THE EFFECT OF BACTERIAL ENDOTOXIN ON THE PLACENTA OF THE RAT

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The fact that bacterial endotoxin is capable of inducing abortion in experimental animals has been known for some time. Among the first to study this reaction were Zahl and Bjerknes [1] as early as 1943. Takeda and Tsuchiya [2,3] observed abortion, hemorrhage into the decidua and placental separation in pregnant mice and rabbits given bacterial endotoxin. They postulated that this reaction represented a placental Shwartzman reaction. Rieder and Thomas [4] took issue with the latter interpretation and presented the view that endotoxin-induced abortion was a specific reaction with significant differences from other endotoxin-induced lesions and was not analogous to a Shwartzman reaction in the placenta. On the other hand, two recent studies by Wong [5] and Kaley, Demopoulos and Zweifach [6] have demonstrated that one injection of bacterial endotoxin induced the generalized Shwartzman reaction in pregnant rats. Both authors noted a high incidence of intra-uterine fetal death (abortion) in animals developing the Shwartzman reaction. Thus, the question remains of the pathogenesis of abortion in animals exposed to endotoxin and its relationship to the generalized Shwartzman reaction.

In attempting to unravel the mechanisms behind the process of abortion, a careful study of the pathologic alterations in the placenta would seem an essential part of the analysis. It is the purpose of this report to describe the histologic changes in the placenta of the rat when the animal is exposed to an intravenous injection of bacterial endotoxin and develops simultaneously the generalized Shwartzman reaction.

MATERIAL AND METHODS

Forty-four pregnant albino rats of the Sprague-Dawley strain were included in this study. All rats were obtained from a single local breeder where they had been fed Purina Fox Checkers. In the laboratory they were fed the same diet and had free access to water. Most of the animals were between the 17th and 18th days of gestation at the time endotoxin was injected.

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TABLE I

GENERAL BIOLOGIC EFFECTS OF ENDOTOXIN ON THE PREGNANT RAT

Endotoxin (mg.)	Rat no.	Body wt. (gm.)	Gestation (days)	Spontaneous death after injection	Sacrificed 48 hr.	Glomerular thrombi	Bilateral renal cortical necrosis	Delivery after injection (hr.)	No. of macerated fetuses	No. of placentas examined
	S119	350	15–16		+				13	3
	S120	360	17–18		+					3
	S121	360	17–18		+	+	+		2	4
	S122	380	18–19	4 hr.		+				3
0.25	S124	420	18–19	3 hr.						3
	S125	380	15–16	24 hr.					3	4
	S126	310	17–18		+	+	+			6
	S127	400	18–19		+			44		3
	S128	430	17–18		+				7	9
	S129	330	18–19	6–18 hr.		+	+			3
	S64	330	18–19		+			35		6
	S65	330	17–18		+					4
	S66	370	18–19		+			35		3
	S67	370	17–18		+	+	+		6	13
0.30	S68	310	17–18	3–15 hr.		+	+			3
	S69	360	18–19		+			44		3
	S70	360	18–19		+			44		3
	S71	400	17–18	27 hr.		+	+		13	13
	S79	310	15–16	5 hr.		+				2
	S80	300	15–16	4 hr.						4

	S72	350	17–18	21 hr.		+	+		10	10
	S73	370	16–17	3 hr.		+				4
	S74	320	14–15		+				10	3
	S75	320	15–16		+			35		4
0.35	S91	310	17–18	16–19 hr.					13	5
	S92	320	18–19		+			44		3
	S93	320	17–18		+				3	7
	S94	330	18–19		+				3	5
	S95	330	17–18	4 hr.		+				3
	S96	320	18–19	21 hr.		+	+		9	9
	S60	290	16–17	3½ hr.		+				4
0.40	S61	310	16–17	4 hr.		+				4
	S62	310	17–18	14 hr.		+	+			4
	S63	310	16–17	4 hr.		+				3
	S56	290	17–18	2–15 hr.						6
0.50	S57	300	17–18	2–15 hr.		+				5
	S58	300	18-19	2-15 hr.		+				3
	S59	330	17–18	24 hr.					6	8
	S101	360	18–19		+					4
	S102	360	18–19		+					4
Con-	S103	360	18–19		+					4
trol	S104	340	18-19		+					3
	S105	410	18-19		+					3
	S106	310	18–19		+					4

Used throughout this study was *Escherichia coli* 0127:B8 lipopolysaccharide endotoxin, obtained from Difco Laboratories, Detroit, Michigan. This was dissolved in sterile physiologic saline solution in such a concentration that the indicated dosage of endotoxin would be contained within the range of 0.8 to 1 ml. Each rat was given an intravenous injection of either endotoxin or physiologic saline solution by way of the lateral tail vein. The amount of endotoxin given varied from 0.25 mg. to 0.5 mg. Animals that died as a result of the injection were necropsied, and the lung, liver, spleen, adrenal, kidney, and placenta were examined microscopically. All animals that survived the endotoxin injection were sacrificed at 48 hours and necropsied in the same manner. There were 38 animals in the endotoxin group and 6 in the control group which received physiologic saline injection.

Tissues were fixed in 10 per cent buffered neutral formalin and standard paraffin blocks were cut at 6 μ. Sections were stained with hematoxylin and eosin and phosphotungstic acid hematoxylin.

