active than a single dose of erythropoietin in saline; however, when the latter was given in divided doses, the activity approached that of the single dose of the serum diluted material. However, erythropoietin in serum was not given in divided doses as a control. In the present study, fractionation of the erythropoietin in serum showed even greater enhancement of activity, with the activity of the serum diluted material being greater than the saline diluted material in any given dosage schedule. While these studies in no way eliminate an adjuvant effect of serum merely causing delayed absorption, they do seem to indicate a definite potentiating effect of serum which is consistent with the work cited above.

Summary. Erythropoietin (urinary fraction II + III) was diluted with either saline or normal serum and injected into assay animals as a single dose or as 2 or 4 fractional doses either intraperitoneally or subcutaneously. Erythropoietic activity increased as the dose was fractionated; however, the erythropoietin plus serum showed greater activity than erythropoietin plus saline in all dosage schedules. This suggests that normal serum actually enhances the activity of erythropoietin rather than merely acting as an adjuvant causing delayed absorption of the material.

Grateful acknowledgement is made to Miss Bettie Williams and Mrs. Carol Cope for fine technical assistance, to Dr. Charles B. Bragassa, Mrs. Rebecca Bedingfield and Mr. Bob Ellis of the Computer Center, Medical College of Georgia, for statistical analyses and to Mrs. Margaret Hiers and Mrs. Shirl Melton for secretarial assistance.

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Received September 6, 1966. P.S.E.B.M., 1966, v123.

Absence of Transferable Endogenous Substances Altering Vascular Reactivity in Endotoxin Shock.* (31561)

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Late phase of endotoxin shock in the rabbit is consistently accompanied by hyperreactivity of pulmonary vasoconstrictor mechanisms to acetylcholine (Ach) (1,2,3). A latent period of 10 to 70 minutes is found between injection of endotoxin and onset of hyperreactivity to Ach(1). It is conceivable that substances produced by tissue, either from endotoxin or in response to endotoxin, are responsible for the alteration in pulmonary vascular response to Ach, rather than endotoxin *per se*. The latent period would then correspond with the slow formation of such products. In such a case, one might expect that blood incubated with endotoxin *in vitro* or *in vivo* would contain substances capable of inducing hyperreactivity to Ach in a recipient rabbit after a significantly shorter latent period than that observed after injection of

* This work was sponsored by the Office of Naval Research and the Bureau of Medicine and Surgery, U. S. Navy, under a contract between Office of Naval Research and the Regents of Univ. of California.

endotoxin. In the present paper we describe experiments designed to detect the presence of such substances in rabbit blood incubated with endotoxin *in vitro* or *in vivo*.

Materials and methods. New Zealand white female rabbits, weighing from 2.3 to 3.7 kg were used. The animals were anesthetized by intravenous (iv) injection of sodium phenobarbital (100 mg/kg) and sodium pentobarbital (15 mg/kg). Heparin (Panheprin, Abbott Laboratories) was used as anticoagulant in doses of 1500 units/kg.

Methods for recording of systemic arterial pressure (P_A) and right ventricular pressure (P_{RV}) and techniques used in the heart-lung preparation (H-L) have been described(1,2). The total blood volume in the H-L reservoir was reduced to a minimum, prior to addition of the incubated blood, to reduce the dilution factor.

The response in P_{RV} to Ach was tested at intervals of 3 to 10 minutes with doses of Ach too small to elicit a pressor response during the control period. A pressor response to Ach was interpreted as hyperreactivity of pulmonary vascular muscle to Ach for reasons previously discussed(3). Injections of endotoxin and of Ach, and transfusions of incubated blood, were made into the left jugular vein of rabbits. In the H-L, endotoxin and Ach were injected into the inferior vena cava and the incubated blood was added to the reservoir.

Endotoxin obtained from *Pseudomonas pseudomallei* was prepared as previously described[†](1).

Incubation of blood and endotoxin in vitro. Rabbit blood obtained from unanesthetized rabbits by heart puncture or from the H-L reservoir, was incubated *in vitro* with endotoxin. The concentration of endotoxin used (1 to 3 μg/ml blood) corresponded to that required in the blood stream to induce hyperreactivity to Ach(1,2). The endotoxin-blood mixture was incubated at 39°C for a period of 30 to 40 minutes, equal to the length of the latent period usually observed in rabbits before hyperreactivity to Ach(1,2). The mixture was subsequently tested for presence of metabolites in recipient rabbits or H-L.

