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Saline and Methionine-Saline Effects on Survival Rate of Rats Receiving Standardized Burn Shock.*

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McCarthy and Bodkin[1] have reported that the oral administration of 160 mg of methionine to rats, 2 hours before a standard thermal injury significantly increased the 48-hour survival rate. Methionine administered at varying periods following thermal injury failed to influence the survival. In 1945 we investigated the effect of methionine and saline in rats receiving standarized burns. Due to unavoidable circumstances, these experiments were interrupted, and only recently have they been completed. The methionine effects are reported here as largely confirmatory to the data of McCarthy and Bodkin.[1]

Experimental. Adult rats of the Sprague-Dawley and Wistar strains were used. Previous to the experimental procedure, the animals received a commercial stock ration and distilled water *ad libitum*. The standard burn was produced by the technic of Rosenthal,[2] modified as described below. The water bath was maintained within ± 0.5° of the desired temperature and the duration of the immersion was determined with a stopwatch. The animals were not shaven and were completely immersed to the center of the neck region. Fifteen minutes before immersion 0.1 cc of sodium pentobarbital solution (6 mg) per 100 g body weight was injected intraperitoneally. In preliminary experiments pentobarbital appeared to be superior to ether in that a more uniform depth of anesthesia was obtained and the anesthesia persisted for a longer time following the burn. Immediately after immersion, the animals were wrapped in a dry towel to remove excess water and then placed in individual cages with access to food and water. They were observed at 5 hour intervals for 50 hours. Animals dying during a 5 hour period were recorded as dying at the end of that period. The experimental animals received by intraperitoneal injection, 2.50 or 1.25 cc of a 1.5% solution of methionine in isotonic saline per 100 g of body weight. The controls received comparable volumes of saline. We found the results of this simplified method of producing a standard burn to be highly reproducible from day to day.

Results. The data are summarized in the table. Apparent differences were analyzed statistically and only those in which the difference was 3 or more times the standard error of the differences were considered significant. All animals injected one hour before immersing for 15 sec. at 70° C survived. Next, the temperature was elevated to 75°, with the immersion period remaining at 15 sec. The 5° elevation in temperature was sufficient to decrease the survival rate of the animals injected with saline one hour before burning from 100.0 to 33.3%. The methionine under these conditions gave a highly significant increase in the survival rate, increasing the rate from 33.3 to 91.7%. In the experiments of McCarthy and Bodkin[1] the animals received approximately 80 mg of methionine per 100 g body weight, whereas in the present study 37.5 mg per 100 g produced a significant effect. These results confirm the findings of McCarthy and Bodkin.[1] A comparison of the uninjected controls with those receiving saline one hour before burning indicates that the saline was without effect. Prinzmetal and co-workers[3] have reported that

* Preliminary experiments were carried out by the junior author in the Biochemistry Laboratory, Southwestern Medical College, Dallas.

1 McCarthy, M. D., and Bodkin, R. E., Proc. Soc. Exp. Biol. and Med., 1946, **63**, 377.

2 Rosenthal, S. M., *U. S. Pub. Health Rep.*, 1942, **57**, 1923.

3 Prinzmetal, M., Hechter, O., Margolis, C., and Feigen, G., *J. Am. Med. Assn.*, 1943, **122**, 720.

TABLE I.
Effect of Saline and Methionine-Saline Injections on the 50-Hour Survival Rate of Rats Receiving Standardized Burn Shock.

Bath temperature, °C	Duration of immersion, sec.	No. of rats	Injection		Survival, %
			Solution*	Volume,† cc	
		Uninjected controls.			
75	15	22			13.6
		Injected 1 hour before burning.			
70	15	12	S	2.5	100.0
70	15	12	MS	2.5	100.0
75	15	12	S	2.5	33.3
75	15	12	MS	2.5	91.7
		Injected at time of burning.			
75	15	11	S	2.5	72.7
75	15	11	MS	2.5	72.7
75	20	8	S	2.5	62.5
80	15	8	S	2.5	50.0
80	15	22	S	1.25	13.6
80	15	21	MS	1.25	28.6
		Injected 1 hour after burning.			
75	15	34	S	2.5	79.4
75	15	35	MS	2.5	68.6
75	15	19	S	1.25	26.3
75	15	22	MS	1.25	13.6
80	15	12	S	2.5	58.3
80	15	12	MS	2.5	41.7

* S signifies isotonic saline; MS, methionine-saline.
† Volume injected per 100 g body weight.