Results

General Biologic Effects

The general biologic effects of endotoxin in the pregnant rat are listed in Table I. Of 38 animals, 18 (47 per cent) developed the generalized Shwartzman reaction following one injection of bacterial endotoxin. Within the range of 0.25 to 0.4 mg. of endotoxin, the incidence of the Shwartzman reaction remained the same. However, only 2 of the animals given 0.5 mg. showed glomerular thrombi. The rest in this group died of endotoxin shock and died too soon to exhibit the Shwartzman reaction. Of the total group of 38 animals, 16 (42 per cent) had macerated fetuses in the litter. Since some of the rats died before evidence of maceration could be expected to evolve, the 16 animals with macerated fetuses should be related to 22 animals with this possibility, thus giving an incidence of 73 per cent.

With the use of Difco lipopolysaccharide, the amount of 0.4 mg. appeared to be optimal for the production of the generalized Shwartzman reaction since all the animals in this group were affected. It is of interest to note a dissociation between the 3 major effects of endotoxin in this group of experiments. The optimal dosage for producing abortion (intrauterine fetal death) was 0.35 mg. of endotoxin, whereas the optimal dosage for the Shwartzman reaction was 0.4 mg. and that for death in endotoxin shock was 0.5 mg. (Table II).

In the animals given between 0.25 and 0.35 mg. of endotoxin there were 150 fetuses, of which 92 were macerated, an incidence of 61 per cent in each litter.

Pathologic Effects on Placentas

Early Changes. Seventeen of the 38 animals in this study died spontaneously at varying intervals after exposure to bacterial endotoxin. Nine died within 3 to 5 hours and 8 died within 14 to 27 hours, and these

TABLE II

MAJOR EFFECTS OF ENDOTOXIN IN RELATION TO DOSAGE

Endotoxin (mg.)	No. of animals	Macerated fetuses	Shwartzman reaction	Death in endotoxin shock
0.25	10	4	4	4
0.30	10	4	4	4
0.35	10	7	4	5
0.40	4	0	4	4
0.50	4	1	2	4

2 groups have been arbitrarily separated for purposes of analysis. The pathologic changes in the placentas are presented in Table III A.

Among the animals dying within 3 to 5 hours, the earliest histologic changes in the placentas consisted of (a) degeneration of the labyrinthine trophoblast; (b) thrombosis of the maternal blood spaces in the labyrinth and giant cell layer; (c) necrosis of the "clear cells" in the giant cell layer; (d) congestion of the maternal blood spaces in the labyrinth and (e) accumulation of large clumps of platelets in the maternal blood spaces.

Degeneration of the trophoblast of the labyrinth was observed at this early stage in 7 of 9 animals, which included 22 of the 29 placentas examined. This proved to be the most consistent of the early effects of endotoxin (Figs. 1A and B). It was often of focal distribution and was found frequently in association with congestion of that region of the placenta.

Thrombosis of the maternal blood spaces in the labyrinth was present in 4 of 9 animals. It was minimal in amount, focal in distribution and frequently was observed near the "roof" of the placenta, precisely where the central artery pours its blood into the labyrinth (Fig. 2).

Necrosis of the "clear cells" in the giant cell layer was noted in 3 of 9 animals and was usually focal in nature. There was a loss of glycogen from the cytoplasm, a shrinkage of the cell with pyknosis and fragmentation of the nuclei and a separation of the cells (Figs. 3A and B). Not all of the "clear cell" masses participated in this reaction.

One of the most interesting changes from the biologic standpoint was the appearance of large clumps of platelets in the maternal vascular spaces of the labyrinth. These platelet masses can be recognized when they reach the proportions shown in Figure 4, but in all probability occurred on a smaller scale in many of the other placentas.

TABLE III A

PLACENTAL ALTERATIONS IN ANIMALS DYING SPONTANEOUSLY AT VARYING TIMES AFTER EXPOSURE TO ENDOTOXIN

Time of death (hr.)	3	3	3	4	4	4	4	4	5	14	15	18	19	21	21	24	27
Animal number	124	73	60	122	80	95	61	63	79	62	68	129	91	72	96	125	71
Number of placentas examined	3	4	4	3	4	3	4	3	1	4	3	3	6	10	9	3	13
Necrosis of giant cells										+		+	+				+
Congestion of maternal blood spaces in labyrinth			+		+					+	+	+	+	+	+	+	+
Degeneration of labyrinthine trophoblast			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thrombosis of maternal blood spaces in labyrinth	+			+								+	+	+(1)	+		+
Thrombosis of maternal blood spaces in giant cell layer		+						+			+	+					+
Necrosis of yolk sac																	
Necrosis of decidua											+*						
Necrosis of clear cells in giant cell layer			+	+				+		+		+	+				+
Thrombosis of decidual vessels																	
Platelet masses in labyrinth			+		+							+			+	+	

* Minimal, focal.

TABLE III B

PLACENTAL ALTERATIONS IN ANIMALS SACRIFICED AT 48 HOURS AFTER ENDOTOXIN EXPOSURE

	Animal number														Total	Per cent
	119	120	121	126	128	129	64	65	67R	67L	74	75	93	94		
Number of placentas examined	3	3	4	6	9	3	4	4	6	7	3	1	6	3	62	
Necrosis of giant cells		+	+	+	+	+	+	+	+		+	+	+	+	52	85
Congestion of maternal blood spaces in labyrinth		+	+	+	+	+	+	+	+		+		+	+	51	82
Degeneration of labyrinthine trophoblast					+	+	+	+	+	+	+	+	+	+	45	72
Thrombosis of maternal blood spaces in labyrinth		+	+	+	+	+				+		+		+	37	59
Thrombosis of maternal blood spaces in giant cell layer					+	+	+	+	+		+	+	+		36	58
Necrosis of yolk sac			+		+	+				+				+	29	46
Necrosis of decidua				+	+	+			+			+			27	43
Necrosis of clear cells in giant cell layer			+	+	+	+						+		+	24	38
Thrombosis of decidual vessels				+											6	9

During the period 14 to 27 hours the same changes were observed, but for the most part, all alterations were more frequent and more widespread. Congestion became as constant as degeneration of the labyrinthine trophoblast. Clear cell necrosis was observed in one half of all the placentas examined. The incidence and extent of thrombosis in the maternal vascular spaces increased. Disintegration of the large platelet clumps in the labyrinth with the appearance of fibrin strands between the platelet clumps at 21 hours is shown in Figure 5.

