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LIVER GLUTATHIONE AND POLYAMINES IN HEPATOCARCINOGEN-TREATED RATS

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Abstract—Hepatocarcinogens such as 2-acetylaminofluorene and DL-ethionine share with carcinogenic aminoazo dyes the ability to increase male rat liver glutathione content. The effect of various hepatocarcinogens and related compounds on the concentrations of rat liver spermine and spermidine has been examined.

INTRAPERITONEAL injections of carcinogenic aminoazo dyes caused a marked increase in the glutathione (GSH) content of male rat liver.[1] There was in general a close correlation between the extent of this increase and the carcinogenicity of the injected dye.

It has now been found that injections of other hepatocarcinogens such as 2-acetylaminofluorene (2-AAF) and DL-ethionine produced a rise in male rat liver GSH whereas some related noncarcinogenic materials including the natural amino acid L-methionine failed to bring about this increase. As in heptocarcinogenesis by 2-AAF and DL-ethionine, sex differences were found in the response of rat liver GSH to these substances.

In the course of an investigation of the mechanism by which hepatocarcinogens increase rat liver GSH the concentrations of the polyamines spermine (SP) and spermidine (SPD) in the livers of normal and hepatocarcinogen-treated rats were examined. Although the precise origin of these polyamines in mammalian tissues is obscure[2] it seems probable that they may be derived from S-adenosylmethionine as in *E. coli*[3] or from methionine as in chick embryo.[4] According to Tabor *et al.*[3] S-adenosylmethionine undergoes decarboxylation to S-adenosyl(5′)-3-methylmercaptopropylamine in cell-free extracts of *E. coli*. This compound then reacts with putrescine to give spermidine plus 5′-methylthioadenosine. Subsequently spermine is formed by combination of spermidine with a further molecule of S-adenosyl (5′)-3-methylmercaptopropylamine. If similar reactions occur in mammalian tissues it could be expected that the concentrations of SP and SPD would be markedly altered in GSH-rich livers where changes in the levels of sulphur-containing amino acids and S-adenosylmethionine may have occurred.

The SPD content of rat liver was indeed found to be appreciably altered by injections of hepatocarcinogens and related compounds. Although no obvious relationship has yet been observed between GSH increase and the direction of changes in rat liver SPD and SP concentrations it is thought useful to report results which have been obtained so far.

EXPERIMENTAL

Animals and materials

Unless otherwise stated adult stock male albino rats [200–350 g body weight (b.w.)] maintained on water and Oxoid Diet 86 (prepared by the Oxoid Division of Oxo Ltd., London, E.C.4) were used. Pairs of rats were injected intraperitoneally with solutions or fine suspensions of aminoazo dyes, amines etc. in arachis oil (0·6 ml/100 g b.w.) during 10–11 a.m. Control rats received arachis oil only. Aminoazo dyes, which were synthesized and purified by the usual methods, were injected in amounts corresponding on a mole for mole basis with a standard dose of 8·25 mg of 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB; mol. wt. = 239) per 100 g b.w. A new compound, 4′-phenoxy-4-dimethylaminoazobenzene was prepared by coupling dimethylaniline with diazotized 4-aminobiphenylether. After crystallization from ethanol this aminoazo dye melted at 131°. Aromatic amines, N-acetyl derivatives and nitro compounds were commercial preparations with the exception of 4-nitrobiphenylether, 4-amino and 4-acetylamino-biphenylether which were synthesized by the method of Suter.[5] Amines and related compounds were applied at twice the standard 3′-MeDAB dose level (mole for mole basis) since it was found that injection of 2-mole proportions of 2-AAF produced about the same increase in male rat liver GSH as did 1 mole proportion of 3′-MeDAB. DL-Ethionine, L-methionine and related amino acids obtained from Koch-Light Laboratories Ltd. were usually injected in amounts equivalent (mole for mole) to an arbitrary dose of 25 mg of L-cysteine (mol. wt. = 121) per 100 g b.w.

