Serum Transaminase Response to Drum Trauma in Conditioned Animals. (29365)

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When animals are repeatedly exposed to various noxious stimuli, they develop tolerance to the offending agent, if the episodes are appropriately graded in time and intensity. The properly conditioned animal can survive lethal amounts of trauma in the Noble-Collip drum, or bacterial endotoxins or amine releasers such as compound 48/80 which would be lethal for control animals(1, 2,3). There is also a degree of cross tolerance observable among some of these agents (2).

Evaluation of the extent of this conditioning has generally relied on the animal's survival of an appropriate challenge. While the criterion of survival is quite decisive, it provides little or no information on the extent of tolerance above or below the selected challenge dose. Therefore, a means of estimating the gradations of tolerance achieved by various procedures was sought by assay of a serum component such as serum transaminase activity. Transaminase assays have been successfully employed as clinical diagnostic aids in conditions of myocardial or hepatic tissue damage(4), hence the serum glutamic oxalacetic transaminase activity was examined as a possible indicator criterion for assessing changes in resistance to drum trauma effected by several agents.

Methods and materials. 1. *Animals.* Albino rats obtained from the Holtzman Co., Madison, Wisc., were maintained on Purina Lab Chow and water *ad libitum* for a minimum of 4-5 days prior to experiment. Male animals weighing 200 ± 20 g were employed for the experiments.

2. *Drum trauma.* A rotating drum of the design of Noble and Collip(5) was rotated at 40 rpm. The rats' paws were bound with adhesive tape to eliminate variations due to running during rotation.

3. *Induction of tolerance.* Drum trauma tolerance was developed by exposing rats to 150 revolution increments on alternate days until a 750 revolution episode was experienced. Two days later these animals were exposed again to 750 revolutions and were then designated TR-750. Compound 48/80 (a polymer of p-methoxy-N-methyl, phenylethylamine with formaldehyde, from Burroughs-Wellcome) tolerance was induced by administering 100 μg increments in one milliliter physiological saline intraperitoneally for 6 consecutive days. A saline placebo was administered to the control animals. Dibenzyline (phenoxybenzamine of Smith, Kline & French) protection against drum shock was obtained by intravenous administration of 0.5 mg/kg body weight 2 hours prior to rotation in the drum(6).

4. *Blockade of R.E.S.* Thorotrast (24-26% colloidal thorium dioxide suspension, Testagar Co.) was administered i.v. in doses of 0.15-0.90 ml/100 g body weight for the purpose of blocking the reticuloendothelial system function and abolishing tolerance to drum trauma(7).

5. *Glutamic oxalacetic transaminase assay.* The procedure of Reitman and Frankel(8) was employed on plasma from heparinized blood obtained by cardiac puncture or lateral tail vein venipuncture and also on the supernatant of tissue samples homogenized in 0.1 M phosphate buffer, pH 7.4. The blood and tissue samples were obtained 30 minutes after the animals were removed from the drum trauma apparatus. The activity in the blood is expressed as serum glutamic oxalacetic transaminase (SGOT) values in units/ml.

Results and discussion. Fig. 1 illustrates the SGOT response to graded amounts of trauma in the Noble-Collip drum. The lower line of the graph represents the values, with standard deviations, for one group of animals during the drum trauma training program. The upper line illustrates the response of normal, unconditioned, animals to the same number of revolutions in the drum. A

TABLE I. Tissue Levels of Glutamic Oxalacetic Transaminase.

Treatment	Back muscle*	Duodenum*	Heart*	Liver*	Spleen*	Serum†
Control	106 ± 15	65 ± 4	380 ± 11	104 ± 4	46 ± 3	31 ± 12
Normal + 750 rev.	85 ± 13	56 ± 3	376 ± 15	115 ± 6	43 ± 7	2280 ±400
TR-750	101 ± 16	73 ± 4	416 ± 16	98 ± 5	39 ± 4	73 ± 14
TR-750 + 750 rev.	118 ± 8	60 ± 8	420 ± 13	123 ± 9	46 ± 4	660 ± 65

* Units/mg dry tissue.

† Units/ml.

dose of 750 revolutions is an LD_{100}.

Fig. 2 presents the SGOT response of rats on a prolonged training program. After tolerance to 750 revolutions had been demonstrated, the training program was continued with 750 revolution episodes on alternate days for 2½ weeks. A further increase in tolerance was suggested by the decline of SGOT values to a constant value for the same amount of trauma.

Glutamic oxalacetic transaminase activity was estimated in several tissues to observe any differences between normal and trained animals before and after trauma which might be associated with the dramatic difference in serum values (Table I). The blood and tissue samples were obtained thirty minutes after the animals were removed from the drum. Tissues of the drum conditioned animals showed no evidence to suggest a depletion of GOT activity by the trauma during training, and indeed the transaminase in the heart was increased. There was also no evidence of dramatic change in the tissues of either normal or trained animals following trauma, but it must be borne in mind that a small change in tissue content could account for the whole change in serum.