Two additional changes were also noted, i.e., the appearance of focal necrosis of the giant cells and focal necrosis of the decidua. These lesions were minimal in extent but represented the onset of alterations that reached major proportions at 48 hours.

Late Changes. Thirteen* of the animals survived for 48 hours and were sacrificed. The pathologic features in the 62 placentas examined in this group are presented in Table III B, where they are listed in order of decreasing incidence. The most consistent changes were (a) necrosis of giant cells (Fig. 6); (b) congestion of the maternal blood spaces in the labyrinth; (c) degeneration of the labyrinthine trophoblast; and (d) thrombosis of the maternal blood spaces in the labyrinth (Fig. 7).

At 48 hours necrosis of the decidua became more frequent and more extensive (Figs. 8 and 9) and was occasionally associated with thrombosis of decidual vessels. Extravasation of blood into the decidua and between the giant cell layer and the decidua was occasionally noted. Necrosis of the yolk sac epithelium was also found at 48 hours.

INTERPRETATION

Sequence of Pathologic Changes in Placenta

After exposure of the blood stream to bacterial endotoxin, the most constant of the initial alterations in the placenta was degeneration of the labyrinthine trophoblast. Although this was probably the single most important early change from which most of the subsequent damage stemmed, other types of damage occurred concomitantly but less frequently. These were congestion of maternal vascular spaces in the labyrinth, focal thrombosis of maternal blood spaces in the labyrinth and accumulations of platelets in the same location. All of these represented mechanisms causing slowing of or actual obstruction to the flow of blood in the labyrinth which, by depriving the trophoblast of its oxygen supply, inflicted further damage on this tissue.

In time, with the degeneration of the trophoblast lining the vascular spaces through which the maternal blood circulated, more platelets

* Animals that delivered or who died of endotoxin shock have not been included in the analysis of placental changes.

accumulated on this damaged "endothelium." The agglutination of platelets was soon followed by viscous metamorphosis and release of platelet clotting factors. In a short time this led to fibrin deposits and thrombosis of the labyrinthine channels. Within at least 14 hours, the thrombosis spread to the maternal blood channels in the giant cell layer which drain the labyrinth. This caused infarct necrosis of giant cells and a more extensive necrosis of the "clear cells." The thrombotic process extended beyond the giant cell layer into the veins of the decidua where obstruction led to infarct necrosis of the decidua. Infarction and vascular obstruction in the decidua resulted in retroplacental hemorrhage as well as marked congestion of the labyrinth due to back pressure. Eventually some of the placentas were the seat of complete infarction. It is quite likely that necrosis of the yolk sac was secondary to the death of the embryo.

It is worth emphasizing that although this sequence appeared to be initiated by a degeneration of the labyrinthine trophoblast, other changes of a functional nature probably preceded the anatomic change.

Comparison with Placental Damage in the Generalized Shwartzman Reaction Produced by Diet

It has recently been observed that the generalized Shwartzman reaction may be produced in pregnant rats by a diet containing oxidized oil[7] and that in addition to the disseminated intravascular thrombosis which characterized the Shwartzman reaction, there were a variety of anatomic changes in the placentas of these animals.[8] It has seemed important to compare the placental changes with those produced by endotoxin since the end results, both systemic and local, were so similar, despite the differences in the etiologic agents.

Both the oxidized lipid diet and the injection of endotoxin caused (a) degeneration of the labyrinthine trophoblast; (b) thrombosis of lacunas of the giant cell trophoblast; (c) congestion of the labyrinth; (d) fibrin deposits in the labyrinth; (e) retroplacental hemorrhage; (f) decidual necrosis; (g) decidual or uterine vein thrombosis; and (h) macerated fetuses. Another important similarity was the fact that the most constant of the earliest histologic changes in each was the degeneration of the labyrinthine trophoblast.

In spite of these similarities, certain significant differences exist: (1) Animals given injections of endotoxin exhibited no evidence of placentitis whereas 10 per cent of animals on the oxidized lipid diet had a purulent placentitis. (2) The incidence of macerated fetuses was higher in endotoxin-treated animals. (3) Infarct-like necrosis of the giant cell layer and of the "clear cells" was much more extensive in endotoxin-

treated animals. (4) Intra-uterine hemorrhage was more frequent in animals on the oxidized lipid diet. Degeneration of the labyrinthine trophoblast persisted for at least 5 days prior to the development of infarctive lesions in the animals on the oxidized oil diet. On the other hand, it was of only a few hours' duration in endotoxin-treated animals. Although some of the mechanisms of placental damage and intra-uterine fetal death are shared by these two experimental conditions, these differences would indicate that there was not a complete identity of mechanisms.

Mechanisms Involved in Placental Damage

A brief review of the known basic effects of bacterial endotoxin on the animal organism seems appropriate before considering the possible mechanisms of fetal and placental damage. The basic effects of bacterial endotoxin can be grouped under 3 major headings: (a) vasomotor; (b) thrombotic; and (c) metabolic.

