Effects of Trauma on Incorporation of L-[³⁵S]Cysteine into Tissue Glutathione

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When mice, rats or rabbits are subjected to certain procedures which produce stress or injury, there is a fall in the concentration of non-protein-sulphydryl-group compounds of the liver and other tissues (Stolfi, 1936; Beck & Linkenheimer, 1952; Lindan & Work, 1953; Bartlett & Register, 1953; Beck & Rieck, 1958). The literature suggests that such decrease may be due mainly to decreased synthesis of glutathione, the most abundant component of liver non-protein-sulphydryl-group compounds. To test this possibility we have studied effects of tourniquet trauma on incorporation of the radioactivity of L-[³⁵S]cysteine into mouse-tissue glutathione.

METHODS

In each control or trauma experiment, pooled tissue and excreta samples were taken from 10 Carworth Farms Webster strain male mice. In the trauma experiments, the blood supply to both hind legs was occluded for 3 hr.; mice were killed 2.5 hr. after removal of the rubber-band ligatures. L-[³⁵S]Cystine, 5–20 µC/mg., was secured from Schwarz Bioresearch Inc., Mt. Vernon, New York, dissolved in 0.5N-HCl and reduced electrolytically to L-[³⁵S]cysteine, in a model 1930 Desalter (Research Specialties Co., Berkeley, Calif., U.S.A.). A weighed amount, about 0.1 g. (about 1.5 µC) of radioactive L-cysteine solution was injected by tail vein into each mouse 0.75 or 1.5 hr. before killing. Pooled tissue samples were kept frozen on solid carbon dioxide until analysed. Material termed carcass consisted of everything left over after the tissue and excreta samples had been taken. For tissues other than liver, the following procedure was employed. The weighed sample was ground in a mortar with ice-cold 5% trichloroacetic acid, about 5 ml./g. The brei was filtered through Whatman no. 1 paper into a volumetric flask preselected to have a volume not less than 10 and not more than 20 ml./g. of sample. Trichloroacetic acid (5%) was poured into the paper funnel until the flask was full to the mark. Trichloroacetic acid was then removed from a portion of the filtrate by extraction with ice-cold, peroxide-free ether. Three successive extractions were made under nitrogen in a Thunberg tube, each with 2 vol. of ether. Residual ether was removed by evacuation. After removal of trichloroacetic acid, a copper glutathionate precipitate was secured by the method of Hopkins (1929), about 50-fold excess of carrier glutathione (GSH) being used. This precipitate was used to estimate the amount of radioactivity incorporated into GSH of the tissue sample. For each liver sample, the procedures of Waelsch & Rittenberg (1941) were employed

to secure a 2 ml./g. extract in 10% trichloroacetic acid, and from this two copper glutathionate precipitates. One of the precipitates was secured with, the other without, carrier GSH, to permit estimation of liver GSH, by isotopic dilution (Kamen, 1947), as well as total radioactivity incorporated into liver GSH. Each precipitate remaining after the original extraction was re-extracted with 5% trichloroacetic acid, 20 ml./g., to permit estimation of non-protein-SH not extracted by use of 2 ml. of 10% trichloroacetic acid/g. This amounted to about 12–20% of total extracted non-protein-SH (cf. Table 2). Non-protein-SH in extracts was estimated essentially as described by Grunert & Phillips (1951). Losses in non-protein-SH from ether extraction ranged from zero (SH solutions, liver extracts) to 15%. Estimations of radioactivity were made, for both trichloroacetic acid-containing and ether-extracted extracts, by use of P611 counting mixture (Davidson & Feigelson, 1957), for copper glutathionate suspensions by use of thixcin counting gel (Nathan, Davidson, Waggoner & Berlin, 1958). The copper glutathionate was brought into suspension by ultrasonic bombardment. Counting rate for each particular sample-counting mixture (10 or 20 ml.) was obtained with a low-potassium five-dram counting vial (Wheaton Co.) and a Packard Co. Tri-Carb liquid-scintillation spectrometer. Quenching was corrected for by use of internal standards (Davidson & Feigelson, 1957). After allowing for quenching, corresponding trichloroacetic acid-containing and free extracts gave nearly identical counting rates; very little radioactivity was found in ether supernatants.

