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Role of Spleen in Choline Stimulation of Reticuloendothelial System and Resistance to Acute Hemorrhage[1] (40143)

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In 1953, Heller, utilizing mice, demonstrated that choline chloride stimulated reticuloendothelial system (RES) phagocytic function (1). This was later observed by others for rats as well (2, 3). Altura and coworkers demonstrated that choline pretreatment not only enhanced RES function but produced increased spleen size and significantly enhanced survival of rats subjected to hemorrhagic shock (4). In view of these findings, the present studies were undertaken to determine whether the spleen plays a role in choline-induced RES stimulation and protection against hemorrhagic shock.

Materials and methods. Intact female rats (Wistar strain, 135 ± 15 g) were given choline chloride (20 mg/kg, im), obtained from Nutritional Biochemical Corp. (Cleveland, OH), twice daily for three consecutive days. The compound was freshly dissolved each day in sterile, pyrogen-free saline (single use vials without preservative). Three groups of control animals were used; one group of normal animals received sterile, pyrogen-free saline (1.0 ml/kg, im), the second group (sham) also received im saline but was first subjected to a simple laparotomy (mid-line incision from pelvis to diaphragm) under pentobarbital sodium anesthesia (3 mg/100 g Nembutal), while the third group was also laparotomized and received choline chloride (20 mg/kg, im) twice a day for three days. Two other groups of animals were subjected to a complete splenectomy (splenx) under similar pentobarbital anesthesia and sterile conditions; one of these groups of animals received choline chloride (20 mg/kg, im) twice a day for three days, while the other splenx group received sterile, pyrogen-free saline (1.0 ml/kg, im) twice a day for three days similar to normal controls.

Phagocytic index and RES organ weight determinations. Phagocytic indices (K values) were determined in all 6 groups of animals on the fourth day after the start of the pretreatment regimen (i.e., beginning of the experiment) by measuring the rate of clearance of carbon (4 mg in calf-skin gelatin per 100 g bw) similar to that described previously (4). Fresh weights of liver, spleen, and lungs were obtained upon sacrifice (15 min after the iv colloidal carbon injection).

Hemorrhagic shock. Separate groups of normal, sham-operated and splenx pretreated animals (saline controls and choline injected) were subjected to acute hemorrhagic shock similar to that described previously (5). Briefly, the animals were bled over a 20–30 min period via cannulated femoral arteries to a fixed 3% by body weight. The blood was withheld from these animals for 2 hr. After returning the shed blood, intra-arterially, the cannulas were removed and the wounds sutured. All of these animals were observed for survival for 7 days.

Statistical analyses. Mortality data were statistically analyzed using the Chi-square test. Phagocytic indices (K values) and organ weights were compared by means of Student's t test.

Results. Table I shows that choline administration to normal and sham-operated rats resulted in slightly more than a 100% increase in RES phagocytic activity when compared to normal controls or sham-operated animals. However, choline pretreatment of splenectomized animals failed to result in RES phagocytic stimulation. Splenectomy itself (i.e., splenx rats which received saline) did not appear to be able to alter RES phagocytic activity from the level seen in normal control rats (Table I). Choline administration to normal rats caused a significant increase in splenic tissue mass but did not affect liver or lung weights (Table II), results very similar to those reported previously (4). In addition, although not shown, the spleens of choline-

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treated animals contained almost twice the amount of carbon particles seen in the control animals. Splenectomy or administration of choline to splenectomized rats did not significantly alter liver or lung weights (Table II).

As shown in Table III, choline pretreatment of normal, intact rats or sham-operated animals approximately doubled the survival rate in animals subjected to acute hemorrhage. However, choline pretreatment of splenectomized animals failed to result in an increase in survival (Table III). Splenectomized control animals which received saline also did not demonstrate any alteration in survival, over normal controls, when subjected to acute hemorrhage (Table III).