Vasomotor Effects.[9] Although species differences exist, there are certain reactions common to all when the blood stream is exposed to endotoxin. The blood pressure falls to low levels, and in most instances the hypotension may be of such degree that by itself it is a sufficient cause of death. The hypotension results from a drop in cardiac output, which is probably due to a decreased venous return to the heart. The decreased venous return is caused by a redistribution of blood, with a pooling in the splanchnic bed as a result of obstruction to flow in the liver. In certain experiments, the additional factor of a decreased total peripheral resistance may contribute to the hypotension. In general, there is a sequence of early constriction followed by dilation.

The studies by Zweifach and Thomas[10] of the mesenteric vessels have shown several changes in the small vessels after exposure of the blood stream to massive (lethal) doses of bacterial endotoxin. Fifteen to 20 minutes after injection there is an erratic opening and closing of the small arterioles and precapillary sphincters. After one hour the capillary bed appears normal. Following this there appears to be a slowing of the blood, particularly in collecting venules, with no measurable changes in the size of the arterioles or precapillaries. Spontaneous vasomotor movements disappear by the third hour.

The experiments of McKay and Rowe[11] indicate that dilation of capillary beds of the lungs, liver and kidneys occurs in the rabbit. There is considerable variation in the timing of this dilation in the different organs and in the response to two doses of endotoxin. Because of the variation from one organ to another, it is quite likely that the placental and uterine vessels will exhibit their own unique type of response. The

best illustration of the significance of the vasomotor action of endotoxin lies in the recent demonstration by Palmerio, Ming, Frank and Fine[12] that unilateral sympathectomy prevents the renal thrombosis on the denervated side in the generalized Shwartzman reaction. With respect to the placenta, it is of great interest to note that this organ is without a nerve supply of any kind. However, effects on the uterine vessels which are innervated could have a profound influence on placental circulation.

Thrombotic Effects. In the proper dosage, bacterial endotoxin produces disseminated intravascular coagulation[13] with thrombosis of arterioles, capillaries and venules. The lung, liver, spleen and kidney are the organs most frequently involved. The disseminated clotting is accompanied by marked alterations in the hemostatic mechanism of the circulating blood. The effect on platelets probably represents the initial change from which most of the others stem. Platelets are agglutinated *in vitro* and destroyed by bacterial endotoxin. *In vivo,* platelets accumulate in small vessels of the lung and other organs and are subsequently destroyed. With their destruction comes the release of the platelet factors which in combination with plasma factors result in the formation of thromboplastin.

Metabolic Effects. Woods, Landy and Shear[14] have observed that endotoxins exert a stimulatory effect on cell glycolysis that is quite similar to the action of insulin. Endotoxin in concentrations of 0.003 to 0.3 μg. per ml. stimulates the formation of acid aerobically from glucose several fold, while oxygen uptake remains essentially unaffected. Anaerobic glycolysis is stimulated to a lesser extent. Endotoxin, like insulin, is also capable of reversing the inhibitory effect of stress on glycolysis in certain tumors. These effects are not confined to tumors, since Cohn and Morse[15] noted glycolytic stimulation, without respiratory impairment with rabbit polymorphonuclear leukocytes. The increased glycolytic activity is accompanied by an increased phagocytic activity. Yunis and Harrington[16] found that a high concentration of endotoxin causes an increased rate of adenine incorporation into the ribonucleic acid of leukocytes. Giger[17] has demonstrated a stimulation of glycolysis in mitochondrial preparations of melanoma and of brain with endotoxin as well as insulin. Snell[18] has shown that the inhibitory effect of cortisone on chromium phosphate uptake by Kupffer cells in mice *in vivo* can be overcome by bacterial lipopolysaccharides.

Mechanisms of Placental and Fetal Damage

Placental damage can be ascribed with certainty to some of the known basic effects of endotoxin but only in a speculative manner to others.

A sorting out of the known from the possible effects should lead to more definitive studies.

Vasomotor Damage. The occurrence of congestion of the labyrinth implies a vasomotor effect on uterine or placental vessels. In some instances the congestion can be related to organic occlusion of outflow vessels by thrombosis, but this is not a constant accompaniment of congestion. In the immediate period following endotoxin injection, the dilation and congestion of these small maternal vessels of the placenta may be analogous to that seen in other capillary beds, such as those of the lung, liver and kidney.

The possibility that the local pooling of blood in the placenta might be mediated unspecifically by the systemic hypotension was raised by Rieder and Thomas.[4] However, they failed to produce abortion in mice by shock due to intravenous glycogen administration. In addition, they separated the aborting from the lethal effects of endotoxin by giving cortisone, which greatly diminished the lethal action but had no effect on the abortifacient action. Hypotension alone would seem to be an unlikely cause of fetal death, but still might account for local changes in placental blood flow.

The effect of serotonin on the placenta must be taken into account. Shimamoto and associates [19] have shown that bacterial endotoxin causes serotonin to appear in the plasma of the rabbit. Since platelets are the major source of serotonin in this species, platelet destruction could account for the increased plasma levels. Craig has reported that serotonin, in the amount of 10 mg. per kg., causes intra-uterine fetal death in rats.[20] A prompt fall in blood pressure occurs, accompanied by death of the fetuses in half an hour. A demonstrable decrease in the circulation of the placenta is present ½ hour after serotonin injection, and 12 hours later there is a massive infarct-like necrosis of this tissue. The placental fetal damage in these experiments follows intraperitoneal injection. Furthermore, serotonin appears to be released in microgram amounts into the plasma of rabbits but was of milligram proportions in Craig's injections; this might exceed the range from endogenous sources. Whether or not placental damage following intravenous endotoxin administration can be ascribed to serotonin release is problematic.