the administration of isotonic saline in amounts equivalent to 5% of the body weight one-half hour before burn shock significantly increases the survival time. In the animals injected at the time of the burn (75° - 15 sec), the saline was highly protective, increasing the survival rate from 13.6 to 72.7%. The methionine-saline solution gave the same survival rate as saline alone under these conditions. Because of the protective effect of saline alone, the experimental conditions were not satisfactory for demonstration of an effect of methionine, so the conditions were varied to produce a lower survival rate in the saline-injected animals. The best conditions found were 80° C for 15 sec. with injections of 1.25 per 100 g body weight. However, the 28.6% survival rate of the methionine-saline treated rats was not significantly different from the 13.6% observed in the saline controls. Therefore, it was concluded that while isotonic saline was highly protective against burn shock when administered at the time of the burn, the addition of methionine was without effect. The effect of the two solutions one hour after burning was determined under 3 different sets of conditions. In all 3 experiments the methionine-saline solution gave a slightly lower survival rate than the saline alone. However, the differences were not statistically significant. The saline solution was as effective when given one hour after the burn as when given at the time of the burn. Rosenthal[4] has previously reported that isotonic saline lowers the mortality when administered one or more hours following a standard burn. Croft and Peters[5] have shown that methionine has a protein-sparing action when fed to rats following thermal burns.

The explanation for the difference in effect of methionine when given before a burn and at the time or after a burn is not apparent. It is possible that the action of methionine involves preliminary processes in the liver or other organs which are interfered with when the methionine is administered at a later time in relation to the burn. Also, these results do not preclude the possibility that larger

[4] Rosenthal, S. M., *U. S. Pub. Health Rep.*, 1943, **58**, 513.

[5] Croft, P. B., and Peters, R. H., *Lancet*, 1945, **1**, 266.

amounts or repeated doses of methionine might increase the survival rate.

Summary. A simple and convenient method for producing standardized burn shock in rats is described. Isotonic saline injected intraperitoneally one hour before a standardized burn shock did not increase the survival rate; administration at the time of the burn or one hour following significantly increased the survival rate. Methionine-saline solution given one hour before burning significantly increased the survival rate; given at the time of the burn or one hour following, the effect was the same as with saline alone.

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Enhancement of Epinephrine Induced Cardiac Arrhythmias by Tetraethylammonium Chloride (Etamon).*

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In the experiments to be reported herein tetraethylammonium chloride has been shown to intensify the cardiac arrhythmias occurring in dogs during cyclopropane anesthesia. Secondly, experiments on unanesthetized animals suggest that the choice of a pressor agent for use as an antidote after tetraethylammonium chloride may be critical. After tetraethylammonium injection epinephrine resulted in ventricular tachycardia whereas phenylephrine (Neo-Synephrine) caused no arrhythmias.

Cardiac arrhythmias occurring during cyclopropane anesthesia have been demonstrated experimentally to depend upon reflex sensitization of the heart to epinephrine.[1] Cyclopropane stimulates receptors located in the abdominal viscera, producing afferent impulses which pass to the cord by way of fibers travelling with the splanchnics. The reflex center is located in the brain, presumably in the hypothalamus. Efferent impulses pass to the heart by way of cardiac sympathetics. Since interruption of this pathway at any point has prevented the arrhythmias, the tetraethylammonium ion should theoretically block the reflex sensitization. This agent has been reported to interrupt sympathetic impulses by ganglionic blockade[2] and consequently was expected to block the efferent impulses.

When it was found that tetraethylammonium potentiated the cardiac effects of epinephrine under cyclopropane, the cardiac effects of the ion and epinephrine were also investigated in unanesthetized animals. Epinephrine has been recommended as an antidote for the profound hypotension which may follow the clinical administration of tetraethylammonium.[3]

Cyclopropane-epinephrine ventricular tachycardia was produced in the dog by the method described by Meek *et al.*[4] The only modification of this technic was reduction of the standard test dose of epinephrine in order to lower the mortality from ventricular fibrillation. After the control duration of ventricu-

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1 (a) Allen, C. R., Stutzman, J. W., and Meek, W. J., *Anesthesiology*, 1940, **1**, 158; (b) Stutzman, J. W., Murphy, Q., Allen, C. R., and Meek, W. J., *Anesthesiology*, 1947, **8**, 579; (c) Pettinga, F. L., and Stutzman, J. W., *Fed. Proc.*, 1948, **7**, 93.

2 Acheson, G. H., and Moe, G. K., *J. Pharmacol. and Exp. Therap.*, 1946, **87**, 220.

3 Berry, R. L., Campbell, K. N., Lyons, R. H., Moe, G. K., and Sutler, M. L., *Surgery*, 1946, **20**, 525.

4 Meek, W. J., Hathaway, H. R., and Orth, O. S., *J. Pharmacol. and Exp. Therap.*, 1937, **61**, 240.