Incubation of blood and endotoxin in vivo. Rabbit blood obtained from the left femoral or jugular vein of donor rabbits, after hyperreactivity of pulmonary blood vessels to Ach had been induced by injection of endotoxin (50 to 100 μg/kg) in these animals, was tested for presence of metabolites in recipient rabbits or H-L.

In addition, in one experiment, carotid artery-to-femoral vein cross-circulation was established between a donor rabbit and a recipient rabbit after induction of hyperreactivity to Ach in the donor animal by injection of endotoxin.

The criterion for presence of metabolites in blood previously incubated with endotoxin was that of a significant reduction or total absence of latent period after injection of the incubated blood into the recipient rabbit or H-L. When the tested blood did not induce hyperreactivity to Ach, control injections of endotoxin were given to determine the responsiveness of the recipient rabbit or H-L to endotoxin.

Results. *Incubation* in vitro. Thirty ml of blood incubated *in vitro* for 30 minutes with 30 μg endotoxin induced hyperreactivity to Ach 21 minutes after transfusion into a recipient rabbit. Twenty-five ml of blood incubated with 50 μg endotoxin and 60 ml of blood incubated with 180 μg endotoxin for a period of 40 minutes induced hyperreactivity to Ach in H-L, 33 and 40 minutes respectively after addition of the blood-endotoxin mixtures to the reservoir.

Incubation in vivo. Seventy ml of blood obtained from a donor rabbit after induction of hyperreactivity to Ach by injection of 100 μg of endotoxin/kg, were injected into a recipient rabbit. Onset of hyperreactivity to Ach was observed 14 minutes after injection of endotoxin into the donor rabbit and 16 minutes after passive transfusion of the donor's blood into the recipient rabbit.

Cross-circulation between a donor rabbit, injected with 100 μg of endotoxin/kg, and a recipient rabbit was established 12 minutes after injection of endotoxin, at onset of hyperreactivity to Ach in the donor. Onset of

[†] Endotoxin was kindly provided by Dr. M. S. Redfearn, Univ. of California.

TABLE I. Hyperreactivity of Pulmonary Vasoconstrictor Mechanisms to Acetylcholine After Injection of Endotoxin in Donor Rabbit and After Transfer of Blood from Hyperreactive Donors in the Heart-Lung Preparation.

	Onset of hyperreactivity in donor after injection of endotoxin			Onset of hyperreactivity in heart-lung preparation			
				After transfer of blood from hyperreactive donor		After control injection of endotoxin*	
Exp No.	Dose of endotoxin, μg/kg	Onset of hyperreactivity	Time of withdrawal of blood	Vol of blood transferred from donor, ml	Onset of hyperreactivity, min after transfer of blood	Dose of endotoxin, μg/kg	Onset of hyperreactivity, min after inj of endotoxin
		Min after inj of endotoxin					
1	100	20	31	80	>90†	100	none
2	100	27	33	70	>90†	250	63
3	50	25	38	70	44		
4	100	19	26	75	24		
5	100	20	26	40	37		

* In the heart-lung preparations, where blood from the donor had not induced hyperreactivity (Exp 1 and 2), control injections of endotoxin were given.
† No hyperreactivity was observed.

hyperreactivity to Ach in the recipient rabbit was observed 29 minutes after beginning of cross-circulation. The quantity of blood transferred from donor to recipient was not determined.

Blood obtained from 5 hyperreactive rabbits was added to the H-L reservoirs with the results shown in Table I. In 2 experiments, the transferred blood did not induce hyperreactivity to Ach in H-L (Table I, Nos. 1 and 2). In one of these (No. 1), subsequent control injection of 100 μg endotoxin into H-L failed to induce reactivity to Ach, while in the other (Table I, No. 2), the relatively large dose of 250 mμ endotoxin induced hyperreactivity to Ach only after a latent period of 63 minutes. In the remaining 3 experiments (Table I, Nos. 3, 4 and 5, Fig. 1), the onset of hyperreactivity to Ach in H-L occurred 44, 24, and 37 minutes, respectively, after transfer of donor blood to H-L.

