response of uterine tissues to estrogens. In contrast is the marked reduction of plasminogen activator caused by this hormone.

This unexplained suppressive effect of female sex hormones on plasminogen activator may not be limited to the endometrial fluid of mice. Thus, for example, estrogens suppress blood fibrinolytic activity in castrated rats(13). In human pregnancy, blood plasminogen activator decreases as hormones rise, and returns to normal after placental delivery(14,15).

Sex hormones may, therefore, suppress blood fibrinolytic activator activity similarly to the suppression demonstrated in uterine fluid of the mouse by both estrogen and progesterone.

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Effect of Pretreatment with Aggregate Albumin on Reticuloendothelial System Activity and Survival After Experimental Shock.* (31358)

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There is considerable evidence from studies of adaptive protection against shock to indicate that a direct functional relationship exists between the level of reticuloendothelial system (RES) activity and the tolerance of laboratory animals to various types of experimental shock(1,2,3,4,5). However most of the methods used to induce adaptation or resistance to shock *via* RES stimulation are not compatible for potential use in patients; *i.e.*, repeated trauma, saccharated iron oxide, endotoxins, thorium oxide, highly denatured serum proteins, etc. In these protection studies RES function was measured experimentally by its phagocytic capacity to clear the peripheral blood of known amounts of injected colloids.

Our laboratory has been exploring materials(6) which, from reported experience, should be safe to use in man and which could be expected to stimulate the RES to determine whether such stimulation did, in fact, occur and whether this also influenced survival after various types of experimental shock. The data reported here cover preliminary observations with a mildly denatured human serum albumin developed for use with an

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I^{131} tag for liver photoscans.†. The albumin aggregates in this preparation are sub-micron (10-20 mμ) in size(7). Use was made of the "cold" colloid which has been given to patients in relatively large loading doses often repeated for multiple scans over variable intervals of 3 weeks to 18 months without evidence of anaphylactoid reactions, positive skin tests reactions or detectable albumin antibodies(8,9).

Methods. There were 2 groups of experiments: 1. The effects of the albumin aggregate on RES phagocytic function were determined. 2. Rats pretreated with albumin were subjected to hemorrhage, intestinal ischemia, trauma or endotoxemia and their survival rates were determined.

Group 1. Female Wistar strain rats, 150 ± 15 g body wt, received i.v. injections of 21.6 mg/kg of albumin twice daily for 3 consecutive days. On the fourth day, during light pentobarbital anesthesia (3.0 mg/100 g, i.m.), phagocytic indices (K values) were determined and also corrected for organ weights (α values‡) by obtaining blood clearance rates for colloidal carbon, 8 mg/100 g body wt, (suspended in calf skin gelatin) essentially using the method of Biozzi *et al* (10). Normal animals and rats which received pyrogen free saline without preservative (single use vials) served as controls. Precisely timed, serial blood samples of 0.025 ml were obtained from tail cuts, hemolyzed in 3.0 ml of 0.1% Na_2CO_3 (1:120 dilution) and carbon concentrations determined photometrically (Bausch & Lomb Spectronic "20") at 675 mμ. The slope of the line through the plot $\log_{10}$ carbon concentration (C) vs time (t) in minutes was calculated as the phagocytic index (K). Fresh weights of liver and spleen were obtained upon sacrifice 15 minutes after carbon injection and their combined weights (Wls.) calculated as % body weight for determination of α values.

$$K = \frac{\log C_1 - \log C_2}{t_2 - t_1} \qquad \alpha = \frac{W}{Wls} \sqrt[3]{K}$$

The means and standard errors of the mean were determined for K and α which were statistically analyzed using the Student's *t* test.

Group 2. Rats prepared identically as in Group 1 were subjected to one of the 4 types of shock on the fourth day. Carbon clearances were not done. All animals were anesthetized. Aseptic technic was used. No therapy was given during or after the shock episode. Survival was determined at 48 hours. Controls as in Group 1 were simultaneously subjected to identical shock procedures. Survival data in controls and albumin pretreated animals were statistically compared using the Chi-square test.

a. Hemorrhagic shock was induced by rapid graded bleeding (15 minutes), *via* a cannulated femoral artery to a mean B.P. of 35-40 mm Hg using a previously described (11) automatic bleed-out device incorporating a heparinized saline reservoir and conventional mercury manometer. This B.P. level was maintained for 2 hours with additional small bleedings when necessary. But no blood was reinfused during this period after which all shed blood was returned I.A., vessels ligated and incisions closed.

b. Intestinal ischemia shock (SMA) was instituted by temporary ligation of the superior mesenteric artery for 90 minutes using a previously described(12) technic. The abdominal incision was closed following release of the arterial ligature.

c. Trauma was produced by Noble-Collip drum using 725 revolutions at 40 rpm.

d. Endotoxemia was produced with *Salmonella enteritidis* endotoxin (Difco Labs., No. 12047), 2.0 mg/100 g, i.v.