Another vasomotor-active agent, i.e., histamine, is also present in platelets, and with their destruction is released into the plasma. The possible additive effects of serotonin and histamine on the vascular beds must be considered.

Thrombotic Damage. Although it is clear enough that thrombosis of the placental and uterine vasculature is a major effect of endotoxin and is responsible for much of the ultimate necrosis, the question as to

whether it is a primary or secondary phenomenon remains to be elucidated. Thrombosis in the placenta can be attributed to two factors: (a) the clot-promoting effect of endotoxins on the general circulation with lodgment of platelets and fibrin in the maternal vascular spaces, and (b) damage to the trophoblast, which in essence amounts to damage of the maternal "endothelial" lining of the placenta, with local deposits of platelets and fibrin. The focal deposits of platelets and fibrin in the first 3 to 5 hours after endotoxin are probably due to the general blood coagulative effect of endotoxin. These deposits for the most part do not appear to be extensive enough to be responsible for the death of the embryo. Rieder and Thomas[4] have shown that heparin does not prevent intra-uterine fetal death in pregnant mice injected with endotoxin. Parenthetically, this is quite in contrast to the fact that heparin prevents endotoxin damage to the liver, lung, spleen and kidney. The weight of evidence supports the idea that the early minimal thrombosis is not the cause of intra-uterine fetal death, and that the extensive thrombosis and consequent extensive infarction of placental tissue after 24 hours is secondary to an initial local damage to the trophoblast.

Metabolic Damage. Several observations suggest that much of the early damage to the placenta is mediated by the metabolic effects of endotoxin. As a preface, the duration of endotoxin in the circulation and its fate in the body deserve attention. The distribution of injected endotoxin has been studied by use of endotoxin labeled with Cr^{51}, P^{32} and fluorescein.[21-23] Chromium[51] tagged endotoxin injected into rabbits is rapidly concentrated in the buffy coat of the plasma and is not found in the red cells. After 1 hour the plasma level falls to $\frac{3}{5}$ and by 2 hours to $\frac{1}{12}$ of the starting level. Sixty to 75 per cent of P^{32} tagged endotoxin disappears in 8 minutes from the blood of mice or guinea pigs, while the remainder persists for hours. Ultimately, tagged endotoxin is found largely in the liver, spleen and lung. The reticuloendothelial system appears to be active in the plasma clearance of endotoxin. Obviously endotoxin persists for long enough periods in the circulating blood to allow many passages through the placenta and to permit a thorough direct exposure of the trophoblast to the toxin.

It is possible that the early degeneration of the labyrinthine trophoblast within the first 5 hours is due to a direct toxic effect of endotoxin. This degeneration is the most consistent early change in the placenta and may be analogous to the damage inflicted by endotoxin on tumor cells.[24] The fact that endotoxin exerts an anaerobic as well as aerobic glycolytic effect is of particular interest with respect to the "clear cells" of the giant cell layer. The cytoplasm of these cells is filled with glycogen, and under the influence of endotoxin the glycogen is lost, the cytoplasm

shrinks, and the cells become separated and ultimately undergo necrosis. The implication that the metabolic effects of endotoxin may be responsible for degeneration of the trophoblast applies only to the early period of 3 to 5 hours after endotoxin exposure. It is quite obvious that much of the necrosis of these cells seen at 48 hours is due to thrombosis and ischemic infarction.

Death of the Fetus. It is possible that the major action of endotoxin in producing abortion is a direct lethal effect on the fetus. Although Zahl and Bjerknes[1] were unable to demonstrate pathologic changes in fetuses of endotoxin-treated mice, and although young animals are generally resistant to the lethal effects of endotoxin, the possibility remains. Endotoxin is capable of exerting its lethal effects without leaving a histologic trace, and the amount of endotoxin required to kill a fetus *in utero* is not known. The major unanswered question is whether or not endotoxin can traverse the "placental barrier." Since endotoxin is a lipopolysaccharide of large molecular weight, this would seem unlikely. Also, it is well known that foreign antigens generally are incapable of crossing the trophoblast from maternal to fetal circulation. It is of some consequence that McKell, Helseth and Brunson[25] have shown that endotoxin changes the permeability of the placenta so that macromolecules can enter the fetal circulation after endotoxin injection into the maternal blood stream. Trypan blue and colloidal iron injected into the aorta of pregnant rabbits are not found in the fetal circulation in normal animals. In those given endotoxin, the dyes are found 4 hours later in the liver, kidney and cerebral capillaries of the fetus. It is conceivable that damage to the trophoblast by endotoxin might allow passage of the endotoxin molecule into the fetal circulation in the same manner.

These observations leave unanswered the questions of whether or not bacterial endotoxin crosses the placental trophoblast, whether the platelets damaged by the endotoxin release enough serotonin or histamine to cause fetal death, and the precise time when intra-uterine fetal death occurs after endotoxin administration.

Summary

The injection of bacterial endotoxin into the veins of pregnant rats caused death in endotoxin shock, the generalized Shwartzman reaction and intra-uterine fetal death, depending on the dose of endotoxin given. In the animals dying within 3 to 5 hours, the earliest histologic changes in the placenta consisted of (a) degeneration of the labyrinthine trophoblast; (b) minimal focal thrombosis of the maternal blood spaces in the labyrinth and giant cell layer; (c) focal necrosis of the "clear" cells in

the giant cell layer; (d) congestion of the maternal blood spaces in the labyrinth; and (e) accumulation of clumps of platelets in the maternal blood spaces. Between 14 to 27 hours these changes increased in incidence and extent. At 48 hours the surviving animals were necropsied. By this time extensive thrombosis and necrosis of the placental and decidual tissue was found.