The rats were killed with ether about 24 hr after injection and the livers were perfused *in situ* with ice-cold normal saline, removed, blotted and frozen immediately in solid carbon dioxide.

GSH *estimation*

One-gramme samples of frozen liver from each of a pair of identically-treated rats were pooled and GSH determination was carried out as already described[1] using Saville's method.[6]

Bound-dye estimation

One-gramme samples of frozen liver from each of a pair of identically-treated rats were pooled and dried liver powder was prepared from the pooled material according to the method of Miller and Miller.[7] A 50 mg sample of powder was used for bound dye (B.D.) determination (E_{520} mμ) as already described.[1, 7]

Spermine and spermidine estimations

SP and SPD were determined by the method of Raina[4] with some modifications. One-gramme samples of frozen liver from each of a pair of identically-treated rats were pooled and homogenized with 2 × 4 ml of 0·1 N HCl in an all-glass homogenizer. To the homogenate was added 10 ml of 10% trichloroacetic acid (TCA) solution and after stirring thoroughly and keeping for 1–2 hr at room temperature the mixture was centrifuged for 5 min at 2500 rev/min. The supernatant was extracted four times with 16-ml lots of ether to remove TCA and the last traces of ether were removed by warming. After keeping the supernatant overnight at −15°, 4 g of sodium sulphate—tribasic sodium phosphate (62·5 g anhydrous sodium sulphate + 9 g trisodium orthophosphate) salt mixture was added, followed by 0·8 ml of 4 N NaOH.

The alkaline solution was then shaken vigorously and repeatedly by hand during ½–1 hr with 16 ml of *n*-butanol in a glass-stoppered tube. After standing for 1 to 2 hr, the *n*-butanol layer was removed by pipette, four drops of conc. HCl added and the acidified butanol extract was taken to dryness under reduced pressure on a hot water bath. Drying was completed by storing the residue *in vacuo* over phosphorous pentoxide overnight.

The residues containing SP and SPD were dissolved in 0·4 ml of 0·1 N HCl and 0·02 ml aliquots were applied to the centre line (four simultaneous separations) of moist Whatman No. 1 chromatography paper (50 cm × 12 cm) which had been wetted with citric acid–sodium hydroxide solution (21 g of citric acid + 20 ml of 4 N NaOH made up to 1 l. with deionized water) and blotted. The paper was suspended in the form of an inverted V between two glass tanks (capacity 800 ml each) containing citric acid–sodium hydroxide buffer and platinum electrodes to which a potential of 350 V from a D.C. power pack (output 10 mA) was applied for 3 hr. After drying the paper for 1 hr at room temperature, SP and SPD spots were revealed by dipping the paper in a saturated solution of Amido Black, drying for 1 hr at room temperature and eluting the excess Amido Black by the methanol–acetic acid washing procedure described by Raina.[4] Each spot was cut out and placed in 5 ml of 0·1 N NaOH for 30 min. The extinction of the resulting Amido Black solution was read at 615 mμ in 1 cm cells in a Unicam Spectrophotometer (Model SP 500) against deionised water. Correction for background colour was made by eluting blank areas of the electropherogram similar in size to the SP and SPD spots with 5 ml of 0·1 N NaOH, reading at 615 mμ against water and subtracting these values from the extinction readings for SP and SPD extracts.

Stock solutions of SPD (100 mg in 100 ml of 0·1 N HCl) or of spermine hydrochloride (175 mg in 100 ml of 0·1 N HCl) were diluted with 0·1 N HCl to give solutions containing 10, 7·5, 5 and 2·5 μg of each polyamine in 0·02 ml aliquots. After electrophoresis of these aliquots, staining the spots and correcting the extinction readings with blanks, the following E_{615} values (SP, SPD) were obtained for the stated quantities of polyamines: 10μg (0·867, 1·029); 7·5 μg (0·617, 0·804); 5 μg (0·405, 0·498) and 2·5 μg (0·202, 0·214).