Results. Table I indicates K and α values found in Group 1 and shows that the albumin aggregate resulted in significant RES phagocytic stimulation. The K and α values in the albumin pretreated rats indicate stimulation to approximately 180% and 60% respectively, over those of normal controls. All of the treated animals appeared entirely

† The authors are indebted to Dr. Paul Numerof, Squibb Inst. for Medical Research, for generously providing the albumin aggregate used in these studies.

‡ Alpha values.

TABLE I. Effect of Colloidal Albumin on RES Phagocytic Index in Rats.

Group	No. of rats	Phagocytic index (K)† (mean ± S.E.)	Corrected phagocytic index (α) (mean ± S.E.)
Controls	29	.025 ± .002	5.36 ± .13
Saline	14	.020 ± .004	5.71 ± .20
Albumin*	14	.065 ± .010‡	8.46 ± .36‡

* Colloidal aggregate albumin (100-200 A. Diam.) —21.6 mg/kg, i.v., 2× day for 3 consecutive days.
† Clearances done on 4th day. Test dose carbon = 8 mg/100 g body wt.
‡ P <.001 (Student's t test) compared with control and saline animals.

TABLE II. Effect of Colloidal Albumin on RES Organ Weights in Rats.

Group	No. of rats	Organ weights (% body wt) (mean ± S.E.) Liver	Spleen	Lung
Controls	33	4.23 ± .06	.47 ± .03	.73 ± .03
Saline	14	4.24 ± .10	.40 ± .04	.74 ± .04
Albumin*	14	4.49 ± .14	.34 ± .02†	.72 ± .06

* Colloidal aggregate albumin—21.6 mg/kg, i.v., 2× day for 3 consecutive days.
† P <.05 (Student's t test) compared with control and saline animals.

normal, without weight loss, at the time of the clearance determinations.

Fig. 1. illustrates carbon clearance curves in Group 1. The points on the lines through which each curve is drawn represent the averaged values (log) for carbon concentration obtained from each animal in each of the 3 sub-groups: A,B,C.

Table II lists the RES wet organ weights in controls and albumin pretreated rats 24 hours after the last injection. The only significant change is a small decrease in spleen weight in the albumin sub-group.

Table III summarizes the 48 hour survival rates in Group 2. A significant increase in resistance to trauma, SMA and endotoxemia is evident in the albumin pretreated animals when compared with the saline control subgroups. Survival after hermorrhage is not significantly altered.

Discussion. Prior to any consideration of these data in relation to shock mechanisms or therapy several facts should be noted. 1. These observations are limited to a single dose-duration regime of therapy in a single species. Any implications, particularly clinical, must be regarded as tenuous pending examination of other regimes, other species and specific search for inherent toxicity of the therapy. 2. It is well established that while

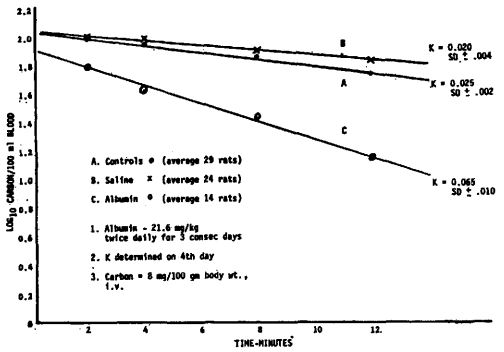

FIG. 1. Influence of albumin aggregate on clearance of carbon from blood of rats.

TABLE III. Influence of Colloidal Albumin Pretreatment* on Survival After Experimental Shock in Rats.

Type of shock		Survivors/Total rats	% Survivors	Bl. loss (% body wt)
Hemorrhage	Saline	20/34	59	3.60
	Albumin	21/31	68	3.73
Intestinal ischemia	Saline	0/19	0	
	Albumin	6/31	19	(P <.001 Chi-sq)
Trauma	Saline	4/22	17	
	Albumin	10/20	50	(P <.001 Chi-sq)
Endotoxin	Saline	10/16	63	
	Albumin	44/46	96	(P <.001 Chi-sq)

* Colloidal aggregate albumin—21.6 mg/kg, i.v., 2× day for 3 consecutive days. Controls received pyrogen-free saline. Shock induced on 4th day.
Mean BP maintained at 35-40 mm Hg for 2 hr, then shed blood returned.
Temporary ligation sup. mesent. art. for 90 min.
Noble-Collip Drum, 725 rev. @ 40 rpm.
Salmonella enteritidis endotoxin, 2.0 mg/100 g body wt, i.v.

stimulation of phagocytosis and resistance tc shock are often parallel, either may occur without the other(1,3) phagocytosis being but one of many RES homeostatic functions.

The present findings indicate that this very small colloidal albumin aggregate results in significant stimulation of RES phagocytic function. The absence of an increase in weight of the RES organs suggests that this change in RES function is due to an increase in tissue activity per unit mass(10). But this change may also be a reflection of increased RES organ blood flow(13), a factor not delineated in these experiments. The data also indicate that pretreatment with this potentially non-toxic colloid, selectively taken up by the liver(7), also results in increased tolerance to some types of shock to an extent comparable with that previously found to be produced by several frankly toxic materials (1) rapidly taken up by the RES which they adaptively stimulate. There is no clear explanation, however, for the lack of protection observed after hemorrhage in these stimulated animals since the basic mechanisms involved in adaptive protection, as an experimental entity, are obscure(3). This finding, however, may be additional evidence of the particular susceptibility of the liver to injury during hemorrhagic hypovolemia(14,15) a circumstance which may have negated the influence of preexisting hepatic RES hyperactivity.