Discussion. The present studies reconfirm previous observations of many other investigators and indicate that short-term administration of choline chloride can enhance RES phagocytic function in rodents (1–4). Our observations also indicate that choline can induce resistance to acute hemorrhage. Both of these phenomena are, however, obviated by splenectomy.

The spleen is usually thought to play a minor role in global RES phagocytosis (i.e., usually no more than 5–10% of the total RES phagocytic activity) (6, 7). But previous (4) and present observations indicate: i) choline induces hypertrophy of the spleen; (ii) choline-induced RES hyperfunction is absent in splenectomized animals; and (iii) choline-induced protection against hemorrhagic shock is lost in splenectomized, but not laparotomized, animals suggesting that, under certain conditions, the spleen can play a very important role in RES phagocytic function.

Although the spleen is widely known to play an important "reservoir function" in certain animals (e.g., dogs, cats) when they are subjected to shock and trauma (8, 9), such a release (or auto-transfusion) of red blood cells does not take place in rodents. A "reservoir function" can not, therefore, account for the enhanced resistance of choline-stimulated intact rats to hemorrhage. Other mechanisms must be entertained.

A considerable body of information is available which indicates that either normally occurring or chemically-induced hyperfunction of the RES is usually equated with resistance to acute hemorrhage in a variety of laboratory animals, including rodents; hypofunction of the RES is usually associated with susceptibility to hemorrhage (5, 10–22). In this context, it should be noted that when the choline chloride dosage is increased 10-fold

TABLE I. EFFECT OF CHOLINE ON CARBON CLEARANCE IN NORMAL, SPLENECTOMIZED AND SHAM-OPERATED RATS[a]

Group	No. of rats	Phagocytic Index (K) (mean ± SE)
Normal Controls[b]	9	0.035 ± 0.006
Shams[b]	6	0.037 ± 0.008
Normals + Choline[c]	10	0.079 ± 0.012[d]
Splenx + Choline[c]	8	0.031 ± 0 005
Splenx + Saline	9	0.028 ± 0.003
Shams + Choline[e]	8	0.074 ± 0.010[d]

[a] Clearances done on fourth day. Test dose carbon = 4 mg/100 g bw.

[b] Controls and sham-operated animals received saline intramuscularly twice daily for 3 consecutive days.

[c] Received choline chloride (20 mg/kg), intramuscularly, in saline (20 mg/ml) twice daily for 3 consecutive days.

[d] Significantly different from all other groups ($P < 0.02$).

[e] Subjected to laparotomy and received choline chloride (20 mg/kg), im, in saline (20 mg/ml) twice daily for 3 consecutive days.

TABLE II. INFLUENCE OF CHOLINE ON RE ORGAN WEIGHTS IN NORMAL, SPLENECTOMIZED, AND SHAM-OPERATED RATS.

Group	No. of rats	% Organ weights (mean ± SE)		
		Liver	Lungs	Spleen
Normal Controls[a]	9	4.27 ± 0.08	0.75 ± 0.04	0.39 ± 0.04
Shams[a]	6	4.18 ± 0.08	0.72 ± 0.06	0.40 ± 0.05
Normals + Choline[b]	10	4.14 ± 0.06	0.74 ± 0.05	0.60 ± 0.07[c]
Splenx + Choline[b]	8	4.45 ± 0.17	0.71 ± 0.05	—
Splenx + Saline	9	4.67 ± 0.16	0.95 ± 0.11	—

[a] Received saline intramuscularly twice daily for 3 consecutive days.

[b] Received choline chloride (20 mg/kg), intramuscularly, in saline twice daily for 3 consecutive days.

[c] Significantly different from normal controls and shams ($P < 0.03$).

