Occlusive Vascular Lesions Induced by Bacterial Endotoxin in Kidneys of Pregnant Rats.* (27235)

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Two appropriately spaced, intravenous injections of bacterial endotoxin produce in kidneys of rabbits cortical necrosis and deposition of fibrinoid in the glomerular capillaries, characteristic of the generalized Shwartzman reaction(1). Comparable pathologic alterations can be obtained by a single injection of endotoxin into pregnant rabbits(2,3), suggesting that pregnancy may represent a state of increased reactivity similar to that produced by the preparatory dose of endotoxin in production of the generalized Shwartzman reaction. Although rats are normally resistant to endotoxin, our previous observations indicate that rats with desoxycorticosterone hypertension manifest increased renal vascular reactivity to endotoxin resulting in development of fibrinoid thrombi in glomerular capillaries(4).

The above considerations led us to investigate the effects of endotoxin in the pregnant rat. The present report is based upon initial observations made during this study of the increased susceptibility of pregnant rats to some of the effects of endotoxin.

Materials and methods. Primagravida Carworth Farm Nelson rats and non-pregnant females of the same strain and age were used throughout the experiment. Animals were used either on the 12th day (second trimester) or on the 18th day (third trimester) of their pregnancy. The bacterial endotoxin employed was commercially prepared *E. coli* lipopolysaccharide (# 0-111 B:4, Difco)§, the biological activity of which was previously established in our laboratory. Endotoxin was dissolved in physiological saline and administered intravenously *via* the tail vein, in doses of 800 μg per rat in 0.5 ml saline, whereas control animals received saline in similar amounts. Rats that died after injection were autopsied immediately. Surviving animals were killed and autopsied 24 hours after injection. Animals exhibiting vaginal bleeding, intra-uterine hemorrhage or dead foetuses were considered to have aborted. Tissues were fixed in Zenker's solution and sections were stained with hematoxylin-eosin and the periodic acid-Schiff (PAS) stains.

Results. Experimental results are summarzied in Table I. All rats given 800 μg of *E. coli* endotoxin exhibited prostration and diarrhea shortly after injection. While a majority of normal rats and all of the animals in the second trimester of pregnancy survived, 12 of 18 animals in the third trimester of pregnancy died within 24 hours after endotoxin administration. A high incidence of abortions also was noted in the latter group.

Grossly the kidneys were pale in all rats that received endotoxin. In addition, all animals in the second trimester of pregnancy exhibited a moderately cyanotic uterine wall; the foetuses, however, were alive and active. Inconstant gross morphologic changes observed in animals treated in the third trimester of pregnancy included congested liver, severely congested lungs with focal hemorrhages, and moderate focal hemorrhages in the wall of the gut. A majority of animals within this latter group revealed extensive hemorrhage into the uterine lumen, placentas which were detached from the uterine wall, and dead foetuses in varying stages of maceration.

Microscopic examination revealed no renal lesions in any of the control rats, in pregnant animals which had received saline, or in animals injected with endotoxin on the 12th day of pregnancy. Conversely, a striking glome-

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TABLE I. Influence of Pregnancy on Response to Endotoxin.

Time of pregnancy (day)	No. of animals	Treatment	No. of deaths	No. of abortions	Occurrence of occlusive glomerular lesions
18*	18	800 μg *E. coli* LPS	12	15	10
12†	16	*Idem*	0	0	0
18	6	Saline	0	0	0
12	6	"	0	0	0
Control (non-pregnant)	20	800 μg *E. coli* LPS	2	—	0

* Third trimester. † Second trimester.

rular lesion was demonstrable in a significant number of gravid animals given injections of endotoxin on the 18th day of pregnancy (Fig. 1). Widespread deposits of fibrinoid material, occluding glomerular capillary lumina were conspicuous. The distended capillaries were occluded with an eosinophilic acellular homogeneous substance which was PAS positive and lacked fibrillar or granular quality. Similar material was present in the lumina of a few afferent arterioles. The remaining portions of the nephrons, collecting tubules and larger blood vessels showed no pertinent pathology. Proteinaceous tubular casts were

