that some factor from the placenta with the same biologic activity as bacterial endotoxin may be responsible for this unique tissue reaction.

SUMMARY

The generalized Shwartzman reaction can be produced in pregnant rats by dietary means in the absence of injection of exogenous bacterial endotoxin. The experiment consists of a period of exposure to a diet low in tocopherol followed by exposure to a diet containing oxidized lipids during the gestation period. Pregnancy near term is an essential requirement for the development of the generalized Shwartzman reaction in these experiments. Fibrin thrombi were found in the renal glomeruli in 88.5 per cent, in the lungs in 94.2 per cent, in the liver in 11.4 per cent, in the spleen in 11.4 per cent, and in the adrenal in 40 per cent of animals that died spontaneously. There were pathologic alterations in the placenta which consisted of degeneration of the trophoblast, thrombosis of maternal blood channels in the giant cell trophoblast layer and in the labyrinth, congestion of the labyrinth, hemorrhage into the uterine cavity, placental separation, intra-uterine fetal death, decidual and uterine vein thrombosis, and placentitis in 10 per cent of the cases.

The mechanism by which the diet is instrumental in causing this reaction remains to be demonstrated.

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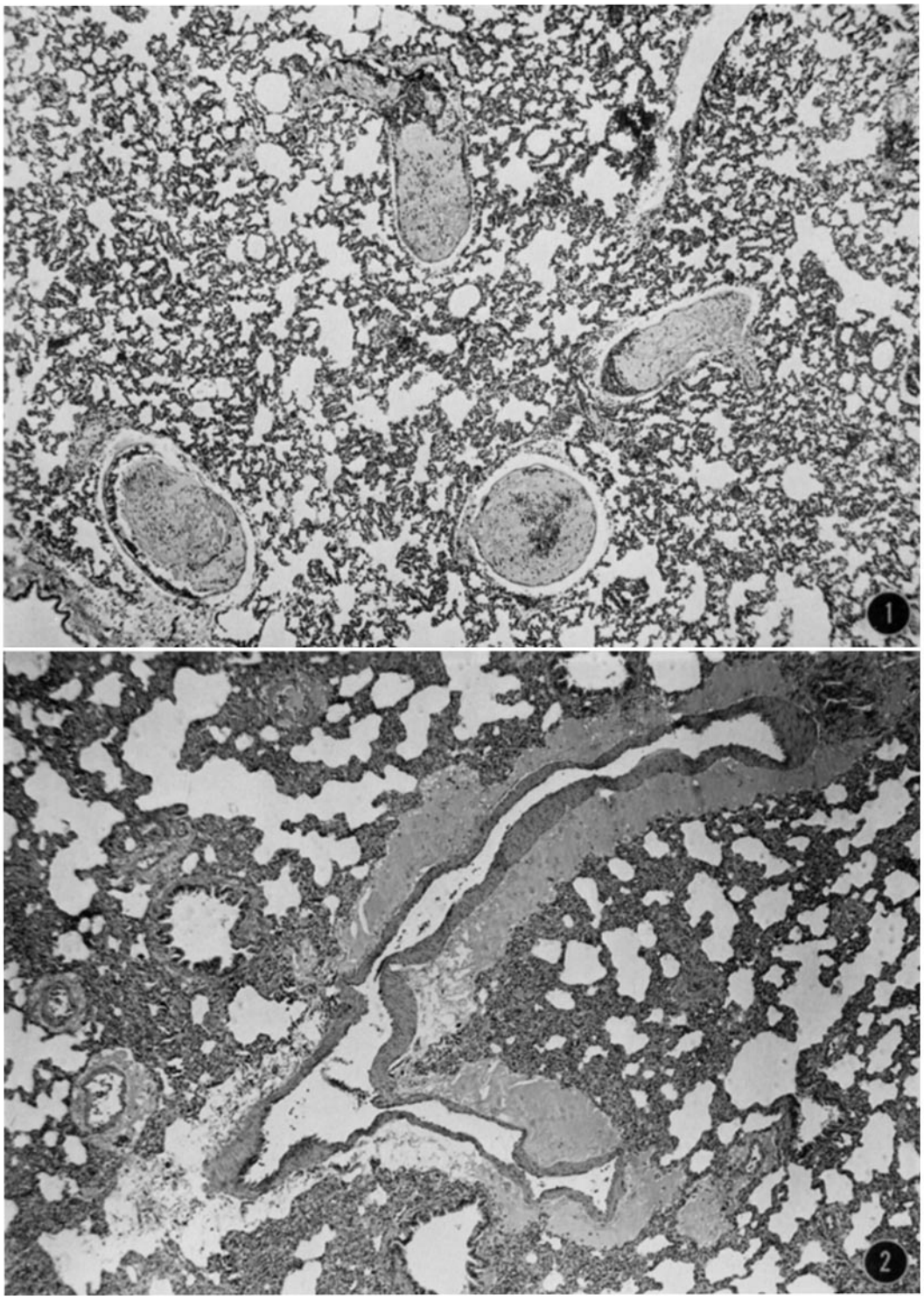
EXPLANATION OF PLATES

PLATE 119

FIG. 1. Thrombi dilate and occlude the lumen of many of the small arteries of the lung. The obstruction of these vessels is probably the immediate cause of death in the majority of these animals. Hematoxylin and eosin. × 50.

