crease in resistance to lethal challenge, while the tolerance was reduced or abolished in animals receiving Thorotrast only 3 hours prior to challenge. The survival figures for Groups I and II in Table III include the additional hazard of the heart puncture employed in obtaining the blood samples for the transaminase measurement, but they nevertheless are in agreement with the other groups which were used for survival studies alone.

Summary. A means of assessing degree of tolerance to traumatic shock was sought in the serum transaminase response to trauma induced by a Noble-Collip drum. Normal rats exposed to increments of trauma exhibited a proportional increase in serum transaminase activity. Similarly treated trauma resistant animals which had been conditioned in the drum exhibited significantly lower serum enzyme activity. The lower transaminase response in these animals was not altered by blockade of the reticuloendothelial system which abolished their tolerance to drum trauma. Tolerance to drum trauma which was induced by other procedures such as compound 48/80 or dibenzyline treatment was not accompanied by a lower transaminase response. These observations indicate that the transaminase response is not applicable as a general indicator of resistance to trauma. The generalized trauma manifested by the Noble Collip drum did produce a dramatic elevation of the serum transaminase activity but no significant change in tissue level was observed. As noted earlier, however, only a very small change in transaminase content of a large tissue mass would easily account for the increase observed in the serum activity. As a general criterion of trauma tolerance, SGOT has fallen short, but it may be useful in studies involving trauma to restricted areas such as in limb ischemia and the separation of local and systemic aspects of traumatic shock.

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Effect of Phenoxybenzamine on Phagocytic Activity and Shock Mortality in the Rat.* (29366)

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It is well known that administration of adrenergic blocking agents such as phenoxybenzamine (PBZ) will lower the mortality in various forms of experimental shock(1,2, 3); the mechanism of this effect is however not certain(4). There is considerable evidence to indicate that 'blockade' of the reticuloendothelial system (RES) will, on the other hand, increase the mortality incident to standard trauma or other forms of stress (5,6).

It would assist our understanding of shock if it were known whether the ultimate results of autonomic blockade and of RES depression were effected *via* different mechanisms or through diverse tendencies within a common mechanism. It has been suggested, for example, that the protective action of PBZ

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involves the support of Kupffer cells phagocytosis in the shock-depressed liver(4).

It seemed fruitful to explore at least one aspect of such a possible relationship and the present study had the specific objectives of: 1) contrasting the effects of phenoxybenzamine and carbon blockade—and their interrelation—on tumbling shock in the rat, and 2) investigating the possibility that the protective effect of phenoxybenzamine involves the support of the phagocytic function of the RES.

Methods and procedure. Experiments were performed on male Sprague-Dawley albino rats (230 ± 20 g) maintained on a standard diet and water *ad lib.* RE blockade was induced through injection into the dorsal vein of the penis of 320 mg/kg colloidal carbon in 2% gel (C11/1431a, Pelikan-Werke, Hanover, Germany). 'Sham-blocked' controls received an equivalent volume (10 ml/kg) of a 1% phenol in 2% gel solution which was the vehicle for the carbon. Phenoxybenzamine (Dibenzyline) was administered iv at 0.5 mg/kg, this dose having previously been shown to be effective in reducing shock mortality(7). Phagocytic indices were determined in the intact unanesthetized rat by measuring the clearance rate of a test dose (80 mg/kg) of colloidal carbon in gel using a slight modification of the technique of Biozzi, Benacerraf and Halpern(8).

Drum shock was produced by a modified Noble-Collip technique which was standardized as previously described(7). Unanesthetized rats with front and hind legs taped together were tumbled in individual closed cylindrical drums equipped with 2 baffles and revolving at 33 revolutions per minute. Stress levels of 325 and 425 total revolutions were found consistently to induce moderate and high mortality rates respectively. Controls of matched weight were tumbled simultaneously. The animals were closely observed for several hours after drumming and those living after 24 hours were considered survivals.

Gross post-mortem examinations were performed on all fatalities. Animals dying with the characteristic intestinal congestion and luminal hemorrhage but with no apparent damage to the brain or visceral organs were classified as shock mortalities. PBZ pretreatment was found to cause some increase in 'acute' deaths *i.e.,* those during or within 15 minutes after drumming. Such deaths were usually associated with rupture of liver or spleen and intraperitoneal hemorrhage. Nickerson and Carter(9) reported a similar phenomenon but distinguished between 'early' deaths and those due to rupture of a viscus; in our experiments most acute deaths were accompanied by trauma or rupture of an abdominal organ and thus no such distinction was made.

