treatment on a random basis, DDS was found to have therapeutic value in lepromatous leprosy as compared to untreated controls(4). Cycloserine was later found about as effective as DDS(5). Results with PAS(4) were ambiguous (PAS was found to be as effective as diasone and less effective than DDS in the one group of patients in which PAS was tried; in other groups diasone and DDS had the same activity). Other published clinical opinions of PAS are not unanimous and appear to be based on experience with small numbers of patients without concurrent controls.

Davidson(6) reported that lepromatous patients responded favorably to INH for 6 to 9 months and then either failed to improve or deteriorated. This course has been interpreted as the result of selection of resistant forms of *M. leprae*. The very great numbers of bacilli present in lepromatous patients and the long period of observation in clinical trials would be more favorable to the observation of resistant forms than are the small number and shorter time in the experiment described here.

The critical question as to whether the acid-fast bacilli in this experimental system are indeed leprosy bacilli has been discussed elsewhere(1,2). The evidence is most simply explained by the hypothesis that they are leprosy bacilli (*i.e.*, that they are the acid-fast bacteria of the patient's tissues). Other hypotheses would need to be unattractively complex. For example, a hypothesized contaminant from the patients would need to be endowed with the following properties: non-cultivable, present in all patients' tissues and present in numbers proportional to the number of leprosy bacilli, capable of slow growth in mouse foot-pads to produce a large round cell response without necrosis and with occasional invasion of nerves.

Summary. 1. A comparison of the activity of 4 drugs was carried out in mice inoculated into the foot-pad with human leprosy bacilli. The drug was administered in the diet, starting on the day of inoculation. 2. Multiplication of the bacilli was completely suppressed by DDS, INH, and PAS, and it was delayed and partially suppressed by cycloserine.

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Impairment of Net Synthesis of Glutathione in Mouse Liver After Tourniquet Trauma.* (27294)

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Numerous metabolic imbalances occur as a part of the syndrome induced by tourniquet trauma. One of the imbalances studied in this laboratory(1,2,3,4) has been the depletion of the non-protein sulfhydryl (NPSH) fraction of various tissues: liver, kidney and muscle. A lowered incorporation of S^{35}-L-cysteine into the major non-protein sulfhydryl component of liver, *i.e.*, glutathione (GSH), has been observed(3,4). This led directly to the present work. Decreased incorporation of L-cysteine into GSH might be due to decreased GSH synthesis, to increased GSH degradation, or to both. Synthesis, relatively

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uninfluenced by degradation, may be studied by inducing a very rapid net synthesis of GSH. Eden *et al.*(5) have shown that this can be accomplished by first depleting rat liver GSH by the use of bromobenzene, followed by oral administration of L-cystine. Their procedure, slightly modified, was used to study net synthesis of S^{35}-L-cysteine into GSH in mice. In the present experiments, mice have been compared as to ability to use intraperitoneally administered S^{35}-L-cysteine for: (I) net synthesis of liver GSH, and (II) incorporation of S^{35}-L-cysteine into liver GSH, when the initial depletion had been obtained by: (a) bromobenzene administration, (b) tourniquet trauma, or (c) both.

Methods and materials. Male Carworth Farms Webster strain mice (20 to 26 g), maintained on Rockland Rat Diet, were used. Four experimental groups were studied: control (*C*); trauma (*T*); bromobenzene (*BB*); and bromobenzene plus trauma (*BBT*). Each of these groups was subdivided into 2 groups, one of which did and one of which did not receive an injection of S^{35}-L-cysteine. In each of the groups receiving S^{35}-L-cysteine, the symbol S^{35} was added to the designations assigned above, *i.e.*, TS^{35} for the trauma group receiving cysteine, etc. Seven to 10 mice were used in each of the 8 groups studied. Sixteen hours before sacrifice, food was removed from the cage, but water was allowed *ad libitum*. At this time, all mice were injected i.m. with 0.01 ml/g of either peanut oil alone, or bromobenzene, 750 mg/kg, in peanut oil (1 ml bromobenzene plus 19 ml peanut oil). Groups *C*, CS^{35}, *T*, TS^{35} were injected with peanut oil; groups *BB*, BBS^{35}, *BBT*, $BBTS^{35}$ were injected with the bromobenzene. Mice receiving trauma (*T*, TS^{35}, *BBT*, $BBTS^{35}$) had rubber band tourniquets applied to both hind legs(6) for 3 hours; they were killed 2.5 hours after removal of the tourniquets. Mice in the S^{35} groups were injected i.p. 45 minutes prior to sacrifice with 0.01 ml/g of a 1.5% aqueous solution of S^{35}-L-cysteine (w/v) to give a dose of 150 mg/kg and 10 to 20 μc/kg. The S^{35}-L-cysteine was prepared from S^{35}-L-cystine (Schwarz BioResearch Inc., Mt. Vernon, N.Y.) by electrolytic reduction with a RECO DESALTER. This radioactive cysteine was diluted with sufficient non-radioactive L-cysteine hydrochloride and neutralized with $NaHCO_3$ to give the 1.5% solution described above.