E_{615} values for SP and SPD are quoted in the present paper. Estimates of the polyamine concentrations (μg/g frozen tissue) can be made from the calibration data, assuming linear extrapolation above 10 μg for some of the higher SP values.

RESULTS

Effects of various aromatic amines, N-acetyl derivatives, nitro and azo compounds on male rat liver GSH *content*

As shown in Table 1, 2-aminofluorene produced a rise in liver GSH content of male albino rats as did the N-acetyl derivative of 2-aminofluorene and 2-nitrofluorene. Also, 2-naphthylamine and 2-nitronaphthalene strongly increased rat liver GSH content, whereas 1-naphthylamine had little or no activity. The powerful carcinogen benzidine increased rat liver GSH considerably but the non-carcinogenic amines *p*-aminophenol and *p*-anisidine had no activity.

4-Aminobiphenylether (4-ABPE) caused a substantial increase in liver GSH. This amine is not known to be carcinogenic. Contrary to the findings with 2-aminofluorene derivatives, the N-acetyl derivative of 4-ABPE was less active in increasing rat liver

GSH content than was the parent amine while 4-nitrobiphenylether was quite inactive. The 4′-chloro-derivative of 4-ABPE produced only a slight increase in liver GSH. The compound, 4′-phenoxy-4-dimethylaminoazobenzene had no effect on rat liver GSH and produced no bound dye with rat liver protein ($E_{520} = 0{\cdot}009$ compared with $E_{520} = 0{\cdot}007$ for control liver powder). These results suggest that 4′-phenoxy-4-dimethylaminoazobenzene would lack carcinogenic activity. It is interesting that the

TABLE 1. EFFECT OF CARCINOGENIC AMINES AND RELATED COMPOUNDS ON MALE RAT LIVER GSH CONTENT 24 HR AFTER INJECTION

Compound	Hepatocarcinogenicity*	Dose (mg/100 g b.w.)	No. of pairs of rats used	Liver GSH (mg%)
2-Aminofluorene	+	12·5	2	248
2-Acetylaminofluorene	+	15·4	3	274
2-Nitrofluorene	+	14·6	1	266
2-Naphthylamine		9·9	1	235
2-Nitronaphthalene		11·9	1	281
1-Naphthylamine		9·9	1	199
Benzidine	+	12·7	1	272
p-Aminophenol	−	6·4	1	184
p-Anisidine	−	8·25	1	190
4-Aminobiphenylether		12·8	2	257
4-Acetylaminobiphenylether		15·7	2	219
4-Nitrobiphenylether		12·5	2	178
4′-Chloro-4-aminobiphenylether		15·2	2	203
4′-Phenoxy-4-dimethylaminoazobenzene		10·9	1	171
4-Aminobiphenyl	+	11·7	1	296
4-Aminobiphenyl		11·7	1	195
4-Acetylaminobiphenyl	+	14·6	1	188
4-Acetylaminobiphenyl		14·6	1	236
4′-Phenyl-4-dimethyl-aminoazobenzene	−	10·4	1	165
Controls†			6	182

* + = compound known to be carcinogenic for rat liver; − = compound without carcinogenic activity for rat liver. Where no rating is given compounds have either not been tested or their hepatocarcinogenic activity for the rat is in doubt. 2-Naphthylamine is said to produce hepatomas in mice. For a recent comprehensive review article on chemical carcinogenesis see ref. 28.

† Range = 165–206.
S.E.M. = ± 5·2.

azo dye 4′-phenyl-4-dimethylaminoazobenzene which is said to be non-carcinogenic[8] failed to increase rat liver GSH content and did not bind to rat liver ($E_{520} = 0{\cdot}032$). Another dye, 4′-benzeneazo-4-dimethylaminoazobenzene prepared by the method of Sawicki[9] produced a slight increase in rat liver GSH (208·3 mg%) but gave no bound dye ($E_{520} = 0{\cdot}025$).

