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Received February 16, 1960. P.S.E.B.M., 1960, v103.

Further Studies on Passive Transfer of Protection Against Lethality of Endotoxin (25700)

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We have recently described passive transfer, by serum or plasma of endotoxin-tolerant rabbits, of protection against lethality of homologous and heterologous endotoxin in the mouse, and of tolerance to pyrogenicity of endotoxin in the rabbit(1,2). Passive transfer from no-longer tolerant donors, after reticuloendothelial system blockade, and failure to obtain protection when tolerant serum and endotoxin challenge are mixed and given as a single injection, were also demonstrated (2,3). These findings argue against mediation of tolerance by antibody, circulating or fixed. Like endotoxin tolerance itself, transfer appears related to the demonstrated stimulation of RES of recipient(3). Investigations to identify the humoral mediator have eliminated certain obvious possibilities. The present report describes experiments ruling out adrenal cortical hormone, properdin, and phagocytosis-promoting factor. Studies showing protection afforded by normal donor serum under limited experimental conditions, and effect of heat treatment of donor blood are included.

Materials and methods. Preparation and treatment of donor rabbits and recipient mice, and precautions against pyrogen contamination, were as previously described(1,2). Endotoxins, Difco lipopolysaccharides of *S. typhosa* 0901 and *E. coli* 0127:B8, were given *i.p.* for challenge. Aliquots of donor serum were adjusted to pH 5.5 with N HCl, placed into boiling water bath 5 minutes, filtered, and the coagulum washed with saline. The combined filtrate was brought to original serum volume and adjusted to pH 7 with N NaOH. Sera were treated with zymosan (Mann Res. Labs, Lot #4393) both for preparation of "RPb" by 2-stage adsorption at 17 and 37°C, and by exhaustive exposure at 37°C which promotes destruction of C'3, and the zymosan eluted for properdin(4,5). Hydrocortisone was given *i.p.* in 0.2 ml volume at 1 and 5 mg 3 hours before challenge. Adrenal weight was determined for normal and endotoxin-tolerant donor rabbits at sacrifice. Corticosterone was estimated in extracts of normal and tolerant donor plasma by absorption in methanol at 240 mμ. Possible appearance of 17-OH corticoid from stimulation of pituitary-adrenal axis by endotoxin treatment for inducing tolerance was measured by Porter-Silber method(6). Phagocytosis-promoting factor (PPF) of serum was adsorbed on $BaSO_4$ and eluted with citrate(7). In all instances serum or plasma was given *i.p.* in 1 ml volume. Schedules for treatments and challenge are given in the Tables.

Results. The data eliminating adrenal cortical hormone, properdin, and PPF, as mediators of the protective effect of endotoxin-tolerant donor blood administered 3 hours before endotoxin challenge are given in Table I. Steroid, in several thousand times the content of donor plasma (<0.5 μg/ml), did not protect in these experiments. Mean adrenal weight for 19 endotoxin-tolerant rabbits, ob-

TABLE I. Failure of Adrenal Cortical Hormone, Properdin, or Phagocytosis Promoting Factor to Account for Protection against Endotoxin Lethality in Mice by *S. typhosa* Endotoxin Tolerant Rabbit Serum.

Group*	No. exp.	Dead/Total	% dead
Controls (LD_{75})	5	40/50	80
Hydrocortisone, 1 mg	2	13/20	65
" ,5 "	1	7/10	70
Tolerant donor serum	3	6/30	20†
Idem, zymosan-adsorbed‡	1	1/10	10†
" , " " §	2	5/20	25†
" , $BaSO_4$ "	1	2/10	20†

* Treatments given *i.p.* 3 hr before *i.p.* LD_{75} *S. typhosa* endotoxin.
† $p < .001$.
‡ 2-stage adsorption, 17° then 37°C.
§ 37°C adsorption, 1 hr.

tained 24 hours after the last of 6 daily injections of endotoxin, was 183 mg, and for 15 normal rabbits was 187 mg. No difference in total steroid absorbing at 240 mμ between normal and tolerant rabbit plasma was found, and no evidence for appearance of Porter-Silber reacting steroid was obtained. Zymosan-adsorbed tolerant serum retained its potency; it is worth noting that from preparation of RPb the zymosan eluate, which did not give protection, contained protein (280 mμ absorption) many times the total properdin content of serum. Adsorption of tolerant serum with $BaSO_4$, for removal of PPF, was also without effect; the eluate did not protect.

The limited protection afforded by normal donor serum or plasma, when given 30 minutes before homologous or heterologous endotoxin, is shown in Table II. Degree of protection given by tolerant donor blood exceeded that obtained with normal. Heating completely removed activity from normal serum, but not, apparently, from tolerant. With neither endotoxin challenge did the mixture of normal serum and endotoxin, prepared just before injection, give protection.