Each mouse was killed by spinal dislocation. The liver was excised, frozen at once between 2 cakes of dry ice and held frozen to time of analysis next morning. For each of the S^{35} groups (CS^{35}, TS^{35}, BBS^{35}, $BBTS^{35}$) the livers were pooled and weighed, and a single extract was prepared using 10% trichloracetic acid (TCA), by a procedure patterned on that of Waelsch and Rittenberg(7) but modified so that the final volume of each extract was 25 ml. This extract was used, appropriately diluted, for estimation of: (a) radioactivity present in liver non-protein compounds, (b) liver cysteine + cystine(8) and (c) total liver non-protein sulfhydryl (NPSH) compounds, including GSH, by the non-specific nitroprusside method(9). The same extract was used undiluted, for estimation of radioactivity and concentration of liver GSH. The concentration value was obtained by a "reverse" isotope dilution method, in which known amounts of non-radioactive GSH were added to unknown amounts of radioactive GSH in the liver extract. Two 1 ml aliquots of extract were used, 2.5 and 5.0 mg of carrier GSH being added to the respective 1 ml samples prior to isolation of the glutathione as its copper mercaptide(7). Glutathione, undiluted by carrier GSH, was isolated by the same method from the remaining extract (19-20 ml). From the specific activity values obtained for the 3 glutathionate powders isolated per extract, concentration of GSH in the liver was calculated. For mice not injected with S^{35}-L-cysteine, a separate TCA extract was prepared from each liver to permit calculation of standard errors of the (average) NPSH values obtained for the *C*, *T*, *BB* and *BBT* groups. This was the only determination made on these groups.

Radioactivity was determined by use of a Packard Tri-Carb liquid scintillation spectrometer at high voltage setting of 1040 v, pulse height setting of 10 to 50 v, and temperature of −5 to −10°C. Each sample was counted for 30 minutes or longer if this was necessary to secure 5,000 counts. The non-protein TCA extract was prepared for count-

TABLE I. Effects of Bromobenzene, Trauma and S^{35}-L-Cysteine on Mouse Liver Glutathione.

Data line	Factor estimated for mouse liver	Group of mice			
	Mice NOT inj. with S^{35}-L-cysteine				
		C	*T*	*BB*	*BBT*
a	NPSH, mmol S/kg, ± S.E.	4.60 ±.40	2.33 ±.10	.94 ±.42	.88 ±.20
	Mice inj. with S^{35}-L-cysteine				
		CS^{35}	TS^{35}	BBS^{35}	$BBTS^{35}$
b	NPSH, mmol S/kg	4.27	2.79	5.56	3.96
c	GSH by isotopic dilution, meq S/kg	4.19	2.22	4.16	2.77
d	$GS^{35}H$, mmol S/kg*	.80	.35	2.47	1.49
e	% of GSH formed from inj. L-cysteine (100% × $GS^{35}H$/GSH)	19	16	59	54
f	% of inj. S^{35} found in GSH	3.2	1.4	10.9	6.2
g	% of inj. S^{35} found in all non-protein epds.	11.1	13.4	18.0	14.4
h	% of inj. S^{35} found in epds. other than GSH	7.9	12.0	7.1	8.2
i	% incorporation (100% × f/g)	29	10	61	43
k	Cysteine + cystine (mmol S/kg)	1.38	.97	.77	1.08

* $GS^{35}H$ = mmol S^{35} inj./kg liver × (f/100).

(In the present experiments the ratio mmol S^{35} inj./kg liver ranged from 23 to 25.)

ing by adding 8.0 ml polyether 611 and 1.0 ml Hyamine(9) to 1.0 ml of liver extract in a 5 dram counting vial made of low potassium borosilicate glass. The polyether 611 was composed of 1.2 g diphenyloxazole and 0.05 g 1,4-bis-2(5-phenyloxyzolyl) benzene dissolved in 80 ml of a mixture of p-dioxane, anisole and dimethoxyethane (6:1:1). The copper glutathionate was prepared for counting by dissolving a weighed amount (0.5 to 4 mg) with 1.0 ml of 2×10^{-3} M EDTA as its trisodium salt in 10% TCA. To this solution was then added 8.0 ml polyether 611 and 1.0 ml Hyamine. Corrections for quenching were made by addition of internal standards and recounting of the samples.

