pears to have no specific protective action against shock. (4) Possible mechanisms are discussed.

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Control Mechanisms for Trauma Induced DLSH Reaction (Decrease in Mouse Liver Non-Protein Sulfhydryl).* (24078)

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The DLSH reaction is a term used to refer to a statistically significant decrease in liver non-protein sulfhydryl (NPSH) or glutathione (GSH) which occurs within a few hours after subjection of animals to certain experimental procedures. This reaction has been found to occur in mice and/or rats in association with severe trauma(1,2,3), severe cold(1,2,4,5), or administration of toxic or pharmacologic amounts of bacterial polysaccharide(2), insulin(6,7) or epinephrine(8). Register and Bartlett(5,8) have postulated that such a reaction occurring in rats subjected to severe cold, is actually due to stressor activation of the sympatho-adrenal system, particularly to release of epinephrine from the adrenal medulla. Our data indicate that in the DLSH reaction induced in mice by tourniquet trauma, both the sympathetic nervous system and the adrenal cortex play essential roles, whereas the adrenal medulla and the islets of Langerhans do not.

Methods. Mice were made diabetic by injection of alloxan, 100 mg/kg, into the tail vein (cf. Waisbren(9)). In bilateral adrenalectomies each kidney and attached adrenal gland was gently exteriorized through incision immediately lateral to backbone; effectiveness of adrenalectomy was checked in each mouse by autopsy at time of sacrifice. Exteriorization of the kidney and attached adrenal gland also proved extremely useful in adrenal demedullations, performed by an adaptation of the method of Evans(10). Effectiveness of adrenal demedullation procedure was checked for each group of mice by chemical estimation of adrenal medullary hormone content of the pooled adrenals. Tourniquet trauma was performed by the double hind leg ligation method of Rosenthal(11). The ligation period was 2 hours, and sacrifice was usually 2 hours after

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TABLE I. Effect of Insulin on Mouse Liver NPSH and Blood Sugar.

Treatment of mice*					
18 hr fast	Insulin only, subcut.	Insulin & glucose, subcut.	Blood sugar, mg %†	Liver, NPSH, mg %, GSH equiv.†	Insulin action on mice‡
No	No	No	138	249	
Yes	"	"	73	166	
			104	156	
"	Yes	"	20	99	+
			58	105	—
"	No	Yes	16	86	+
			30	103	+
			45	99	—
			63	152	—

* Insulin in amount of 10 units/kg was inj. subcut. 4 hr before sacrifice, *i.e.*, in fasted mice at 14 hr after beginning of fast. 2.5 or 15% glucose was inj. subcut., singly or repeatedly, the total dose being 1.5 mg/kg.

† Values are for samples pooled from 2 or 4 mice.

‡ Convulsions and/or prostration prior to sacrifice.

removal of ligatures. In each experiment, injections and/or hind leg ligations of individual mice were so scheduled that the *average* time of day at which sacrifice occurred was practically the same for all groups. This elaborate scheduling was employed to reduce to a minimum, possible effects of diurnal variation in liver NPSH which occurs in mice and rats (12). P values of Tables were secured by use of Student's t table in Peters and Van Voorhis(13). The methods for estimating liver NPSH and glutathione have been described(12). Blood sugar was estimated by the combined method of Nelson(14) and Somogyi(15). The iodine oxidation method of v. Euler and Hamberg(16) for estimating total medullary hormone content of adrenals was adapted to mouse adrenals, as follows: Adrenals pooled from 10 mice were weighed, and homogenized with 3 ml of 5% trichloracetic acid. Solid material was removed by centrifuging. Fat and trichloracetic acid were removed by 3 separate shakings with ether, about 6 ml/extraction. The adrenal medullary hormones in 1 ml of the extract were completely oxidized, using iodine at pH 6.0 for 3 minutes. A blank not containing adrenochrome was prepared by first mixing all reagents together, so that the iodine was reduced only by thiosulfate, and then adding 1 ml of extract. Finally the optical density of the adrenochrome-containing mixture was read against the blank at 529 mμ using a Beckman spectrophotometer.

Results. Insulin and mouse liver NPSH. Eli Lilly Iletin was diluted with either water, 2.5% or 15% glucose to yield solutions which gave doses of 1, 3, 10 or 30 units/kg when 0.01 ml/g was injected subcutaneously. Four hours later mice were sacrificed and the excised livers were frozen on dry ice until analyzed. For both normally fed and 18 hour starved mice, the minimum dose of insulin required to lower mouse liver NPSH appreciably was 10 units/kg. Extent of insulin induced decrease in liver NPSH was positively correlated with both decrease in blood sugar and likelihood that symptoms of hyperinsulinism would be exhibited (Table I). Glucose *as administered in these experiments* did not prevent hypoglycemia, symptoms of hyperinsulinism, or insulin induced decrease in liver NPSH. Findings made with glucagon-free insulin† were similar to those made with Iletin. Mice injected with insulin-free glucagon† exhibited liver NPSH values within the normal range.