To study the phenomenon of phagocytosis more closely and to minimize the effects of blood flow, phagocytic clearance experiments were also performed on the *in situ* perfused rat liver. The technique used was similar to that described by Howard and Wardlaw(10) except that in the present experiments a perfusion pump was set to deliver a constant flow of 8.5 ml/min. Portal pressure was monitored and found to vary between 6 to 10 cm of saline. The perfusate was a warm suspension of carbon (C11/1431a) in Ringer-Locke's solution at a concentration of 50 mg/liter. Perfusion was usually carried out for about 8 to 10 minutes and samples collected at one-minute intervals. Carbon uptake was measured spectrophotometrically and calculated as micrograms removed per gram of liver tissue per minute or also expressed as the carbon extraction ratio $(Conc._{afferent}-Conc._{efferent}/Con._{afferent})$.

Mortality data were analyzed with the Chi square test and corrected for continuity error by Yates' correction factor; phagocytic determinations were compared using Student's "t" test.

Results. A. Effect of phenoxybenzamine and carbon blockade on traumatic shock mortality. As shown in Table I, PBZ given either one or six hours before a tumbling stress caused the expected sharp reduction in shock mortality. The tendency toward fewer deaths in the 6-hour *vs* the one-hour pretreatment group is not statistically significant. The existence of adrenergic blockade after PBZ was verified by the epinephrine reversal response in an independent study.

TABLE I. Effect of Phenoxybenzamine on Traumatic Shock Mortality (Stress—425 Revs).

Exp groups	No. of rats	Hr between inj and shock	% 'Acute' mortality	% Shock mortality	% Total mortality
(A) Saline control	38	1	0	84.2	84.2
(B) PBZ (.5 mg/kg)	31	1	16.1	6.5*	22.6*
(C) *Idem*	12	6	0	0 *	0 *

* A p value <.001 as compared to control group using Chi square analysis.

As other investigators have reported, an RE blocking dose of carbon gel will temporarily increase the mortality to a subsequent tumbling stress (Table II).

TABLE II. Effect of Carbon Blockade on Traumatic Shock Mortality (Stress—325 Revs).

Exp groups	No. of rats	Hr between blockade and shock	% Shock mortality
(A) Sham blockade	8	1	37
Carbon blockade	8	1	100*
(B) Sham blockade	15	4–6	40
Carbon blockade	20	4–6	100*
(C) Sham blockade	12	12	50
Carbon blockade	20	12	35
(D) Sham blockade	8	24	50
Carbon blockade	8	24	63

* A p value <.001 as compared to respective sham blockade group.

B. Combined PBZ pretreatment and carbon blockade. In an effort to determine the relative predominance of the 2 divergent effects, carbon blockade and PBZ were both given in the same animal but in alternating sequence. The time lapses employed were intended to permit each agent to achieve near-maximum effect for the particular dose used.

The results indicate that when combined with sham blockade, PBZ had its usual effect on shock mortality (Group A, Table III).

The protective effect against 'true' or 'delayed' shock was undiminished if the PBZ was given prior to carbon blockade (Group C) but was lost if given after the carbon blockade (Group B). The 'acute' mortality to tumbling was notably heightened however if the animal was subjected to carbon blockade after administration of PBZ. Neglecting for the moment the rise in acute mortality of Group C, it thus appeared that at the dose levels and conditions of these experiments, either the PBZ or the carbon blockade effect may predominate over the other from the standpoint of mortality depending primarily upon which agent is administered first.

C. Effect of traumatic shock, phenoxybenzamine and RES blockade on phagocytic activity in the intact rat. As noted in Table IV, Group C, there was a fall in K values after drum shock; PBZ pretreatment had no significant effect in the non-shocked controls (Group B) or after tumbling shock (Group D). The difference between the post-shock K values (Table IV) was not statistically significant. Six and twelve hours after carbon blockade (Table V) the phagocytic indices were significantly reduced as might be anticipated.

TABLE III. Effect of Combined Phenoxybenzamine Pretreatment (0.5 mg/kg) and Carbon 'Blockade' (320 mg/kg) on Subsequent Traumatic Shock (Stress—325 Revs).