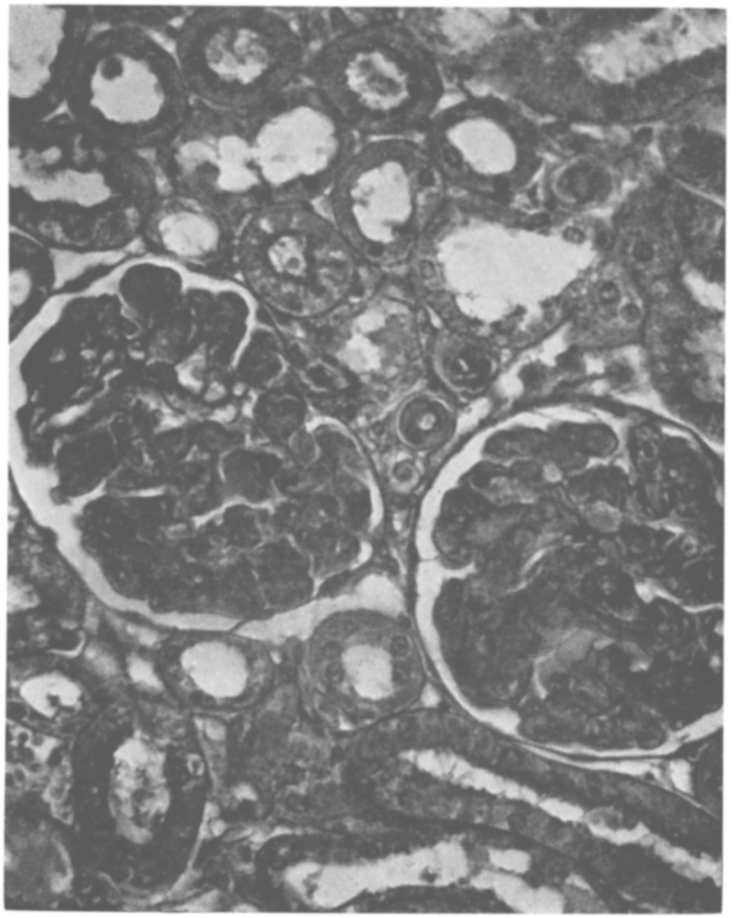

FIG. 1. Section of kidney from pregnant rat, killed 24 hr after intrav. administration of 800 μg endotoxin, on 18th day of pregnancy. Glomerular capillary lumina are occluded by fibrinoid thrombi. PAS, × 400.

present in large numbers. All gravid, endotoxin-treated animals showed depletion of hepatic glycogen; furthermore, some of the animals with renal lesions exhibited focal necrosis of hepatic cells with neutrophil infiltration.

Renal involvement was not necessarily associated with lethality, as evidenced by the fact that occasional animals with severe glomerular damage survived, while some of the animals that did not develop the lesions died shortly after endotoxin administration. Fibrinoid thrombi appeared in renal glomeruli of rats as early as 3 hours after injection of endotoxin.

Discussion. Although the rat is highly resistant to the lethal effects of bacterial endotoxin, our results clearly indicate that in a high percentage of pregnant rats injection of a single dose of endotoxin can produce renal lesions similar to those obtained in the rabbit by 2 properly spaced injections. It is of interest that the "classic" generalized Shwartzman reaction has not been induced in the rat. Renal alterations bearing some similarity to it, however, have been produced in the above species by injection of endotoxin in conjunction with sodium polyanetholsulfonate (Liquoid)(5), or by simultaneous or spaced injections of nephrotoxic serum and endotoxin(6). In these instances bilateral cortical necrosis has been noted together with fibrinoid deposits in glomerular capillaries. The absence of tubular damage in kidneys of pregnant rats may argue against the concept that the morphologic changes observed represent a bona fide Shwartzman reaction. It should be pointed out, however, that glomerular capillary occlusion by fibrinoid presumably precedes cortical necrosis and that, in the present experiments, anmials died or were sacrificed within 24 hours after injection of endotoxin, perhaps too short a time for cortical necrosis to develop.

