(17) are strikingly similar to our experimental results.

*Summary.* Autorepopulation studies using a radiobiological method provide evidence that endogenous hemopoietic stem cell proliferation in the shielded mouse occurs during the first 5 days after irradiation. Exposure to hypoxia, sufficient to produce elevated levels of endogenous erythropoietin, failed to induce erythroid differentiation during the early proliferative phase of marrow repopulation. The data support the concept that the replication of stem cells, *per se*, renders them refractory to the induction of erythroid differentiation by erythropoietin.

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## Effect of Saline Infusion and Norepinephrine on Response of the Kidney to Bacterial Endotoxin.* (32472)

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Exposure of the blood stream to endotoxin derived from gram negative bacteria results in a progressive elevation of circulating catecholamines(1,2,3). One of the early indications that the adrenergic system is involved in the pathogenesis of the generalized Shwartzman reaction came from the experiments of Palmerio *et al*(4) who prevented the reaction in rabbits in the kidney which had been subjected to sympathetic denervation of the renal pedicle. Further support came from the experiments of Müller-Berghaus, when he showed that two alpha adrenergic blocking agents, dibenamine and dibenzyline, significantly reduced the incidence of glomerular thrombosis in pregnant rats exposed to a single small dose of bacterial endotoxin(5).

These experiments indicate that stimulation of the alpha adrenergic receptor sites in the kidney are necessary for localization of fibrin thrombi in the glomeruli in the evolution of the generalized Shwartzman reaction.

If this is true then it follows that it should be possible by the appropriate experiment to use alpha adrenergic stimulation to localize the thrombotic process in the glomeruli after triggering the clotting mechanism with bacterial endotoxin(6) in an animal not

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ordinarily susceptible to the generalized Shwarzman phenomena.

To test this hypothesis the rat was chosen as the experimental animal for two reasons: 1) Alpha adrenergic blockade prevented the generalized Shwartzman reaction in pregnant rats given one injection of endotoxin and 2) non-pregnant rats do not develop the generalized Shwartzman reaction after one dose of endotoxin and reportedly do not do so even after two appropriately spaced injections.

*Materials and methods.* Non-pregnant female Columbia Sherman rats, weighing approximately 200 g and maintained on a normal Rockland pellet diet received in varying combinations:

1. *E. coli* endotoxin purified lipopolysaccharide.‡ It was dissolved in normal saline to a concentration of 0.2 mg/ml. Rats received 0.2 mg injections intravenously *via* the tail vein over a period of 10 seconds.

2. Five percent dextrose in normal saline, 40 ml, intravenously *via* the tail vein, over a 4 hour period.

3. Norepinephrine (as levarterenol bitartrate). One-tenth base was dissolved in 40 ml of 5% dextrose in saline and infused over a 4 hour period. Norepinephrine concentration varied from 0.025 mg to 4.0 mg/40 ml.

4. Dibenzyline (phenoxybenzymine hydrochloride§) was diluted from vials of 100 mg/2 ml to 0.5 mg/ml. Rats received 0.5 mg/kg body weight intravenously *via* tail vein 1-2 hours prior to the endotoxin injection. Dibenzyline was infused over a period of one minute.

5. Ether anesthesia was used during the 4 hour infusions of intravenous fluid.

Histologic examination of the kidneys, lungs, spleen and liver utilizing hematoxylin-eosin stain was performed on all rats.

The following groups were studied:

1. *Endotoxin alone.* Eight animals were given 0.2 mg of endotoxin intravenously in 10 seconds and were sacrificed at the end of 4 hours.

2. *Ether anesthesia plus endotoxin.* Eight animals were given 0.2 mg of endotoxin intravenously in 10 seconds and then were anesthetized with ether over a 4 hour period, at the end of which they were sacrificed and autopsied.

3. *Norepinephrine infusion.* a. Six animals were infused with 0.025 mg of norepinephrine in 40 ml of dextrose/saline for 4 hours, at which time they were sacrificed and autopsied.

b. Four animals were infused with 0.50 mg of norepinephrine in 40 ml of dextrose/saline for 4 hours and then autopsied.

c, d, and e. Eleven animals were infused with 1.00, 2.00 and 4.00 mg of norepinephrine in 40 ml of dextrose/saline. Most of these animals died between 9 to 60 minutes after onset of the infusion and were autopsied immediately.

4. *Ether, endotoxin, norepinephrine and dextrose/saline.* Thirty-six animals were anesthetized with ether, received 0.2 mg endotoxin in 10 seconds, and an intravenous infusion of 0.025 mg norepinephrine in 40 ml dextrose/saline over a 4 hour period at the end of which they were sacrificed.

5. *Pretreatment with dibenzyline.* Nine animals were pretreated with 0.5 mg of dibenzyline 60-90 minutes prior to injection of endotoxin and were otherwise treated the same as group 4.