Groups	Exp sequence	No. of rats	% 'Acute' mortality	% Shock mortality	% Total mortality
A	0 time—sham blockade 4th hr—PBZ 5th hr—traumatic shock	12	7.7	0	7.7
B	0 time—carbon blockade 4th hr—PBZ 5th hr—traumatic shock	16	12.5	75*	87.5*
C	0 time—PBZ 1st hr—carbon blockade 5th hr—traumatic shock	30	46.7*	0	46.7*

* A p value <.001 as compared to Group A.

TABLE IV. Effect of Traumatic Shock and Phenoxybenzamine on Phagocytic Activity in Intact Rat (Carbon Test Dose—80 mg/kg).

Groups	Exp sequence	No. of rats	Phagocytic index (K) mean ± S.D.
(A) Saline controls	0 time—saline (1 ml/kg) 1st hr—carbon clearance test	13	.036 ± .013
(B) PBZ controls	0 time—PBZ (.5 mg/kg) 1st hr—carbon clearance test	10	.033 ± .014
(C) Traumatic shock after saline	0 time—saline (1 ml/kg) 1st hr—traumatic shock (425 revs) 1½ hr—carbon clearance test	10	.017 ± .010†
(D) Traumatic shock after PBZ	0 time—PBZ (.5 mg/kg) 1st hr—traumatic shock (425 revs) 1½ hr—carbon clearance test	12	.023 ± .018*

* † p values of $<.05$ and .001 respectively as compared to non-shock controls.

TABLE V. Effect of Carbon Blockade (320 mg/kg) and Combined Carbon Blockade–PBZ Pretreatment (0.5 mg/kg) on Phagocytic Activity in Intact Rat (Carbon Test Dose—80 mg/kg).

Exp groups		No. of rats	Phagocytic index (K) mean ± S.D.
(A) Blockade 4-6 hr before test	Sham blockade	10	.040 ± .023
	Carbon blockade	17	.018 ± .009*
(B) Blockade 12 hr before test	Sham blockade	9	.030 ± .010
	Carbon blockade	9	.011 ± .006*
(C) PBZ after carbon blockade	0 time—carbon blockade 4th hr—PBZ 5th hr—carbon clearance test	5	.016 ± .007*
(D) PBZ before carbon blockade	0 time—PBZ 1st hr—carbon blockade 5th hr—carbon clearance test	6	.016 ± .014*

* A p value $<.001$ as compared with respective sham blockade; in case of Groups C and D to sham blockade of Group A.

The K values after combined administration of carbon and PBZ were much decreased compared to the sham blockade of Groups A and B (Table V) but the degree of phagocytic depression was unaffected by the sequence of the procedures and was fully as severe as that after traumatic shock (Table IV, Group C).

D. Carbon clearance in in situ *perfused liver.* The dose of carbon used as a clearance test agent in the *in vivo* experiments was sufficiently large to exceed the critical dose beyond which liver blood flow is presumably a limiting factor. The controlled perfusion of a semi-isolated organ would however further increase the likelihood that the removal rate is truly a measure of phagocytic efficiency per unit mass of tissue.

Such measurements were made in 4 groups of animals and the results (Table VI) indicate in the control animals a constant removal rate with an extraction ratio of about 19%. There was a sharp decline in phagocytic activity of the isolated liver after tumbling shock. PBZ exerted no beneficial effect in this respect—indeed PBZ animals tended toward an even lesser post-shock phagocytic ability than controls.

Discussion. The protective effect of PBZ is again clearly illustrated in these experiments; equally clear was the marked, albeit temporary, detrimental action of RE blockade previously reported(5,6). In this study the mortality-heightening effect of a large dose of colloidal carbon was evident at one hour but had disappeared in 12 hours.

When RE blockade was induced prior to PBZ administration, the shock-protective effect of PBZ was much diminished, further illustrating the apparent inability of any

TABLE VI. Effect of Traumatic Shock and Phenoxybenzamine on Phagocytic Activity in Perfused Rat Liver.

Groups	Exp sequence	No. of rats	Carbon removal rate (μg/g liver/min) mean ± S.D.	Carbon extraction ratio mean ± S.D.
(A) Saline controls	0 time—saline (1 ml/kg)	13		
	1st hr—carbon clearance in perfused liver		9.3 ± 1.7	.194 ± .030
(B) PBZ controls	0 time—PBZ (.5 mg/kg)	8		
	1st hr—carbon clearance in perfused liver		9.0 ± 1.3	.188 ± .031
(C) Traumatic shock after saline	0 time—saline (1 ml/kg)	8		
	1st hr—traumatic shock (425 revs)			
	1½ hr—carbon clearance in perfused liver		5.7 ± 1.4*	.116 ± .019*
(D) Traumatic shock after PBZ	0 time—PBZ (.5 mg/kg)	8		
	1st hr—traumatic shock (425 revs)			
	1½ hr—carbon clearance in perfused liver		4.6 ± 1.1*	.101 ± .028*

* A p value $<.001$ as compared to non-shock controls.

agent to nullify or reduce the deleterious effect of an RES blocker during this immediate post-injection period.