TABLE III. INFLUENCE OF CHOLINE PRETREATMENT ON SURVIVAL AFTER ACUTE HEMORRHAGE IN NORMAL AND SPLENECTOMIZED RATS.[a]

Group	Survivors/ Total rats	Survivors (%)
Normal Controls[b]	9/20	45
Normals + Choline[c]	17/20	85[d]
Splenx + Choline[c]	7/20	35
Splenx + Saline[b]	4/12	33
Shams + Choline[e]	15/18	83[d]

[a] All animals were subjected to an acute hemorrhagic episode (3% blood loss by bw). Blood was withheld from animals for 2 hr. Survival was determined at 7 days.

[b] Received saline intramuscularly twice daily for 3 consecutive days.

[c] Choline chloride—20 mg/kg, intramuscularly, in saline (20 mg/ml), twice daily for 3 consecutive days.

[d] Significantly different from all other groups ($P < 0.05$).

[e] Subjected to laparotomy and received choline chloride (20 mg/kg), im, in saline, twice daily for 3 consecutive days.

to 200 mg/kg, and given to rats twice daily for 3 days, it depresses the RES and makes such animals more susceptable to circulatory shock (4). Since previous calculations, the increased number of carbon particles in the spleen, and corrected phagocytic indices (α-values) (4) suggested that most of the choline-induced elevation in RES phagocytic activity was: (i) probably derived from the splenic tissue; and (ii) representative of increased tissue activity per unit mass of this tissue rather than hypertrophy, one is tempted to conclude that choline administration not only produces the latter but may result in an elaboration of some humoral substance(s) which induces protection against hemorrhagic shock. In this context, it is of considerable interest to note that Fine and his coworkers have found that either denervation of the spleen alone or injection of a splenic extract from donor dogs results in significant protection against several forms of circulatory shock (23). Reichard has demonstrated that extracts of spleens and plasma, prepared from rats made resistant to trauma, conferred protection against shock when transferred to normal rats (24). This worker also noted that extracts of spleens and plasma prepared from animals which were pretreated with a variety of RES stimulants, including choline, also would confer trauma protection when transferred to unstimulated rats (24). Other investigators, working with splenectomized dogs and human subjects, have reported a marked decrease in serum activity of the phagocytosis stimulating peptide, tuftsin (25). Lastly, it must be entertained that choline administration confers shock protection by either stabilizing or enhancing splenic blood flow; the splenic circulation is known to be severely compromised in both experimental and clinical forms of circulatory shock (8).

Although it is clear that choline induces protection against hemorrhage via some action on the spleen, the present data do not allow one to specifically infer whether the conferred RES stimulation, *per se* or some other splenic-based cellular mechanism is responsible. One can not eliminate the possibility that the spleen participates by formation of an antibody or opsonin. Since plasma opsonins appear to play an important role in RES phagocytosis in circulatory shock and trauma (17–19), it is possible that the enhanced phagocytic state induced by choline could be mediated by such an action.

Even though we have not elucidated the specific choline–splenic interactions, the present observations could be quite important in view of the clinical implications for prophylactic therapy (e.g., prior to extensive surgery), suggested by these findings. Choline chloride represents an example of an RES stimulant which is compatible for human use (26).

Summary. Experiments were undertaken, with rats, to determine whether the spleen plays a role in choline chloride-induced reticuloendothelial system (RES) phagocytic stimulation and protection against circulatory shock. Choline pretreatment of normal, intact, but not splenectomized rats, resulted in heightened RES phagocytic stimulation and protection against acute hemorrhagic shock. Choline pretreatment significantly increased spleen size in normal intact rats. Wet liver or lung weights were not significantly affected by choline pretreatment of either normal or splenectomized rats. Untreated, splenectomized animals did not demonstrate any significant alterations in either RES phagocytic activity or RE organ wet weights when compared to normal, intact controls or to sham-operated animals. Although the results of these experiments make it clear that the presence of a functioning spleen is necessary for choline-induced RES stimulation and shock protection, it is not clear as to just how these

actions are brought about. Further exploration is warranted in view of the clinical implications of these findings.

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