6. *Ether anesthesia, endotoxin and saline infusion.* Twenty-one animals were anesthetized with ether, given 0.2 mg of endotoxin intravenously in 10 seconds and infused with 40 ml dextrose/saline over a 4 hour period.

7. *Pretreatment with dibenzyline.* Fourteen animals were pretreated with dibenzyline and then treated the same as group 6.

8. *Twenty-four hour survival.* Thirteen animals were treated the same as group 4 but allowed to live 24 hours to determine whether the glomerular thrombosis was transient or persistent.

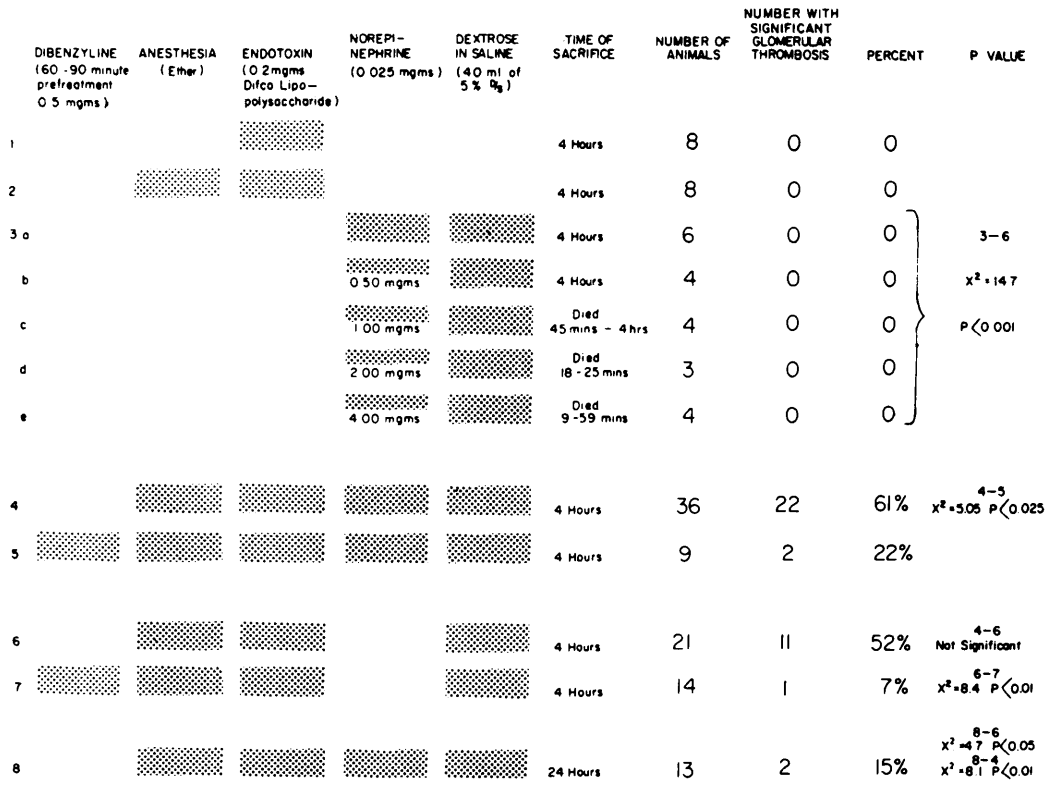
*Results.* The results of these experiments are presented in Table I.

None of the animals given endotoxin alone, or ether anesthesia plus endotoxin, developed the generalized Shwartzman reaction. However, one animal in each group revealed fibrin thrombi in one or two glomeruli. (The generalized Shwartzman reaction is characterized by fibrin thrombi in virtually all glomeruli of both kidneys.)

None of the animals given norepinephrine

‡ Difco Laboratories, Detroit, Mich.

§ By courtesy of Smith, Kline and French Laboratories, Philadelphia, Pa.



| | DIBENZYLINE (60 -90 minute prefreatment 0 5 mgms ) | ANESTHESIA ( Ether ) | ENDOTOXIN (0 2mgms Difco Lipo– polysaccharide ) | NOREPI– NEPHRINE (0 025 mgms ) | DEXTROSE IN SALINE (40 ml of 5 % $D_5$ ) | TIME OF SACRIFICE | NUMBER OF ANIMALS | NUMBER WITH SIGNIFICANT GLOMERULAR THROMBOSIS | PERCENT | P VALUE |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | 4 Hours | 8 | 0 | 0 | |
| 2 | | | | | | 4 Hours | 8 | 0 | 0 | |
| 3 a | | | | | | 4 Hours | 6 | 0 | 0 | 3–6 |
| b | | | | 0 50 mgms | | 4 Hours | 4 | 0 | 0 | $x^2 = 14.7$ |
| c | | | | 1 00 mgms | | Died 45 mins – 4 hrs | 4 | 0 | 0 | $P < 0.001$ |
| d | | | | 2 00 mgms | | Died 18 - 25 mins | 3 | 0 | 0 | |
| e | | | | 4 00 mgms | | Died 9 -59 mins | 4 | 0 | 0 | |
| 4 | | | | | | 4 Hours | 36 | 22 | 61% | 4–5 $x^2 = 5.05$ $P < 0.025$ |
| 5 | | | | | | 4 Hours | 9 | 2 | 22% | |
| 6 | | | | | | 4 Hours | 21 | 11 | 52% | 4–6 Not Significant |
| 7 | | | | | | 4 Hours | 14 | 1 | 7% | 6–7 $x^2 = 8.4$ $P < 0.01$ |
| 8 | | | | | | 24 Hours | 13 | 2 | 15% | 8–6 $x^2 = 4.7$ $P < 0.05$ 8–4 $x^2 = 8.1$ $P < 0.01$ |