Trauma and liver NPSH of alloxan diabetic mice. Of 18 mice which survived alloxan administration, 7 which exhibited unusually large water intakes were subjected to tourniquet trauma. The average liver NPSH value for these 7 mice was 103 ± S.E. 8 mg% GSH (glutathione) equivalent. Their blood sugar

† Donated by Eli Lilly Co.

TABLE II. Adrenal Glands and Tourniquet Trauma Induced DLSH Reaction.

Operation	Days between operation and exp.	Mean liver NPSH values, mg %, GSH equiv., ± S.E.		P values for significance of differences
		Untreated mice	Traumatized mice	
None		245 ± 10 (6)*	116 ± 7 (8)*	<.0001
		201 ± 9 (7)	116 ± 6 (10)	"
		199 ± 6 (10)	122 ± 6 (10)	"
Sham	0	208 ± 9 (11)	118 ± 4 (20)	"
		204 ± 7 (7)	144 ± 7 (8)	"
	4	272 ± 14 (12)	140 ± 6 (12)	"
Adrenal demedullation	35	193 ± 6 (10)	105 ± 6 (9)	"
		191 ± 5 (9)	112 ± 8 (10)	"
Adrenalectomy	0	204 ± 7 (15)	196 ± 8 (15)	NS
		219 ± 10 (16)	171 ± 9 (14)	.004
	4	215 ± 10 (10)	191 ± 8 (9)	NS
		212 ± 5 (9)	186 ± 12 (10)	"
		230 ± 10 (8)	191 ± 9 (8)	.02
		231 ± 13 (15)	182 ± 14 (12)	.04
	39†	238 ± 9 (10)	231 ± 11 (11)	NS

* No. of livers analyzed shown in parentheses.
† Maintained with fluorohydrocortisone up to 4 days before exp.

values ranged from 300 to 800 mg% glucose equivalent. The remaining 11 mice were sacrificed without being subjected to trauma. The average liver NPSH value for these 11 mice was 237 ± S.E. 14 mg%, GSH equivalent: their blood sugar values ranged from 180 to 590 mg%. It is apparent that a DLSH reaction was induced by tourniquet trauma in mice which had been made severely diabetic by use of alloxan.

Influence of certain surgical procedures on trauma-induced DLSH reaction. Data for 15 experiments are shown in Table II. Trauma-induced DLSH reactions occurring in sham operated and adrenal demedullated mice did not differ appreciably from those occurring in non-operated mice. On the other hand, only slight decreases in liver NPSH occurred in adrenalectomized mice in association with tourniquet trauma. The following data were obtained in analyses for adrenal medullary hormones of pooled mouse adrenals (10 mice/group) by the iodine oxidation method: non-operated mice, 110-130 mg of tissue, about 1 mg/kg of epinephrine (E) plus nor-epinephrine (NE); adrenal demedullated mice 35 days after operation, 60-70 mg of tissue, <0.05 mg/kg of (E) plus (NE) (less than could be estimated by procedure employed).

Effect of l-epinephrine (E) and l-norepinephrine (NE) on liver NPSH of non-operated, adrenal demedullated and adrenalectomized mice. The data (Table III) indicate that the smallest dose of either (NE) or (E), administered over a 5 hour period, which regularly induced a DLSH reaction in non-operated mice was 2.5 mg/kg. The reactions obtained with 2.5 and 5 mg/kg of (NE) and (E) were not as great as those obtained routinely with tourniquet trauma. Higher doses of (NE) and (E) were not tested, since occasional deaths occurred in experiments using (E) at 5 mg/kg. Both (NE) and (E) induced DLSH reactions in demedullated mice. On the other hand, mice adrenalectomized 4 days before experimentation failed to exhibit DLSH reactions on administration of (NE), 2.5 or 5 mg/kg or (E), 2.5 mg/kg. (E), 5 mg/kg proved to be so lethal to adrenalectomized mice that tests at this dosage were discontinued.

Counteraction of trauma-induced decreases in mouse liver NPSH by a ganglion-blocking drug. The drug selected for study was chlorisondamine dimethochloride(17) which exerts a prompt, effective and relatively long-lasting ganglion-blocking action with minimum side effects. Four groups of 10 mice each were set

TABLE III. Adrenal Glands and DLSH Reaction Induced by Adrenal Medullary Hormones.

