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Effects of Shock and Cold on Mouse Liver Sulfhydryl.* (19853)

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While testing the effects of *S. marcescens* tumor damaging bacterial polysaccharide(2) on concentration of iodine-reducing substances in mouse tissues, one of us noted(1) that toxic doses of this substance induced in mouse liver an appreciable decrease in concentration of non-protein sulfhydryl, measured as the difference between total iodine-reducing substances and iodine reduction due to ascorbic acid. Toxic doses of the polysaccharide markedly depress body temperature, circulation and reactivity of mice(3,4,5), as do also burns(6) and ligation trauma(7). This suggested the desirability of testing for effects of burn shock and traumatic shock on concentration of sulfhydryl compounds in mouse liver. The effects of a non-shock stress situation (exposure to cold) on liver sulfhydryl were also studied.

Materials and methods. Distilled water used: Ordinary distilled water was passed through a LaMotte Filter-ion exchange column, to remove traces of heavy metal ions reactive with sulfhydryl groupings. *Preparation of liver extracts.* A. *Solutions.* M/10 potassium phosphate buffer of pH = 6.8 (KH_2PO_4, K_2HPO_4 1:1); 22% aqueous sulfosalicylic acid. B. *Procedure.* The test mouse was decapitated, the liver promptly excised, blotted to remove excess blood, weighed to within 10 mg, placed in the micro-cup of the Waring blender, and homogenized with 10 cc of ice-cold phosphate buffer for 30 seconds. Additional phosphate buffer was then added, to a total of 19 cc of the buffer for each gram of liver tissue. Homogenization was continued for another 30 seconds. The homogenate was centrifuged at about 500 g for 5 minutes to

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give a supernatant (Extract 1). A 10 cc aliquot of Extract 1 was added to 1 cc of 22% sulfosalicylic acid, and the mixture shaken vigorously, and poured through Whatman No. 1 paper to give a protein-free filtrate (Extract 2). *Amperometric titrations for sulfhydryl concentrations in Extracts 1 and 2*: Except that a vibrating platinum electrode was substituted for the revolving platinum electrode, these titrations were carried out essentially as described by Weissman, Schoenbach and Armistead(8). Diluted methanol, prepared as described by them, was used in all titrations, including those of glutathione standards. Titration of 2 or 4 cc of Extract 1 was started as soon as possible after the centrifuging, to give a value for total sulfhydryl extractable from mouse liver (concentration in liver taken as 20 times that in Extract 1). Five cc of Extract 2 were then titrated, to give a value for non-protein sulfhydryl extractable from mouse liver (concentration in liver taken as 22 times that in Extract 2). Protein sulfhydryl has been estimated as the difference between total and non-protein sulfhydryl. *Protein concentration in Extract 1*: This was estimated by the Biuret method of Robinson and Hogden(9), using a Beckman spectrophotometer set at 560 mμ for the optical density readings. In each case, 2 cc of Extract 1, 8 cc of 3.75% NaOH and 0.25 cc of 20% $CuSO_4 \cdot 5H_2O$ were used. Biuret optical density readings were standardized against casein standards, and against dry weights of material, presumably protein, which was precipitated from Extract 1 by 22% sulfosalicylic acid. *Mice used:* CAF_1 male mice were secured from Jackson Laboratories, Bar Harbor, Maine, and were used when weighing 22-27 g. ABC female mice were secured from Dr. J. J. Bittner, University of Minnesota, Minneapolis, Minn., and used when weighing 26-30 g. *Scalding of mice:* The procedure for inducing burn shock described by Rosenthal(6) was employed. The test mouse was etherized and dipped to the neck in water at 60°C for 10 seconds, or in water at 65°C for 15 seconds. Three, 6 or 18 hours after scalding, the mouse was sacrificed, and liver extracts obtained as described above. Unscalded mice were used as controls. Some of these were untreated, some had been etherized and dipped to the neck at 37°C for 10 or for 15 seconds. *Production of ligation trauma:* The procedure described by Rosenthal(7) for production of traumatic shock was employed. The time between placement of the rubber bands around both hind legs and their removal was set at 2 hours. Livers were secured from test mice 2 hours after removal of bands. *Administration of S. marcescens polysaccharide:* A water solution of polysaccharide(2), preparation P25, was injected intraperitoneally in the amount of .01 cc per gram, to give a dose of 16 mg per kg. Controls were injected with distilled water. The dose of polysaccharide was appreciably greater than the minimum of 2 mg or less per kg found necessary to induce marked hemorrhagic necrosis in mouse Sarcoma 37, implanted not less than 6 days earlier, and somewhat less than the minimum dose at which deaths were encountered. Eighteen hours after injection of polysaccharide or water, the mice were sacrificed and livers excised for sulfhydryl determinations. *Exposure to cold:* Two mice were simultaneously placed in a round glass container having a surface area at the bottom of about 200 sq. cm. The container was placed in a refrigerator just beneath the freezing compartment. Air in the glass container near the mice had a temperature between 5° and 10°C. The mice were sacrificed for liver sulfhydryl determinations 5 hours after having been placed in the refrigerator.