Discussion. Blood incubated *in vitro* or *in vivo* with endotoxin induced hyperreactivity to Ach in recipient rabbits or H-L after latent periods not significantly shorter than those found in previous experiments after injection of endotoxin in rabbits or in H-L (1,2). This suggests that endotoxin contained in the blood, rather than metabolic products of endotoxin or secondary substances

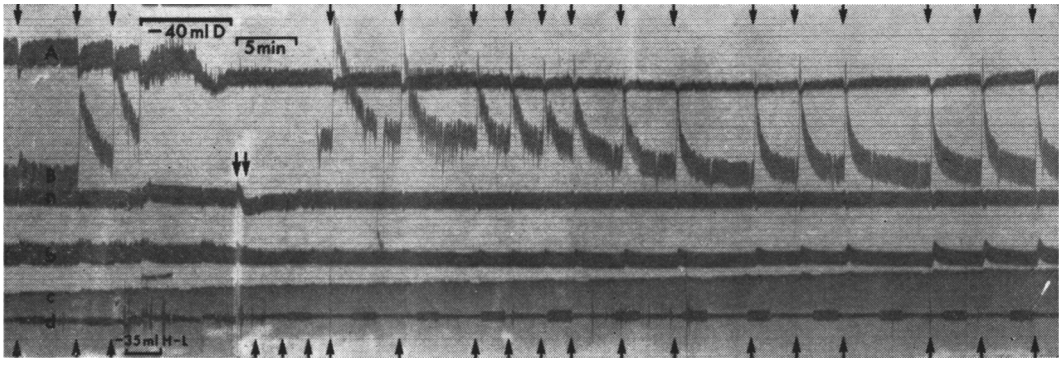

FIG. 1. Hyperreactivity of pulmonary vasoconstrictor mechanisms to acetylcholine (Ach) in the rabbit heart-lung preparation (H-L) after transfer of blood from a hyperreactive donor rabbit. Donor had received 100 μg of endotoxin per kg 26 minutes prior to the withdrawal of blood. *A*: systemic arterial pressure in donor rabbit. *B*: right ventricular pressure in donor rabbit. *a*: arterial pressure in H-L. *b*: right ventricular pressure in H-L. *c*: intratracheal pressure in H-L, and *d*: central venous pressure in H-L. ↓ Injection of 1 μg of Ach per kg into rabbit. ↑ Injection of 1 μg of Ach into H-L. *—40 ml D*: Withdrawal of 40 ml of blood from donor rabbit. *—35 ml H-L*: Withdrawal of 35 ml of blood from H-L reservoir. ↓↓ Transfer of 40 ml donor's blood to H-L.

liberated from the tissues, induced the hyperreactivity to Ach. The validity of this conclusion rests upon the assumption that metabolic products of endotoxin or secondary substances formed by endotoxin in the blood would be capable of rapid induction of alteration of vascular reactivity to Ach. We had to assume further that such products were not fixed rapidly and firmly to the tissues but were present in an active form in the blood and transferable to the recipient. These assumptions appeared reasonable in view of the observations of other authors that secondary pyrogenic products of endotoxin produced in rabbits a significantly faster rise in temperature than the original endotoxin, and that these products were transferable passively with blood(4).

Summary. Transfer of rabbit blood incubated with endotoxin *in vitro* or *in vivo* to a recipient rabbit or a heart-lung preparation induced hyperreactivity of pulmonary blood vessels to acetylcholine after a latent period comparable to that observed after injection of endotoxin. We could not demonstrate the presence of metabolic products of endotoxin or secondary substances capable of inducing immediate alteration of vascular reactivity to acetylcholine after passive transfer to a recipient animal.

The authors wish to express their grateful acknowledgment to Mr. J. Ng for his skillful technical assistance.

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Received September 13, 1966. P.S.E.B.M., 1966, v123.

Hemodynamic Effects of Pericardial Tamponade.* (31562)

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Resuscitation and management of patients in shock from blood loss and traumatic injuries complicated by bleeding into the pericardial cavity (tamponade) pose a difficult problem which has prompted a number of investigations of experimental tamponade in animals(1-6). However, there is a lack of information concerning the quantitative relationship between the stress imposed (*i.e.*, pressure or fluid volume in the pericardial cavity) and the resulting hemodynamic changes in pericardial tamponade. The purpose of the present investigation was to examine the hemodynamic adjustments in splenectomized dogs when the volume of fluid pumped into the pericardial cavity remained unchanged as compared with the adjustments observed when the fluid pressure in the cavity was kept constant.

Materials and methods. Eight successful experiments were completed on mongrel dogs (8.4-17.1 kg body weight) which were splenectomized and prepared with implanted pericardial catheters at least 10 days prior to performing the experiments. The catheter was tied securely into the pericardium, exteriorized through the thoracic wall and the distal

* This investigation was supported by USPHS Research Grant HE-06139 from Nat. Heart Inst., and by U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2272. The generous support of several private donors including Mrs. Alan M. Scaife, Mrs. George W. Perkins, the Alexander Angus McDonnell, Jr. Foundation, and the Maxwell M. Upson Foundation is gatefully acknowledged.

† Supported, in part, by a general research support grant from Nat. Inst. Health.