Operation	Days between operation and exp.	Hormone* and dose (mg/kg)	Mean liver NPSH values, mg %, GSH equiv., ± S.E.		P values for significance of differences
			Saline inj. mice	Hormone inj. mice	
None		NE, 1	198 ± 7 (10)†	176 ±14 (10)†	NS
		5	" "	123 ± 4 (10)	<.0001
		1.25	193 ± 6 (10)	164 ± 8 (10)	.02
		2.5	176 ± 9 (10)	140 ± 4 (12)	.005
		5	186 ± 8 (8)	137 ± 8 (8)	.002
		E, 1.25	171 ± 6 (10)	158 ± 6 (10)	NS
		2.5	" "	140 ± 8 (10)	.02
		5	" "	120 ± 8 (9)	.001
		1.25	193 ± 6 (10)	158 ± 5 (10)	.002
		2.5	176 ± 9 (10)	129 ± 5 (12)	"
Adrenal demedullation	35	NE, 2.5	191 ± 5 (10)	125 ± 7 (9)	<.0001
		5	" "	122 ± 6 (10)	"
		E, 2.5	192 ± 5 (10)	146 ± 6 (10)	.0002
Adrenalectomy	4	NE, 2.5	199 ± 7 (10)	206 ±13 (10)	NS
		5	" "	173 ±11 (9)	"
		2.5	192 ±13 (10)	169 ± 7 (10)	"
		E, 2.5	" "	181 ± 8 (10)	"

* NE = 1-norepinephrine bitartrate; E = synthetic l-epinephrine neutralized with tartaric acid. Total dose shown was given subcut. in 5 parts at hourly intervals, with sacrifice 1 hr after final injection. Dilutions were made immediately before injections using 0.9% NaCl; all injections were .01 ml/g.

† No. of livers analyzed shown in parentheses.

up. All injections were made intraperitoneally 0.01 ml/g, at 4 and again at 2 hours before sacrifice. Mice of Groups A and B were injected with 0.9% NaCl, mice of Groups C and D with the drug at a concentration of 50 mg% in 0.9% NaCl. The total dose of drug received by mice of Groups C and D was 10 mg/kg. Mice of Groups B and D were also subjected to tourniquet trauma. The liver NPSH values secured, expressed as glutathione equivalents, mg%, ± standard errors, were as follows: Group A (saline controls), 183 ± 10; Group B (saline + trauma), 128 ± 7; Group C (drug controls), 190 ± 8; Group D (drug + trauma), 181 ± 10. It is apparent that the ganglion-blocking drug chlorisondamine dimethochloride practically abolished the trauma-induced decrease in mouse liver NPSH.

Discussion. A trauma-induced DLSH reaction was exhibited by non-operated, sham operated, adrenal demedullated and alloxan diabetic mice, but not to any appreciable extent by adrenalectomized mice, or mice previously injected with the ganglion-blocking drug chlorisondamine dimethochloride. These findings are interpreted to mean that both adrenal cortical and sympathetic nervous system activities are necessary for the DLSH reaction to trauma, but not those of endogenous insulin from the islets of Langerhans or epinephrine (E) plus nor-epinephrine (NE) from the adrenal medullae. DLSH reactions also occur following administration of very large doses of insulin(6,7), E(8) or NE (this paper). Both E and NE mimic trauma, in that following administration of either hormone DLSH reactions occurred in non-operated or adrenal demedullated, but not in adrenalectomized mice. However, E is toxic to the point of acute lethality when used at the minimum dose of 5 mg/kg required to induce a DLSH reaction approaching in size that obtained routinely with trauma; this minimum dose of E is well beyond any dose which the literature indicates could be considered physiological. This fact, coupled with the finding that adrenal demedullated mice exhibit as great a trauma-induced DLSH reaction as do non-operated mice, suggests that a DLSH reaction induced by a very large dose of E or NE may be due to achievement throughout the body of E or NE concentrations approximating those of NE plus E which occur discretely in the

liver in association with intense sympathetic nervous system activity, however induced. This in turn suggests that a DLSH reaction induced by a very large dose of insulin may be due to stressor activation of adrenal cortical and sympathetic nervous system mechanisms necessary to any DLSH reaction, rather than to a specific insulin action on liver NPSH.

Summary. A DLSH reaction (statistically significant decrease in liver NPSH) was secured in mice by administration of very large amounts of insulin, epinephrine or nor-epinephrine, or tourniquet traumatization. Trauma was as effective in inducing a DLSH reaction in alloxan diabetic or adrenal demedullated mice as in normal mice; hence the trauma induced DLSH reaction was not attributable to endogenous insulin or adrenal medullary hormones. The trauma induced DLSH reaction was practically abolished by either adrenalectomy or administration of the ganglion blocking drug chlorisondamine dimethochloride. This indicates that both adrenal cortical and sympathetic nervous system activities are required for the reaction.

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Failure of DL-Batyl Alcohol to Prevent Aplastic Anemia in Calves.* (24079)

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This work was prompted by the report of Evans *et al.*(1) that experimentally induced or field cases of Bracken poisoning in cattle could be successfully treated with subcutaneous or intravenous administration of DL-batyl alcohol and comprehensive antibiotic therapy. Hematological aspects and clinical course of Bracken poisoning in cattle are very similar(2) to aplastic anemia induced when certain specimens of trichloroethylene-extracted soybean oil meal (TCESOM) are fed to cattle or calves(3,4). In both instances the intoxication produces a blood dyscrasia characteristic of hypoplastic or, in more severe cases, of aplastic anemia, namely thrombocytopenia, leukopenia, granulocytopenia and a relative lymphocytosis, a moderate degree of anemia, a hemorrhagic syndrome, ter-

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