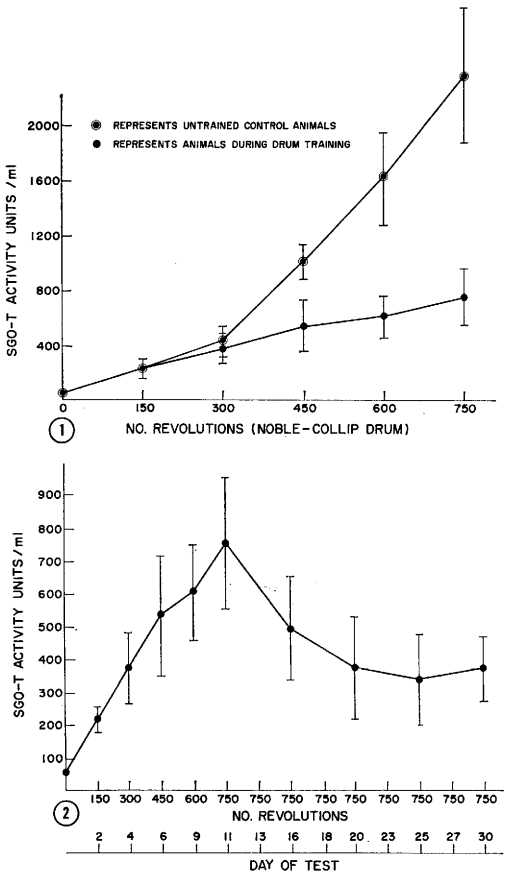

FIG. 1. SGO-T response to drum trauma in untrained animals and during drum training.

FIG. 2. SGO-T activity during trauma training.

The SGOT response to drum trauma was examined in animals which had been conditioned with the amine releaser compound 48/80 or protected by dibenzyline. The results are presented in Table II. The animals were protected by either pretreatment as demonstrated by their high rate of survival. However, the SGOT values of the protected animals were as high as the control animals that failed to survive. The SGOT values failed to reflect the difference in the protection and only suggested that both the control and treated groups experienced the same amount of trauma and tissue damage.

Since administration of the same amount of trauma to all animals was implicit in the drum trauma technique, it then appeared that the lower values exhibited by the drum trained animals might have indicated that they were not receiving the same amount of trauma as the control animals. Although the animal's paws were taped to eliminate running and insure more uniform tumbling and trauma, this was not completely effective. The drum conditioned animals became sufficiently accustomed to the characteristics of

TABLE II. Serum Transaminase Response to Drum Trauma in Animals Protected by Other Agents.

Treatment	No./group	ΔT (hr)*	No. of revolutions	Units SGOT ± S.D.	Survival, %
Saline placebo	6	1–2	600	1720 ± 500	0
Dibenzyline	6	1–2	600	1400 ± 400	83
Saline placebo	6	24	600	1370 ± 305	17
Compound 48/80	6	24	600	1360 ± 385	100

* ΔT = Time interval between medication and challenge.

the drum's rotation during their training to be able to compensate for the drum's action. When the animals were observed through the transparent drum covers during rotation, the drum trained animals appeared to land less frequently on their head and back than untrained control animals. The extent to which this might have accounted for the lower values in the drum trained animals was examined with animals under light anesthesia. A group of TR-750 rats was anesthetized with Nembutal®, 30 mg/kg i.p., and challenged with 450 revolutions in the drum. The lower challenge was employed because it had been reported by Noble(5) that animals drummed under light anesthesia were more susceptible to shock and rupture of liver and spleen. The SGOT values in this group were 305 ± 90 u/ml which is significantly lower than untrained animals exposed to a similar challenge (Fig. 1) and overlaps the range of unanesthetized partially trained rats. In a control experiment, untrained animals received the same dose of Nembutal and trauma and their SGOT value was 1416 ± 319. In saline treated animals which were run simultaneously, the SGOT value was 1187 ± 505. Thus the lower SGOT values for drum conditioned rats did not result from conscious cooperation of the animals, but were indeed a result of physical conditioning.

Local tissue resistance(9) and the reticuloendothelial system(2) are but 2 of the factors implicated in development of resistance to traumatic shock. If the SGOT response to drum trauma is lower in the drum trained animal due primarily to a mechanism such as local tissue adaptation, then treatments affecting subsequent links in the chain of the body's defense mechanisms would have little or no effect on it. One of the links examined was the reticuloendothelial system. Resistance to trauma can be greatly reduced or abolished by agents which alter the functional capacity of the reticuloendothelial system, R.E.S(7). A colloidal dispersion of thorium dioxide is one of the agents which have been used to "blockade" the R.E.S. and concomitantly abolish tolerance to trauma. The correlation between capacity of animals to withstand lethal drum trauma and their serum transaminase level was studied in R.E.S. blockaded rats. The results are presented in Table III.

There was no difference in SGOT values of the Thorotrast treated and control group one-half hour after a lethal drum challenge, but the survival of the former group was significantly reduced. Further evidence of R.E.S. blockade at this level of Thorotrast was obtained from the decreased rate of clearance of administered colloidal carbon from the circulation(10). The transient effect of R.E.S. blockade on trauma resistance reported by McKenna and Zweifach(7) was confirmed in these experiments. Trauma resistant animals receiving Thorotrast 24 hours prior to challenge exhibited no de-

TABLE III. Transaminase Response to Trauma in R.E.S. Blockaded TR-750 Rats.

Group	Thoro-trast	ΔT*	SGOT, units/ml	Survival 24 hr
I	—	—	510 ± 55	7/8
II	+	3	550 ± 100	1/8
III	+	3		3/12
IV	+	24		6/6

* ΔT = Time interval between Thorotrast administration (.9 ml/100 g i.v.) and drum challenge of 750 revolutions.

Groups I-IV are TR-750 rats which received either a placebo or Thorotrast injection (.9 ml/100 g i.v.) and were then challenged with 750 revolutions after an interval of 3 or 24 hr.

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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