A number of RES mediated functions have been implicated in both the pathogenesis of shock and the mechanisms involved in experimental adaptive protection against shock based, in large measure, on data involving SMA and endotoxin shock models(3,5). These types of shock presumably emphasize hepatointestinal and anti-bacterial factors which, if not identical with each other, undoubtedly have much in common. Zweifach(1), Fine (16) and others(17) have postulated that the RES may represent the homeostatic system serving as a critical common pathway in both pathogenesis and host adaptation in some shock syndromes. The fact that this RES stimulating albumin preparation increased tolerance to both SMA and endotoxin shock tends to add support to the above postulate. It may well be that the so called "gut factor", "hepatic factor" and "bacterial defence factor", each somewhat separately implicated in shock, represent different facets of the same broad adaptive defence system of the body. If this broad system, the RES, can be safely manipulated by non-toxic, simple means a promising approach to prophylactic shock therapy might be developed.

Summary. Rats pretreated with human serum albumin aggregate (10-20 mμ) develop significant stimulation of total RES phagocytic capacity without appreciable increase in RES organ masses. Such pretreated animals show significantly increased survival after traumatic, intestinal ischemia and endotoxin shock but not after hemorrhage. Since RES mediated mechanisms have been implicated in both the pathogenesis of and adaptive resistance to shock, experimental manipulation of the RES by other than the frankly toxic materials used heretofore suggests a potentially compatible approach to prophylactic shock therapy.

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Effect of Isomeres of Triiodothyronine on Erythrokinetics. (31359)

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Accelerated erythropoiesis occurs in hyperthyroid humans(1) and animals with drug induced hyperthyroidism(2-4). This augmentation of erythropoiesis has been attributed to hormonal calorigenesis with a resultant imbalance between total tissue oxygen requirement and oxygen transport capacity of the red blood cell(2-3). In prior studies levo-triiodothyronine and its calorigenically inactive dextroisomere, dextro-triiodothyronine, produced equal increases in erythrocyte radioiron incorporation. Oxygen utilization, however, was significantly greater in the LT_3 treated animals(4). This has suggested that the erythropoietic acceleration following triiodothyronine administration was not completely dependent upon alterations in oxygen need. The present study constitutes an attempt to characterize further the effect of thyroactive substances on erythrokinetics and explore the mechanisms involved.

Methods and materials. All animals utilized in these experiments were maintained on a diet of Purina Laboratory Chow* with tap water allowed *ad libitum*. Homologous red blood cells were obtained by cardiac puncture during light ether anesthesia from female Sprague-Dawley rats weighing 250-300 g. The blood was centrifuged, the plasma removed, and the red blood cells resuspended in 0.9% saline solution sufficient to produce a hematocrit of 70%. Female Sprague-Dawley rats, 180-200 g, were transfused on 2 successive days with red blood cell suspension equal to one-half of the recipient animals' red blood cell mass. Forty-eight and 96 hours following the last transfusion LT_3† or DT_3‡ in doses of 100 or 250 μg was administered I.P. Control animals were given 0.9% saline solution. Six hours following the last injection of T_3, 1 μc ^{59}Fe/1 μg ^{56}Fe citrate was given *via* the tail vein and the 18-hour erythrocyte radioiron incorporation determined(5).

Female Sprague-Dawley rats, 180-200 g, were given a single I.P. injection of 25, 100 or 250 μg LT_3 or DT_3 or 0.9% saline solution. Groups of rats were given 1 μc ^{59}Fe/1 μg ^{56}Fe citrate I.V. either 24, 48, 72 or 96 hours following the injection of triiodothyronine and the 18-hour erythrocyte radioiron incorporation determined. Pooled plasma obtained either 6, 12, 24, 48 or 72 hours following I.P. administration of a single dose of 100 μg of LT_3 or DT_3 was bioassayed for erythropoietic stimulating activity by modification of the bioassay of Cotes and Bangham (6). Virgin female CF #1 mice weighing 20-25 g were maintained in an altitude chamber at 0.5 atmosphere pressure for a period of 2 weeks. The mice were removed one hour daily for routine care. Four days following removal from the chamber and return to ambient conditions, 0.4 ml saline, 0.4 ml ane-

* Iron content 275 p.p.m.

† Supplied as Cytomel® sodium (Lot #BS-0207) Smith Kline and French Research Laboratories, Philadelphia.

‡ Prepared by Sigma Chemical Co., St. Louis, Mo., by iodination of tyrosines after the manner of Chalmers, Dickson, Elks and Hems(7). Impurity was determined to be less than 1% by single chromatographic spots and a rotation value of + 21.5°.