When phenoxybenzamine was given before carbon blockade, the shock-protective effect of PBZ was apparently undiminished. The increase in 'acute' deaths *i.e.,* those during or immediately after drumming, is reminiscent of the increased incidence of acute deaths which follow pretreatment with higher doses of adrenergic or ganglionic blocking agents(7). If it is true that such early deaths are caused by acute hypotension and respiratory arrest as suggested by Nickerson (9), then it appears that RE blockade, if given after PBZ, in some manner accentuates or reinforces the vasodilator action of the latter.

It is evident from the carbon clearance data that pretreatment with PBZ did not influence phagocytic activity either before or after shock. The liver perfusion experiments, by minimizing the possibility of blood flow effects on RE function, reinforce the notion that PBZ had no apparent effect on carbon clearance in these experiments. If the RES and PBZ have a common denominator with regard to their action in shock, it does not appear to be phagocytosis.

The injection of large doses of colloidal carbon caused in our rats the typical 'blockade' effects described by McKenna and Zweifach(5) *i.e.,* a marked fall in phagocytic activity and increased sensitivity to traumatic shock. Although these two effects are generally related, this relationship is not universal as indicated by the fact that 12 hours after RE blockade shock mortality had returned to control levels (Table II, Group C) but phagocytic function was still greatly diminished (Table V, Group B).

Indeed, the frequency of the dissociation of phagocytic function and shock resistance *e.g.,* the normal clearance properties in rats made more resistant to trauma by sublethal drumming(6) or less resistant by adrenalectomy(11), make it quite clear that tolerance to trauma is not specifically related to the carbon clearance function in the rat. This does not of course preclude the possibility that some other RE function may be closely associated or even causally related to shock resistance.

Summary. Phenoxybenzamine (PBZ—0.5 mg/kg) decreased, and blockade of the reticuloendothelial system with colloidal carbon (320 mg/kg) temporarily increased the mortality of rats to subsequent tumbling shock. The usual protective effect of PBZ was lost if the drug was given after carbon blockade; in

contrast, PBZ maintained its protective effect if given before blockade although 'acute' traumatic deaths incident to drumming increased. PBZ pretreatment had no effect on pre-shock or post-shock carbon clearance activity either in the intact rat or in the *in situ* perfused liver. The results indicate that in the rat carbon clearance activity is not well correlated with the protective action of PBZ in traumatic shock.

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Effects of 1, 3, 5, (10), 16 Estratetraen-3-ol on Mammalian L-Fibroblasts *in vitro*.* (29367)

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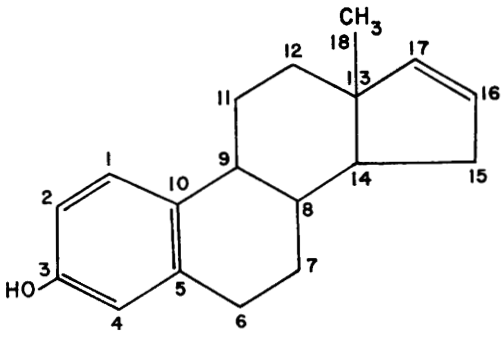
Previous investigations in this laboratory have revealed that concentrations of one-two ppm of 1, 3, 5 (10), 16-estratetraen-3-ol (coded and referred to hereinafter as chemical #742) when used as a culture medium for fish and/or frog embryos caused (tumors) hyperplasia in the tails of these embryos. Preliminary observations of the effects of #742 on cultures of pig kidney cells indicated that mitosis was accelerated.

Chemical #742 is a synthetic Δ^{16} steroid chemically related to the androgens. It was prepared by Huffman(1) from estradiol-3, 16. The chemical formula is



Materials and methods. Standardized stock cultures of L-fibroblasts (mouse) obtained from Dr. Franklin Leach, Oklahoma State University, were cultured in Eagle's medium, supplemented with calf serum, at 37.5°C. Cell suspensions for counting and/or transfer were formed by the use of a "policeman" and vigorous pipette action (20 ×).

Chemical #742 was supplied dissolved in

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