FIG. 2. Perivascular edema involving the interstitial connective tissue. The edema seldom was seen in the alveolar spaces. Hematoxylin and eosin. × 50.

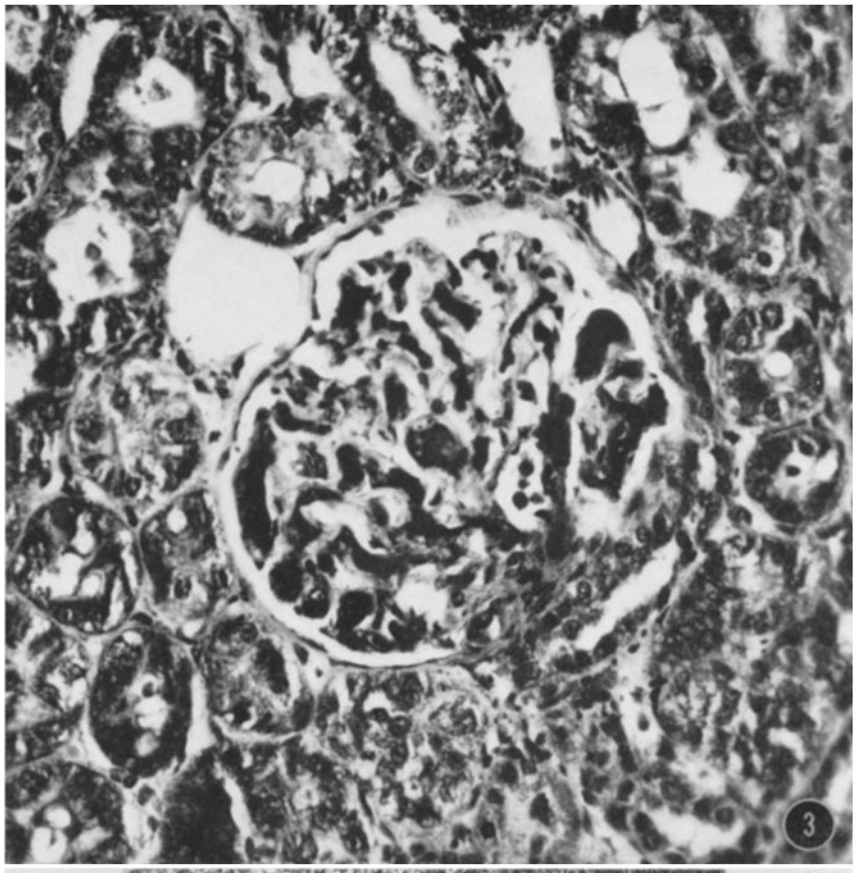


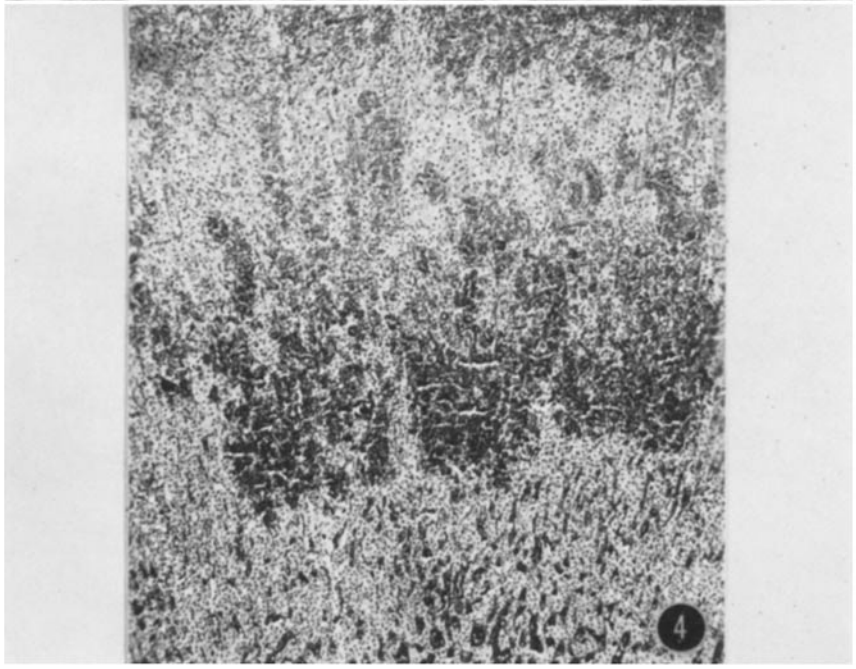
(McKay and Wong: Generalized Shwartzman reaction. I)

PLATE 120

FIG. 3. Fibrin thrombus in renal glomerulus. Phosphotungstic acid hematoxylin. × 200.