RESULTS

The data of Table 1 indicate that decreases in tissue non-protein-SH compounds were induced by trauma in all of the tissues examined, but most markedly in liver and kidney. Differences in liver non-protein-SH compounds between control and traumatized groups of mice were very similar to those found in earlier work (Beck & Linkenheimer, 1952; Beck & Rieck, 1958). In the earlier work, in which liver non-protein-SH was estimated for each mouse used, *P* values for control versus traumatized groups of mice were almost invariably less than 0.001; hence present differences are considered significant. The concentration ratios of Table 1 were calculated to simplify comparisons between control and trauma experiments, for distribution of administered ³⁵S into (a) GSH, and (b) all non-protein compounds:

$$\text{Concentration ratio} = \frac{\text{Counts/min./mg. of tissue used to obtain } ^{35}\text{S in (a) GSH or (b) all non-protein}}{\text{Counts/min. (injected material)/mg. of mouse}}$$

From this definition it follows that if administered ^{35}S were uniformly distributed throughout the entire body and none was excreted, and if all were incorporated into GSH, all numbers of columns (a) and (b) of Table 1 would be 1.0. The concentration ratios obtained for tissue GSH [cf. rows (a) of Table 1] indicate that L- ^{35}S]cysteine incorporation into GSH was: (1) much greater for liver and kidney than for the other tissues examined; (2) greater for tissues of control than of traumatized mice, especially for liver and kidney; (3) greater for a 0.75 hr. than for a 1.5 hr. incorporation time. This last finding suggests that GSH is an early intermediate in the metabolism of L-cysteine.

The concentration ratios obtained for ^{35}S in all non-protein compounds [cf. rows (b), Table 1] indicate that liver and kidney of both control and traumatized mice selectively accumulated L-cysteine or derivative non-protein compounds or both, and to an extent well beyond what would have been expected if the accumulation had been due mainly to GSH synthesis. If, in corresponding control and trauma experiments, the ratio

$$\frac{^{35}\text{S in L-cysteine}}{^{35}\text{S in all non-protein compounds}}$$

were the same for corresponding tissues, then the (b) values of Table 1 would indicate what fraction

of administered L- ^{35}S]cysteine had been available for synthesis of labelled GSH, and (a)/(b) ratios would indicate how efficient the tissues had been in carrying out such synthesis. From Table 1 it is apparent that all liver and kidney (a)/(b) ratios were much lower in trauma experiments than in corresponding control experiments, a finding which strongly suggests that these tissues of traumatized mice were less efficient in GSH synthesis than were corresponding tissues of control mice. The relatively high (b) values found for tissues of traumatized mice, and the greater excretion of ^{35}S found in control than in corresponding trauma experiments, indicate that trauma-induced decrease in tissue synthesis of labelled GSH was not due mainly to trauma-induced decrease in availability of L- ^{35}S]cysteine to the tissues. Skeletal-muscle samples were taken from the hind legs. Therefore it is likely that the much higher (b) values found for muscle in the trauma experiments were due to accumulation of oedema fluid in the hind legs, as a result of the trauma.

In five of the experiments, liver GSH was estimated by isotopic dilution (Kamen, 1947). The data obtained (Table 2) indicate that trauma-induced decrease in liver GSH was of a magnitude sufficient to account for the greater part of trauma-induced decrease in liver non-protein-SH compounds.

Table 1. *Effects of trauma on tissue non-protein-sulphydryl-group compounds and on distribution of L- ^{35}S]cysteine into (a) glutathione and (b) all non-protein compounds*

NPSH, Tissue non-protein SH (m-equiv. of S/kg.); (a) and (b), tissue-concentration ratios for GSH and all non-protein compounds respectively. For definition of concentration ratio, see text. Time between intravenous injection of L- ^{35}S]cysteine and killing, 1.5 or 0.75 hr. C₁, etc., control experiments; T₁, etc., trauma experiments; A₁, experiments in which mice had been adrenalectomized 2 days earlier. Muscle samples were obtained from hind legs, and therefore, in trauma experiments, were subjected to prior occlusion of circulation.