Results: Mice were in shock or a shock-like state at 3 and at 6 hours after scalding, at 2 hours after removal of ligatures from both hind legs, and at 18 hours after injection of polysaccharide. All of these mice were lethargic, indifferent to stimulation, cold to the touch and dyspneic. Mice which had been scalded to the neck at 60°C for 10 seconds, 18 hours earlier, or which had been exposed to cold for 5 hours, were obviously not in shock. They were fairly active, responsive to stimulation and not dyspneic. Although these mice felt warm when held in the hand, those which had been exposed to cold exhibited rectal temperatures which were 1-2°C lower than those exhibited before exposure to

TABLE I. Effects of Scalding on Mouse Liver Sulfhydryl (–SH).

Group No.	Mice used: Strain	Sex	No.	Treatment	Hr between treatment and sacrifice	Liver –SH (GSH equiv., in mg % ± stand. error): Non-protein –SH	Protein –SH
1	CAF_1	♂	7	C:* no treatment	—	210 ± 17	511 ± 19
2			8	C: dipped at 37°C, 10"	3	197 ± 18	505 ± 30
3			7	S* at 60°C, 10"	3	116 ± 11	534 ± 23
4			8	C: dipped at 37°C, 15"	3	201 ± 23	588 ± 24
5			11	S at 65°C, 15"	3	121 ± 10	522 ± 16
6			10	C: no treatment	—	201 ± 7	479 ± 18
7			10	S at 60°C, 10"	18	260 ± 17	426 ± 23
8	ABC	♀	10	C: dipped at 37°C, 10"	3	207 ± 11	486 ± 13
9			10	S at 60°C, 10"	3	141 ± 18	544 ± 22
10			10		6	124 ± 13	470 ± 19
11			10		18	281 ± 10	474 ± 30

* C = Controls; S = Scalded.

TABLE II. Effects of Ligation Trauma, *S. marcescens* Polysaccharide and Exposure to Cold on Mouse Liver Sulfhydryl (–SH).

Group No.	Mice used: Strain	Sex	No.	Treatment	Hr between treatment and sacrifice	Liver –SH (GSH equiv., in mg % ± stand. error): Non-protein –SH	Protein –SH
1	CAF_1	♂	10	C:* no treatment	—	239 ± 11	560 ± 23
2			11	Hind legs ligated, 2 hr	2	112 ± 14	500 ± 13
3			10	*S. marcescens* polysaccharide, IP, 16 mg/kg	18	106 ± 13	448 ± 19
4*			10	C: no treatment	—	201 ± 7	479 ± 18
5			10	At 5-10°C, 5 hr	0	121 ± 14	433 ± 16

* C = Controls.