Results have been summarized in Table I. The data of line a of the Table show that bromobenzene induced much greater depletion of mouse liver NPSH than did trauma. Since the depletion induced by bromobenzene plus trauma was no greater than that induced by bromobenzene alone, it is likely that most of the depletion observed for the *BBT* mice had occurred before the beginning of trauma. On comparison of the data of line b of the Table with that of line a, it is apparent that post-depletion administration of L-cysteine resulted in marked repletion (net synthesis) of liver NPSH, and especially of liver GSH (line c), if the prior depletion had been secured by use of bromobenzene. Such net synthesis was very slight if the prior depletion had occurred consequent to trauma. Livers of mice subjected to both bromobenzene and trauma exhibited net synthesis of GSH more like that of livers from bromobenzene treated than of livers from traumatized mice. Bromobenzene treated mice also exhibited much greater ability to incorporate S^{35}-L-cysteine into liver GSH (line i) than did traumatized mice; a much higher value was obtained for BBS^{35} than for TS^{35} mice in respect to each of the following indices of extent of incorporation of S^{35}-L-cysteine into liver GSH: (I) $GS^{35}H$ formed, line d; (II) % of injected S^{35}-L-cysteine found in liver GSH, line f; (III) % of liver non-protein S^{35} present as $GS^{35}H$, line i. In each case the value obtained from $BBTS^{35}$ mice, subjected to both bromobenzene and trauma, was appreciably closer to that obtained for the bromobenzene treated than for the traumatized mice. Line k of the Table gives data on liver cysteine + cystine concentrations. Although this factor was somewhat lowered in the 3 experimental groups compared to the control, there was no correlation between this factor and either glutathione net synthesis or S^{35}-L-cysteine found in GSH.

Discussion. In the present experiments it was found that net synthesis of liver GSH could be demonstrated for mice with liver GSH previously depleted by use of bromobenzene, but not for mice with liver GSH previously depleted in lesser degree by use of

trauma. From this it may be concluded that trauma inhibits *in vivo* synthesis of GSH. Such decrease in GSH synthesis would account for our earlier findings(1,2,3,4) that severe trauma induced, in mouse tissues, significant decreases in GSH concentration. In the present experiments, for those groups which exhibited prior depletion of liver GSH as a result of bromobenzene and/or trauma action, the extent of S^{35}-L-cysteine incorporation into liver GSH ran parallel to the extent of net synthesis of GSH. This is in accord with expectation if incorporation of S^{35}-L-cysteine into liver GSH is determined more by rate of GSH synthesis than rate of GSH degradation.

Several factors might have been responsible for the decrease in GSH synthesis seen in trauma: (a) lack of cysteine and/or cystine, (b) lack of glutamic acid and/or glycine, or (c) impairment of synthesizing ability of the liver. Livers taken from mice in the TS^{35} group gave somewhat higher analytical values for: (I) cysteine plus cystine, line k, and (II) non-protein S^{35} not in GSH, line h, than did livers taken from BBS^{35} mice. Hence inability of livers of traumatized mice to synthesize GSH after L-cysteine administration cannot be attributed to lack of cysteine and/or cystine. The possibility that trauma induced decrease in liver GSH synthesis might be due to glutamic acid and/or glycine deficiency has not been thoroughly investigated. Total liver amino acids, as estimated by the non-specific B-naphthoquinone-4-sulfonate method(10) were somewhat increased in association with tourniquet trauma (unpublished). Of course this does not insure an unchanged distribution of individual amino acids. In view of the high concentrations of these 2 amino acids in the livers of normal animals(11), it does not appear likely that glutamic acid and/or glycine deficiency are mainly responsible for trauma induced decreases in liver glutathione concentration and synthesis. By elimination, this leaves an impairment in synthesizing ability of the liver after trauma as the most likely explanation. Such impairment could occur as the result of trauma-induced depletion of adenosinetriphosphate (ATP). It has been shown: (a) that both trauma and hemorrhage are capable of inducing tissue ATP depletion(12), and (b) that ATP is required for GSH synthesis(13). It is not necessary to postulate ATP depletion to account for bromobenzene induced depletion of liver GSH, inasmuch as Booth *et al.* (14) have reported that bromobenzene forms a conjugate with liver glutathione which is hydrolyzed enzymatically in the kidney to S-(p-bromobenzyl)-cysteine. Thus ATP stores may still be relatively unimpaired in the *BB* animals, allowing for more rapid resynthesis of GSH.

Summary. Net glutathione synthesis, occurring in mouse liver after S^{35}-L-cysteine administration, was estimated for mice with liver GSH previously depleted by use of bromobenzene or trauma. Evidence was secured that glutathione synthesis was impaired by trauma but not by bromobenzene, and that the trauma impairment was likely due to decreased capability of the liver to synthesize GSH rather than to lack of substrate.

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