No consistent changes in rat liver GSH content were found after injections of the strong carcinogens 4-aminobiphenyl and 4-acetylaminobiphenyl as shown in the two separate experiments with each compound reported in Table 1. Sometimes the value was increased, sometimes unchanged. At the dose levels employed each compound was very toxic. Even 24 hr after injection the rats showed signs of intense methaemoglobinaemia, a condition which might be expected to modify considerably tissue GSH content. With other amines such as 4-ABPE, methaemoglobinaemia was transient.

Some of the compounds such as 4-ABPE and its N-acetyl derivative induced a lethargic condition or even partial anaesthesia in rats. Previously Huggins *et al.*[10] reported anaesthetic effects of 2-AAF in rats.

Effect of 2-AAF, DL-*ethionine,* L-*methionine and a mixture of* DL-*ethionine and* L-*methionine on the* GSH, SP *and* SPD *content of male rat liver*

As shown in Table 2, injection of DL-ethionine produced a substantial increase in liver GSH although it was somewhat less active than 2-AAF in this respect.

TABLE 2. EFFECT OF 2-AAF, DL-ETHIONINE ETC. ON GSH, SPERMINE (SP) AND SPERMIDINE (SPD) CONTENT OF MALE RAT LIVER 24 HR AFTER INJECTION

Compound injected	Dose (mg/100 gb.w.)	No. of pairs of rats used	GSH (mg%)	SP E_{615} mean values ± S.E.M.	SPD E_{615}
2-AAF	15·4	3	276 ± 9·8	0·900 ± 0·54	0·820 ± 0·037
DL-Ethionine	33·7	4	237 ± 13·1	0·810 ± 0·038	0·620 ± 0·012
L-Methionine	30·8	4	165 ± 3·8	0·830 ± 0·047	0·590 ± 0·039
DL-Ethionine + L-methionine	33·7 + 30·8	2	217	0·900	0·571
nil		4	174 ± 4·4	0·890 ± 0·057	0·540 ± 0·054

On the other hand, L-methionine which is presumably devoid of carcinogenic activity (some pathological changes have resulted from its excessive use)[11] decreased the GSH concentration of male rat liver below its normal value. Mixed with DL-ethionine, L-methionine prevented to some extent the rise in liver GSH content due to DL-ethionine. It is known that methionine fed together with DL-ethionine suppresses the carcinogenic activity of the latter.[12]

2-AAF caused an appreciable increase in liver SPD. DL-Ethionine also increased SPD though it was less active than 2-AAF. Livers of rats which received DL-ethionine + L-methionine had practically normal values for SPD and SP.

Effect of 2-AAF *on* GSH, SP *and* SPD *content of livers of male and female rats of about the same age*

Female rats are more resistant to the hepatocarcinogenic action of 2-AAF than are males.[13] If liver GSH increase is closely related to the process of hepatocarcinogenesis it was expected that male rats would show a greater response than female rats to injections of 2-AAF.

Pairs of male and female rats received the stated doses of 2-AAF as shown in Table 3. Each estimation was carried out on the pooled liver sample from one pair of rats.

Evidently 2-AAF at each dose markedly increased the GSH content of male rat liver. There seemed to be an optimal dose of about 4 mg of 2-AAF/100 g b.w. for increasing male rat liver GSH. Only a slight stimulatory effect was noted for female rat liver.

The GSH content of liver of normal (arachis oil-injected) female rats was somewhat less than that of normal male rats. Johnson[14] has stated that normal female rat liver contains 4·9 μmole GSH/g (or 150·4 mg GSH/100 g).