FIG. 4. Areas of hemorrhage and necrosis in the adrenal cortex. Hematoxylin and eosin. × 50.



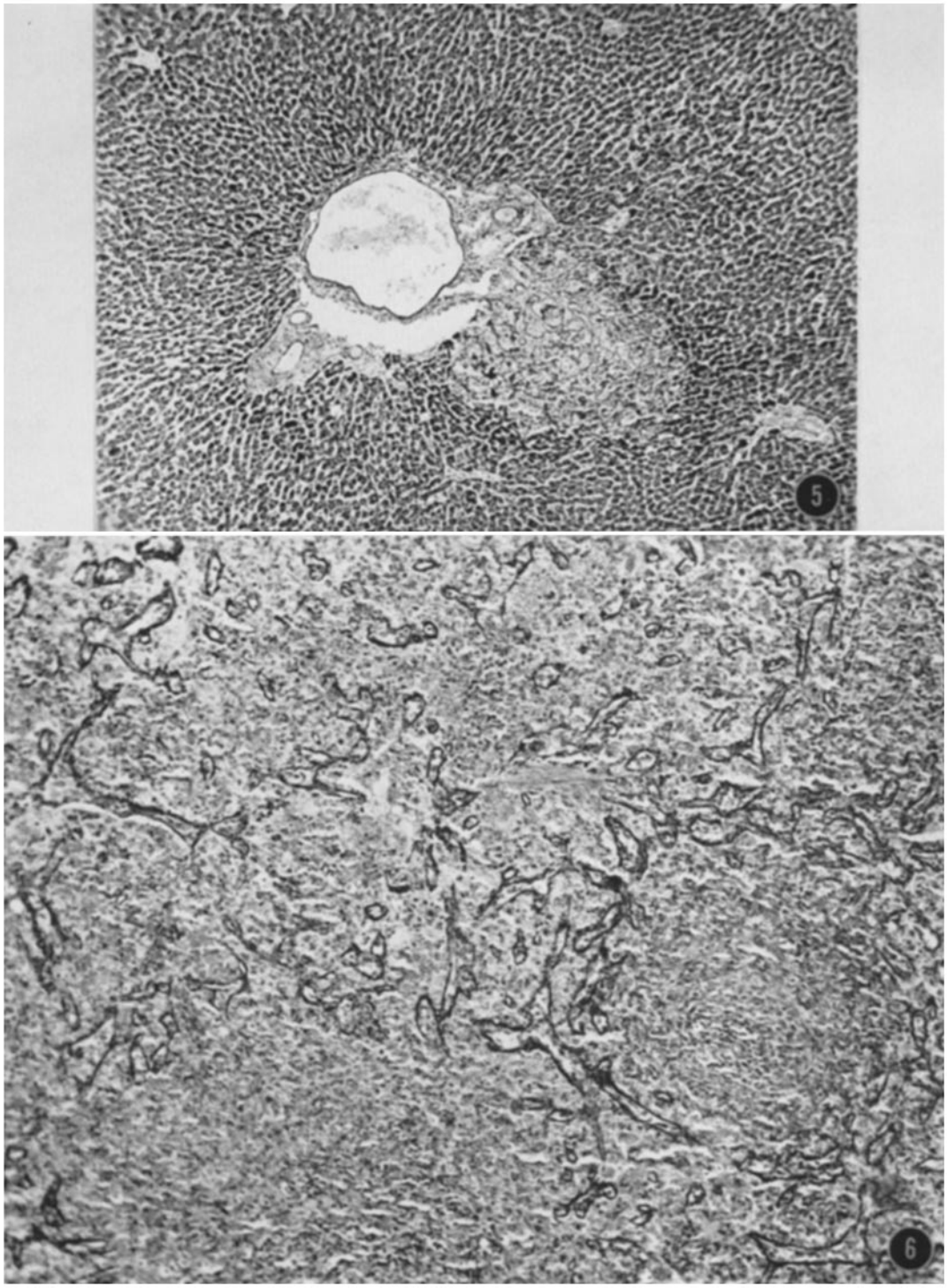


(McKay and Wong: Generalized Shwartzman reaction. I)

PLATE 121

FIG. 5. Periportal focus of necrosis of liver cells with network of fibrin strands in the hemorrhagic area. Phosphotungstic acid hematoxylin. × 50.

FIG. 6. Fibrin coats the lining of the sinuses of the red pulp of the spleen in a few of these animals. Phosphotungstic acid hematoxylin. × 50.



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