A generalized Shwartzman reaction has been described in the pregnant rabbit following a single dose of endotoxin(2,3). According to McKay and his associates(7), this reaction may represent a prototype for the pathogenesis of human eclampsia, a disorder characterized by widespread intravascular deposits of fibrinoid in various organs, including the kidney. Administration of large doses of progesterone to rats in the last third of pregnancy has been reported by Symeonidis(8) to cause an eclampsia-like syndrome, with abortion of the embryos as well as the appearance of glomerular intracapillary thrombi and tubular necrosis in the kidneys of mothers. This finding has not been confirmed by other investigators(9). The increased sensitivity of both pregnant rats and rabbits to bacterial endotoxin would appear to support the suggested significance of the generalized Shwartzman phenomenon in human disease.

How the last third of pregnancy, as opposed to the second trimester, mediates increased reactivity to endotoxin, and whether hormonal changes or an increase in coagulability of blood at this critical stage of gestation account for some of the observations, is not clear. Arndt, *et al.*(10) reported that a single intradermal injection of endotoxin elicited the local Shwartzman reaction in mice with demonstrable Gram-negative infection of the lungs. The possibility exists then that an increase or change in the bacterial flora of pregnant animals may, in the absence of manifest disease, provide for "endogenous" preparation.

An increase in the lethal and abortive effects of endotoxin in the third trimester of pregnancy has previously been observed in mice by Rieder and Thomas(11), who pointed out that "the abortion reaction is not a placental analogue of either the local or generalized Shwartzman reactions." Since in our experiments all of the rats which developed renal lesions also aborted, the possible contribution of placental factors to the causation of renal pathology cannot be disregarded.

Summary. The effects of a single dose of endotoxin on pregnant rats were studied. Animals treated in the second trimester of pregnancy exhibited no abortions, no pertinent renal pathology and no increase in mortality rate as compared with corresponding normal animals. In contrast, a majority of rats given endotoxin during the third trimester of pregnancy aborted and died within

24 hours. In addition 10 of 18 animals within the latter group developed an occlusive glomerular lesion, the microscopic features of which were similar to those seen in the generalized Shwartzman reaction. It is suggested that pregnancy in the rat as well as in the rabbit may substitute for the preparatory phase of this reaction.

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Binding C^{14} Labeled Histamine by Normal Human Serum. (27236)

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It has been observed that normal human serum binds some biologically active amines such as histamine, serotonin, acetylcholine and putrescine(1-3). The amines bound lose their biological activity. The serum constituent responsible for the binding has been identified as a gamma-globulin and given the name plasmapexin I(4). It has also been shown that the binding ability is impaired in the serum of individuals who have shown clinical manifestations of allergy even when these were quiescent. These findings have been confirmed by a number of workers using a variety of technics (reviewed in 5). Kaplan and Davis(6), however, failed to observe the binding of C^{14} labeled histamine by serum using an equilibrium dialysis method. The present paper reports the results of experiments done by a similar technic.

Methods. Partially purified plasmapexin I was prepared from normal human serum. After dilution 1/15 in distilled water, the pH was adjusted to 6.5. The precipitate was discarded and pH reduced to 5.2. The resulting precipitate, containing plasmapexin I, was dissolved in Tyrode's solution and made up to the original volume of serum.

Histamine dihydrochloride labeled with C^{14} in C_2 was used (kindly supplied by Dr. R. W. Schayer and later obtained from The Radiochemical Center, Amersham, England) containing 52 μC per 2 mg with an activity of 11.1×10^8 β/min/2π. Since the counter had an efficiency of about 3% of counts, the real activity of 52 μC of the histamine dihydrochloride was 3.3×10^6 cpm. The solution of labeled histamine was diluted ½ with nonisotopic histamine, adjusted to pH 2.2 and kept at −10°C. Immediately before the experiment the required dilution was made up in Tyrode's solution.

For equilibrium dialysis, 2 ml of a histamine dihydrochloride solution (at concentrations between 1.6×10^{-5} and 2.2×10^{-4} M) were placed in a cellophane bag, together with 1 ml of the plasmapexin I solution containing the same amount of histamine added with 1 ml of Tyrode's solution. Before the experiment, the cellophane was soaked in the histamine solution to eliminate the possibility of small amounts of histamine being adsorbed on it during the dialysis.