TABLE 3. EFFECT OF VARIOUS DOSES OF 2-AAF ON THE GSH, SP AND SPD CONTENT OF LIVERS OF MALE AND FEMALE RATS 24 HR AFTER INJECTION

Dose of 2-AAF (mg/100 g b.w.)	Male rats				Female rats			
	Body weight (g)	GSH (mg%)	E_{615}		Body weight (g)	GSH (mg%)	E_{615}	
			SP	SPD			SP	SPD
nil	225 230	162	0·950	0·540	190 180	141	0·875	0·360
3·9	245 200	262	0·940	0·730	180 160	162	0·920	0·665
7·7	225 245	230	0·925	0·730	165 190	161	1·065	0·870
15·4	220 235	242	0·960	0·820	180 175	173	1·010	0·845

With increasing dose of 2-AAF, the SPD content of male rat liver increased while the SP content was hardly altered. In female livers, SPD showed a marked increase and there was the suggestion of an increase in SP at the higher doses of 2-AAF.

Effect of DL-*ethionine on* GSH, SP *and* SPD *content of livers of male and female rats of about the same age*

In Table 4 are shown GSH, SP and SPD contents of livers of single pairs of rats injected intraperitoneally with 16·9 and 33·7 mg of DL-ethionine/100 g b.w. An

TABLE 4. EFFECT OF DL-ETHIONINE ON THE GSH, SP AND SPD CONTENTS OF LIVERS OF MALE AND FEMALE RATS 24 HR AFTER INJECTION

Dose of DL-ethionine (mg/100 g)	Male rats				Female rats			
	Body weight (g)	GSH (mg%)	E_{615}		Body weight (g)	GSH (mg%)	E_{615}	
			SP	SPD			SP	SPD
nil	255 255	164	0·670	0·455	210 220	152	0·970	0·475
16·9	270 265	211	0·920	0·730	200 185	212	0·930	0·580
33·7	240 260	216	0·675	0·550	205 180	202	0·845	0·660

increase in GSH content of livers of both male and female rats was observed after DL-ethionine injection. According to Farber[15] liver tumour induction by DL-ethionine in rats is uninfluenced by sex. The present results strengthen the view that increase in liver GSH is in some way closely connected with hepatocarcinogenesis.

In both sexes, DL-ethionine produced an increase in liver SPD.

Effect of aminoazo dyes etc. on the GSH, SP *and* SPD *content of male rat liver*

The effects of some carcinogenic and noncarcinogenic aminoazo dyes on the GSH and polyamine content of male rat liver were examined and the results are shown in Table 5.

It was found (Expt. A, Table 5) that whereas carcinogenic aminoazo dyes at the usual dose level (8·25 mg of 3′-MeDAB/100 g b.w.) caused the expected increase in liver GSH content, they decreased strongly the SPD and SP content of the liver. This was in contrast to the simultaneous increase of liver GSH and SPD noted with the carcinogens 2-AAF or DL-ethionine.

TABLE 5. EFFECT OF VARIOUS AMINOAZO DYES ON GSH, SP, SPD AND BOUND DYE (B.D.) CONTENT OF LIVERS OF MALE RATS 24 HR AFTER INJECTION

Expt.	Compound injected	Dose (mg/100 g b.w.)	B.D. E_{520}	GSH (mg.%)	E_{615}	
					SP	SPD
A	4′-EtDAB*	8·73	0·141	358	0·634	0·436
	4′-MeDAB†	8·25	0·065	221	0·714	0·489
	3′-MeDAB	8·25	0·112	261	0·689	0·370
	nil	—	—	166	0·945	0·528
B	3′-MeDAB	16·5	0·157	301	0·977	0·647
	3′-MeDAB	8·25	0·095	262	0·978	0·675
	3′-MeDAB	4·1	0·070	242	0·945	0·712
	3′-MeDAB	2·0	0·038	202	0·912	0·742
	3′-MeDAB	1·0	0·027	176	0·912	0·872
	nil			170	0·958	0·672
C	3′-MeDAB	8·25	0·118	310	0·931	0·596
	2-MeDAB	8·25	0·045	136	1·234	0·764
	2-MeODAB	8·80	0·018	238	0·973	0·704
	nil			193	0·981	0·549
D	3′-MeDAB	8·25	0·116	230	0·955	0·669
	3′-MeDAB + DL-ethionine	8·25 + 33·7	0·057	270	0·817	0·617
	3′-MeDAB + L-methionine	8·25 + 30·8	0·113	245	0·867	0·617
	nil			166	0·977	0·627