These pathologic alterations are similar in many ways to those in the placentas of animals subjected to the diet-induced generalized Shwartzman reaction. An attempt has been made to explain some of the changes on the basis of known effects of endotoxin. These include (a) vasomotor, (b) thrombotic, and (c) metabolic phenomena.

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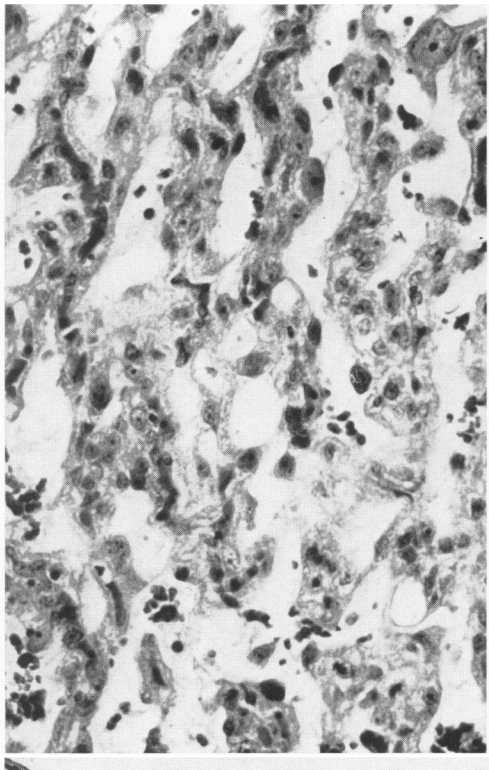
LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

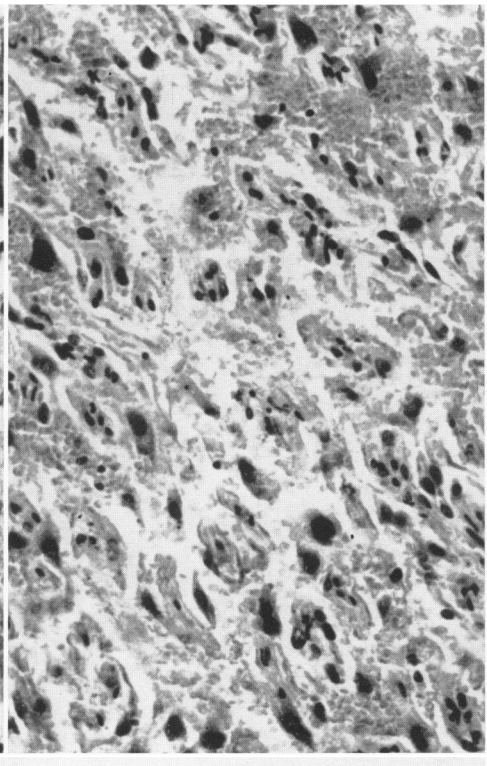
FIG. 1A. Normal labyrinth. Compare with Figure 1B. Rat S119. × 160.

FIG. 1B. Degeneration of labyrinthine trophoblast following endotoxin. The trophoblastic cells lining the maternal blood spaces are shrunken and have pyknotic nuclei. The vascular channels are packed with red blood cells. Rat S64. × 160.

FIG. 2. Thrombosis of labyrinth at the end of the central artery near the roof of the placenta. Rat S121. × 65.