Discussion. Clearly, adrenal cortical hormone content of tolerant donor blood is not responsible for protection against endotoxin lethality. This is not surprising; large amounts of steroid, given close to time of endotoxin challenge, are required for protection (8,9). No evidence was obtained for stimulation of the pituitary-adrenal axis by treatment used to induce endotoxin tolerance in donor rabbits. Tauber and Garson(10) have shown protection against endotoxin lethality in mice by properdin, when the latter was mixed with the toxin and given as a single *i.p.* injection. This required large amounts of properdin, and brings to mind the ability of endotoxin, like zymosan, to bind with properdin(5,11). The protection seen following passive transfer from tolerant donor in our system, not obtained with simultaneous administration(3), is not attributable to properdin content of donor blood. Although a single dose of endotoxin produces alterations of serum properdin(12), the endotoxin-tolerant rabbit(13) and rat(14) had normal properdin titers, indicating development of tolerance to this response. From known effects of endotoxin on leucocytes, PPF was considered; this too is eliminated. Studies on a lipid fraction, isolated from tolerant donor blood, which stimulates RES of normal mice, and protects them against lethality of endotoxin, will be published later.

Limited protection afforded by normal serum is obtained neither when an interval of 3 hours between injections is allowed nor when the challenge is given *i.p.* 30 minutes after *i.v.* administration of normal serum, in contrast with passive transfer from tolerant donor which protects substantially in both circumstances(1). Inactivation of endotoxin by direct reaction with a factor present in

TABLE II. Limited Protection Against Endotoxin Lethality in Mice by Normal Rabbit Serum Given *i.p.* 30 Minutes Before *i.p.* Endotoxin Challenge.

Exp. and group	No. exp.	Dead/Total	% dead
S. typhosa challenge (LD_{75})			
Controls	5	38/46	83
Normal donor serum	2	5/18	28*
Idem, heated	2	16/18	89
Tolerant donor serum	2	0/18	0*
Idem, heated	2	10/18	56
Mixture, normal serum-endotoxin†	1	8/10	80
E. coli challenge (LD_{85})			
Controls	3	25/28	89
Normal donor serum	2	7/20	35*
Mixture, normal serum-endotoxin†	1	8/10	80

* $p < .001$.
† Serum and endotoxin challenge mixed and immediately injected *i.p.*

normal serum seems most unlikely. The "endotoxin-detoxifying component" of normal blood found by Landy and associates(15) and active *in vitro,* can hardly explain our *in vivo* findings in view of the full lethality seen after simultaneous administration of serum and endotoxin extemporaneously mixed. The results suggest need for time for a recipient animal response, and failure with the *i.v.* route with the same 30 minute interval points to an intraperitoneal mechanism. Results with heat-treated serum, to yield a relatively protein-free filtrate, must be interpreted with caution. The protein coagulum formed may, despite washing, trap much non-protein. The results with tolerant donor serum suggest a non-protein moiety. Processing of normal and endotoxin-tolerant donor serum and plasma by the phenol-water partition method for lipopolysaccharide has not yielded endotoxin activity (unpublished experiments).

Summary. The broad protection against lethality of endotoxin obtained by passive transfer from endotoxin-tolerant donors is not mediated by adrenal cortical hormone, properdin, or phagocytosis-promoting factor. Limited protection by normal donor serum given *i.p.* shortly before, but not simultaneously with, inoculation of endotoxin has been demonstrated. The results are not explained by direct inactivation of endotoxin by serum. Heating removes this activity from normal, but not completely from tolerant, donor serum.

The skilled technical assistance of Miriam Graff is gratefully acknowledged.

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Received February 18, 1960. P.S.E.B.M., 1960, v103.

Effect of Tumor Cell Fractions upon Plasma Iron, Liver Catalase and Organ Weights of Rats. (25701)

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A heat stable, water soluble, and alcohol precipitable extract from many different types of tumors contained a factor(s) which depresses liver catalase activity when injected into normal mice(1). Nakagawa and Nakagawa(2) reported that centrifugal fractionation of homogenates of Yoshida sarcoma and ascites hepatoma, yields an active supernatant fraction, which contained soluble protein and microsomes. The nuclear or mitochondrial fraction failed to show clear evidence of liver catalase depressing action. Our previous studies(3,4) indicated that an extract from Walker carcinosarcoma 256 or Flexner-Jobling carcinoma depresses hepatic catalase activity, lowered plasma iron and affected the weights of liver, spleen and thymus. Homogenates of the Walker tumor were, therefore, fractionated by centrifugation to separate the