cold. ABC female mice were apparently in better shape at 18 hours after scalding than were CAF_1 male mice. *Liver sulfhydryl:* Table I, Groups 3, 5, 9 and 10, show that average values for liver *non-protein sulfhydryl* concentration secured for mice apparently in shock, and sacrificed 3 or 6 hours after scalding, were significantly less than those exhibited by mice of corresponding control Groups 1, 2, 4 and 8. (P values less than .01). However, groups of mice recovering from shock, at 18 hours after scalding (Table I, Groups 7 and 11), exhibited average liver *non-protein sulfhydryl* concentrations which were actually significantly greater than those exhibited by corresponding control groups 6 and 8 (P values less than .01). Table II indicates that statistically significant decreases in average values for liver *non-protein sulfhydryl* concentration were also exhibited by mice which had been subjected to: a) ligation trauma (Group 2), b) a toxic dose of *S. marcescens* polysaccharide (Group 3), and exposure to cold (Group 5) (all P values less than .01). Some groups of the experimentally treated mice (Table I, Groups 5 and 7; Table II, Groups 3 and 5) also exhibited moderate but apparently significant decreases in liver *protein sulfhydryl* concentration. The mice of Table I, Group 7, exhibited at 18 hours after scalding an increase in liver *non-protein sulfhydryl* and a *decrease* in liver *protein sulfhydryl* concentration. *Protein extracted from liver with phosphate buffer:* The amount of protein present in Solutions 1, as indicated by Biuret optical density readings and dry weights of material precipitated from Solutions 1 by sulfosalicylic acid, was about 2-2.5%, indicating the presence in mouse liver of extractable protein in the amount of 20-25% of the wet weight of the liver. Values for control and treated mice have not differed

significantly, hence the data for individual Groups have been omitted.

Discussion. The work of Binkley and coworkers(10) has indicated that the non-protein sulfhydryl of rat liver consists mainly of glutathione or precursors of glutathione, *i.e.*, cysteinyl glycine and y-glutamyl cysteine. We have no data as to the nature and amounts of compounds which comprise the non-protein sylfhydryl of mouse liver, nor which ones are concerned in the observed changes in non-protein sulfhydryl occurring as a result of the experimental procedures to which the mice have been subjected. Changes in non-protein sulfhydryl observed in the present experiments probably were due chiefly to changes in relative rates of synthesis and breakdown of glutathione in the liver. Further experimentation may reveal whether decreases in liver non-protein sulfhydryl concentration are a usual concomitant of stress situations and shock, and what mechanisms are involved in the effects noted to date.

Summary. Mice were subjected to severe stress by scalding, ligation trauma, injection of a toxic dose of a bacterial polysaccharide, or exposure to cold. Each of these procedures produced a marked decrease in liver non-protein sulfhydryl concentration. In some experiments, a moderate decrease in liver protein sulfhydryl concentration also occurred. Mice recovering from scalding exhibited marked increases in liver non-protein sulfhydryl concentration to values well above the original control level.

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An Inhibiting Effect Observed in the Course of Normal Wound Healing. (19854)

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The experiments described herein were initiated to attempt to account for the slowing-down of the proliferation of granulation-tissue in the last phase of wound healing prior to closure by epithelization. One possibility is that this slowing-down of the proliferation is caused by the liberation of an inhibiting factor in the organism.

To test this hypothesis, 2 experimental wounds of equal size were produced at different time intervals, so that any inhibiting influence produced in the last phase of the first wound healing process could be made to coincide with the active phase of proliferation of the granulation-tissue in the second wound.

Material and methods. Four series of experiments were performed on male albino rats of about 200 g weight. 1) A circular skin wound, 20 mm in diameter, was produced after depilation on the back of the animal on one side of the dorsal midline, and a second wound of equal size added symmetrically on the other side of the dorsal midline after an interval of 6-7 days (16 animals). The healing wounds were outlined on every second day and the values thus obtained plotted in curves. The exact technic of wound production and measurement has been described

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