1A



1B

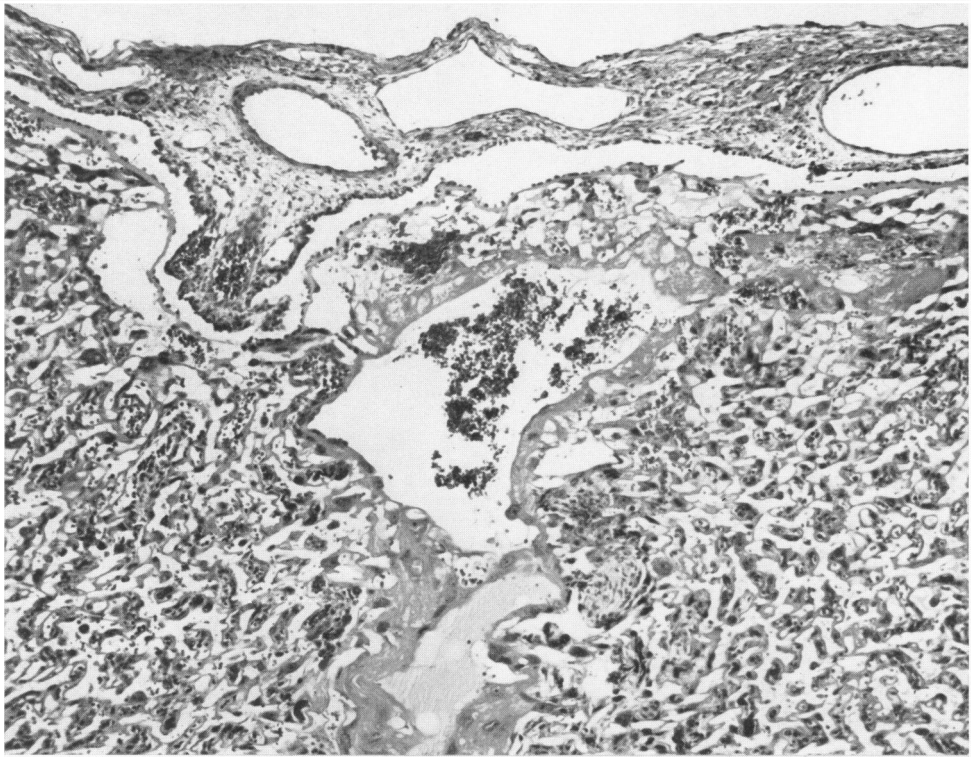


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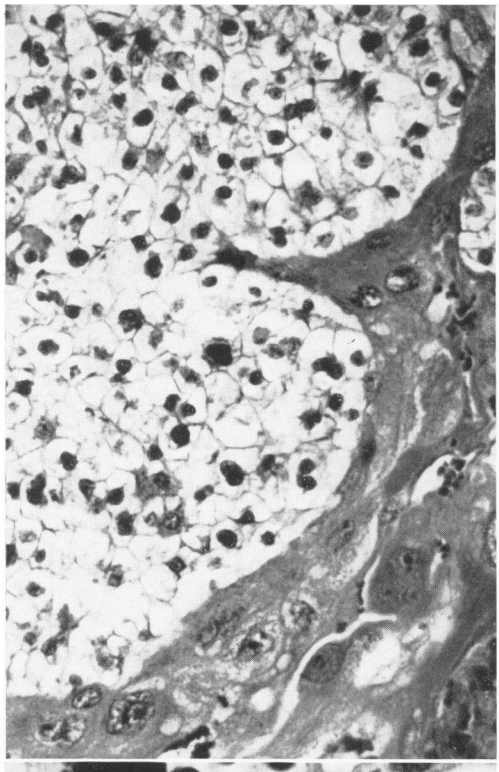
FIG. 3A. Normal giant cell layer with two clusters of "clear cells." Compare with Figure 3B. Rat S119. × 160.

FIG. 3B. Early degeneration of clear cells. The cells have lost their glycogen and present evidence of early necrosis. Rat S122. × 160.

FIG. 4. Platelet clusters in the maternal blood spaces of the labyrinth, 4 hours after endotoxin injection. Rat S60. × 400.

FIG. 5. Twenty-one hours after endotoxin the platelet masses are laced with fibrin strands. Rat S96. × 400.



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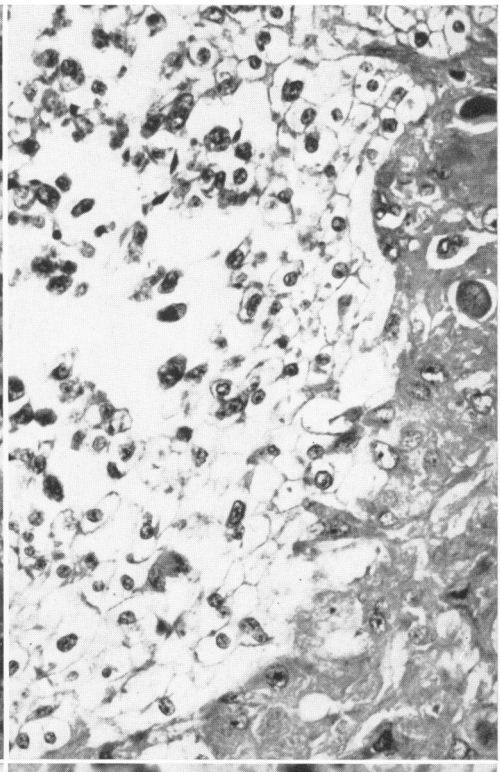

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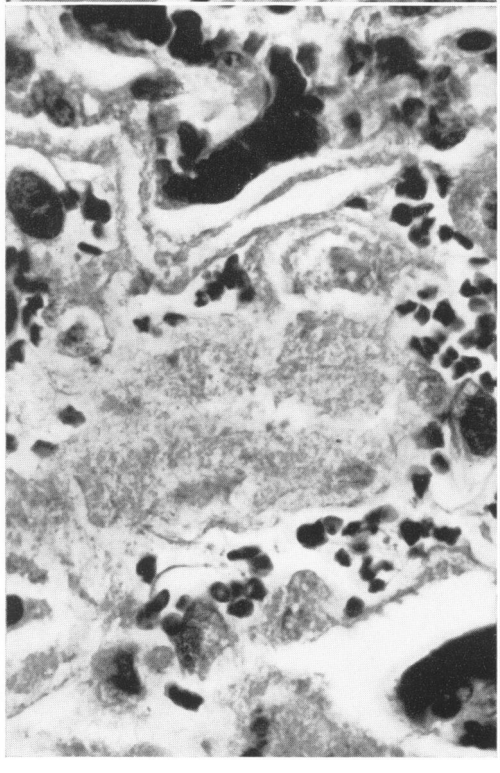

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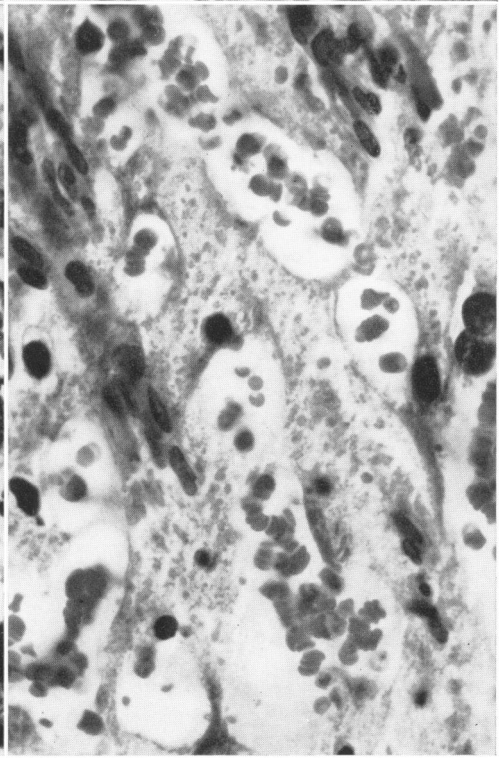