Time interval	1.5 hr.						0.75 hr.		
Estimation made	C ₁	C ₂	C ₃	C ₄	T ₁	T ₂	C ₅	T ₃	A ₁
Liver NPSH	5.80	6.84	6.38	6.56	3.32	3.29	7.22	2.70	7.55
(a)	0.28	0.24	0.27	0.36	0.13	0.13	0.62	0.17	0.83
(b)	1.35	1.39	1.10	1.18	1.34	1.14	1.98	0.98	2.29
Kidney NPSH	4.33	5.11	4.69	3.52	2.93	1.92	4.23	2.28	3.58
(a)	0.47	0.22	0.30	0.25	0.08	0.19	0.59	0.19	0.49
(b)	1.55	1.39	1.33	1.79	1.73	2.58	1.60	2.10	1.08
Mucosa NPSH	3.48	3.29	2.60	2.93	2.80	2.12	3.16	2.41	3.16
(a)	0.12	0.15	0.06	0.10	0.06	0.07	0.20	0.09	0.16
(b)	0.66	0.69	0.52	0.72	0.54	0.36	0.56	0.39	0.43
Carcass NPSH	—	—	—	—	—	—	1.11	0.65	1.11
(a)	0.041	0.026	0.032	0.016	0.022	0.019	0.054	0.038	0.080
(b)	0.265	0.201	0.273	0.200	0.396	0.511	0.307	0.361	0.252
Muscle NPSH	0.81	0.99	0.59	0.39	0.39	0.16	0.59	0.39	0.49
(a)	—	0.010	0.013	0.011	0.009	0.020	0.034	0.021	—
(b)	0.104	0.110	0.080	0.078	0.270	0.473	0.010	0.285	—
S recovered in all non-protein compounds (%)	32	27	33	27	44	55	41	41	37
S recovered in excreta (%)	30	23	28	29	7	3	8	3	3

Table 2. *Effects of trauma on liver glutathione, estimated by isotopic dilution*

GSH and non-protein-SH-group compounds (NPSH₁, NPSH₂) were determined in portions from extract in 10% trichloroacetic acid, 2 ml./g. of liver. The precipitate from this first extract was re-extracted with 5% trichloroacetic acid, 20 ml./g. of liver. NPSH₂ was estimated in this second extract.

Time interval ... Factor estimated (m-equiv. of S/kg.)	1.5 hr.		0.75 hr.		
	C ₄	T ₂	C ₅	T ₃	A ₁
GSH	4.86	2.59	4.73	2.28	4.57
NPSH ₁	5.58	2.64	5.99	2.37	6.38
NPSH ₂	0.98	0.65	1.23	0.33	1.17
NPSH ₁ + NPSH ₂	6.56	3.29	7.22	2.70	7.55

DISCUSSION

Experimentally, each particular tissue of control mice and rats exhibits, within a characteristic range, constancy in GSH concentration and in concentration of non-protein-sulphydryl-group compounds. Since the literature indicates that plasma GSH is very low, it is unlikely that passage of GSH through cell membranes is important in determining GSH concentrations of particular tissues. Rather, the GSH concentration existing in a particular tissue is probably determined mainly by a steady-state equilibrium peculiar to that tissue, in which (a) rate of synthesis and (b) rate of degradation of GSH in the tissue are nearly equal. A trauma-induced decrease in GSH of a particular tissue might be due mainly to decrease in (a) or increase in (b). If trauma reduced (a) to zero, no part of ³⁵S administered as L-cysteine would appear in the tissue GSH. Therefore the much smaller incorporation of L-[³⁵S]cysteine into tissue GSH of traumatized than that of control mice, noted in the present experiments, would appear to be in accord with the hypothesis that trauma-induced decrease in tissue GSH is due mainly to decreased synthesis rather than increased degradation of the compound. Decreased synthesis might be due to decreased efficiency of the GSH-synthesis mechanism or decreased availability of L-[³⁵S]-cysteine for incorporation into GSH or both, as would occur, for example, if trauma markedly increased oxidation of L-cysteine toward sulphate. The literature indicates that decreased efficiency of GSH-synthesis mechanisms may be expected to occur in association with trauma: (a) Bloch (1949) has shown that both adenosine triphosphate and oxygen are necessary for synthesis of GSH in a pigeon-liver preparation *in vitro*; (b) animals in traumatic shock exhibit tissue anoxia, especially in liver and kidney (Wiggers, 1950); (c) Le Page (1946) and McShan, Potter, Goldman & Shipley (1945) have shown that severe trauma or haemorrhage results in depletion in body tissues of adenosine triphosphate and other energy-rich phosphate compounds.

SUMMARY

1. In comparison with controls, mice whose blood supply to the hind legs had been occluded for 3 hr., and which were killed 2.5 hr. after removal of hind-leg ligatures, exhibited in their tissues, especially liver and kidney, appreciably lower values for both concentrations of non-protein-SH-group compounds and incorporation of L-[³⁵S]cysteine into glutathione.

2. This parallelism indicates that trauma induces decreases in tissue non-protein-SH-group compounds mainly by inhibiting synthesis of glutathione *in vivo*.

3. Most of the decrease in liver non-protein-SH-group compounds was accountable for by decrease in glutathione as estimated by isotopic dilution.

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