TABLE I.

alone in a wide range of dosages developed glomerular thrombi.

Of 36 animals given endotoxin and 0.025 mg of norepinephrine in 40 ml of dextrose/saline over 4 hours, 61% developed the generalized Shwartzman reaction. Pretreatment of 9 animals with dibenzyline reduced the incidence of the Shwartzman reaction to 22% ($p<0.025$).

Of 21 rats given endotoxin and 40 ml dextrose/saline over a 4 hour period, 52% developed the Shwartzman reaction. Alpha adrenergic blockade by dibenzyline reduced the incidence to 7% ($p<0.001$).

Of 13 animals allowed to survive 24 hours only 15% exhibited the Shwarztman reaction, indicating that in the majority of instances the thrombi are lysed shortly after they are formed.

*Discussion.* These studies confirm the previous observations that one injection of a small dose of endotoxin does not produce the generalized Shwartzman reaction in nonpregnant rats; that ether anesthesia does not "prepare" this species for the reaction; and that alpha-adrenergic blockade markedly reduces the incidence of the reaction under the conditions of its production in these experiments.

The infusion of norepinephrine alone in varying concentrations was done partly as a control for the experiments in which endotoxin was given but partly to compare the effects of norepinephrine with serotonin. Page (7) and Waugh(8) have shown that glomerular thrombi can be produced in rats infused with high concentrations of serotonin. The failure of norepinephrine to do so in our experiments may be due to the differences in vasomotor effects of the two drugs.

The most interesting and surprising contribution of this study was the demonstration that the generalized Shwartzman reaction developed in 50% of animals given one small dose of endotoxin and infused intravenously with dextrose/saline at the rate of 10 ml per hour for 4 hours. In general terms this means that infusion of dextrose/saline "prepares"

the rat for the Shwartzman reaction. The specific mechanism by which infusion of dextrose/saline in conjuction with bacterial endotoxin acts to localize thrombi in the glomerular capillaries remains to be demonstrated. The observation that alpha-adrenergic blockage interferes with the effect of the infusion plus endotoxin is a start in this direction. It implies that there is a synergism between the two agents, both of which contribute an essential component, to stimulate the alpha-adrenergic receptor sites of the kidney. It seems most likely that such receptor sites are located in the renal vasculature. The contribution of the dextrose/saline infusion could be related to a vasomotor effect of the volume of the infusion or to its content of sodium or glucose.

The other major point of these studies was that in spite of the fact that alpha-adrenergic blockade prevents localization of thrombi in renal glomeruli, the addition of physiologic amounts of exogenous norepinephrine did not increase (or decrease) the incidence of the Shwartzman reaction. The possibility exists that dibenzyline acts by blocking the effect of some other vasomotor active agent on the renal vasculature. It is known to block the effect of serotonin and histamine as well as catecholamines.

Although 15% of the animals showed persistence of glomerular thrombi for 24 hours, the majority had disappeared by this time. We have previously shown that intravenous thrombin infusion into non-pregnant rats results in glomerular thrombi which disappear within one hour of their formation(9). The disappearance of the thrombi was interpreted as lysis by plasmin, since the addition of the proteolytic enzyme inhibitor epsilon-amino caproic acid to the experiment caused the persistence of the thrombi.

*Summary.* The generalized Shwartzman reaction has been produced in the non-pregnant rat by the injection of bacterial endotoxin and infusion of dextrose/saline over a 4 hour period. Intravenous infusion of dextrose/saline acts to "prepare" the rat for the generalized Shwartzman reaction. Alpha-adrenergic blockade by dibenzyline prevents the generalized Shwartzman reaction produced in this way and suggests that the combined effect of endotoxin plus dextrose/saline infusion acts to localize thrombi in glomerular capillaries by stimulation of alpha-adrenergic receptor sites in the kidney. Exogenous norepinephrine infusion has little or no effect on the incidence of glomerular capillary thrombosis under the condition of these experiments.

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