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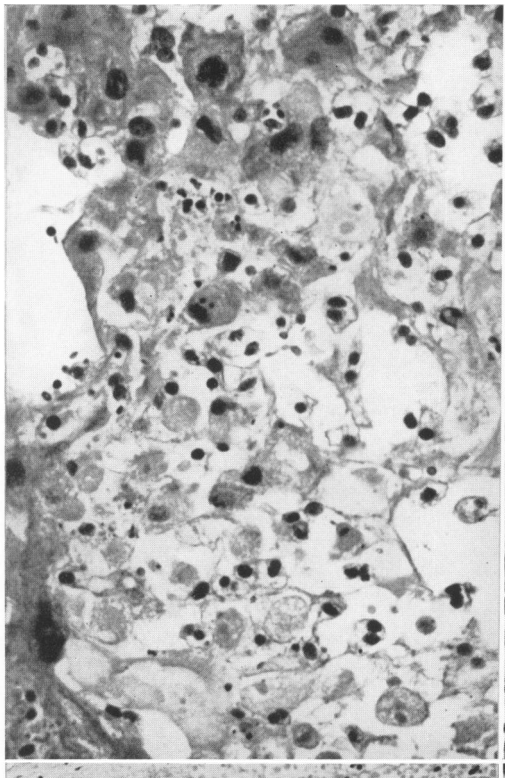
FIG. 6. Infarct-like necrosis of giant cell layer at 48 hours. Rat S126. ×160.

FIG. 7. Thrombosis of the maternal blood spaces of the labyrinth. Rat S121. ×160.

FIG. 8. Necrosis of the decidua. Rat S126. × 65.

FIG. 9. High power view of decidual necrosis, same animal as Figure 8. × 160.



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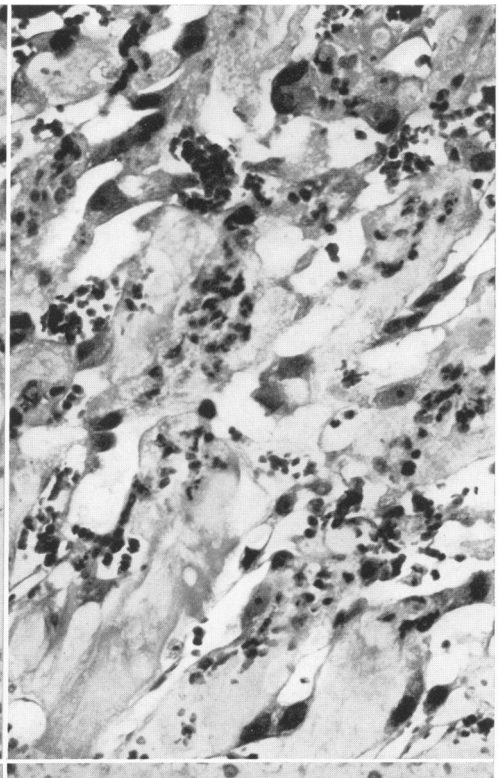

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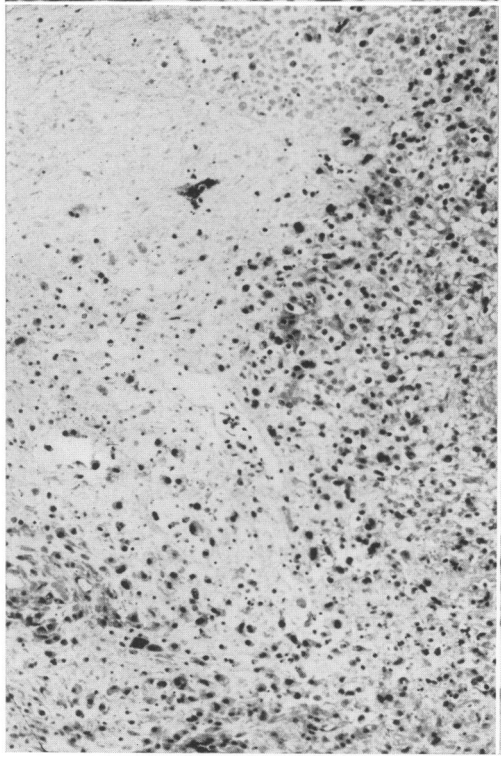

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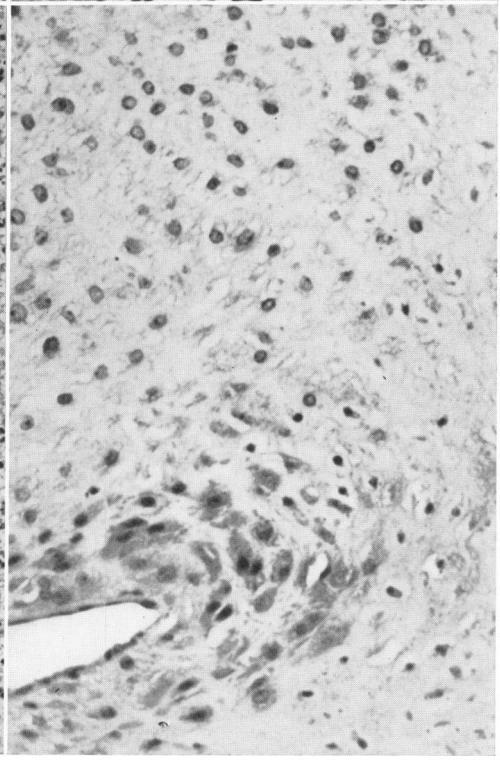

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