* 4′-Ethyl-4-dimethylaminoazobenzene, strong carcinogen.
† 4′-Methyl-4-dimethylaminoazobenzene, weak carcinogen.
Mean values (± S.E.M.) from data in Table 5 for:

Treatment	No. of observations	B.D.	GSH	SP	SPD
3′-MeDAB (8·25 mg/100 g b.w.)	4	0·110(+0·005)	266(±14·3)	0·888(±0·058)	0·578(±0·062)
nil	4	—**	174(± 5·6)	0·965(±0·007)	0·944(±0·049)

** Not done. However in another experiment involving eight pairs of normal rat liver, E_{520} was 0·015 ± 0·002.

In Expt. B (Table 5) pairs of male rats were injected with various doses of 3′-MeDAB. The smaller doses of 3′-MeDAB increased SPD content above the control value. With increasing doses the SPD content of the liver dropped to lower-than-normal values. However no great change was found in SP content throughout the 3′-MeDAB dose range in spite of the marked decline in concentration of this polyamine which had been seen in Expt. A.

In Expt. C (Table 5) the effects of two supposedly non-carcinogenic dyes, 2-methyl- and 2-methoxy-4-dimethylaminoazobenzene (2-MeDAB and 2-MeODAB) were compared with those of the carcinogen, 3′-MeDAB. Previously[1] it had been found that

injection of 2-MeDAB decreased and of 2-MeODAB increased rat liver GSH content and this finding was confirmed in the present experiment. Both 2-MeDAB and 2-MeODAB caused a greater increase in liver SPD than did 3′-MeDAB for which the SPD value was about the same as for control livers. Only in the case of 2-MeDAB was the liver SP content markedly above normal.

It was of interest to determine the effects of L-methionine or DL-methionine on the dye-binding due to 3′-MeDAB and to changes in liver GSH, SP and SPD contents induced by the amino-azo carcinogen. Scribner, *et al.*[16] have found that the linkage between 3′-methyl-4-monomethylaminoazobenzene (a metabolite of 3′-MeDAB) and liver protein takes place at the sulphur atom of a methionine residue which unites at a position ortho to the 4-monomethylamino group. Conceivably, treatment of 3′-MeDAB-injected rats with L-methionine or DL-ethionine could modify this binding reaction. Our results are shown as Expt. D (Table 5).

In agreement with previous studies of Gelboin *et al.*[17] it was found that DL-ethionine considerably depressed the extent of dye binding due to 3′-MeDAB. However, increase in liver GSH content due to the dye was not suppressed. Gelboin *et al.*[17] stated that DL-ethionine together with 3′-MeDAB had little effect on tumour induction due to the azo dye but they noted that other workers[18] had found a synergistic effect on tumour production when DL-ethionine was fed together with 4-dimethylaminoazobenzene. When L-methionine was injected with 3′-MeDAB there was no suppression of dye binding. The effect of L-methionine on azo dye carcinogenesis does not appear to have been studied. Gelboin *et al.*[17] reported that it had a stimulatory effect on dye-binding of 3′-MeDAB. According to the same authors, when L-methionine was applied together with DL-ethionine, the suppressive action of the latter on dye-binding was inhibited.

Effect of other sulphur-containing amino acids on the GSH *content of male rat liver*

In view of the marked activity of DL-ethionine and the absence of activity of L-methionine for producing an increase in the GSH content of male rat liver (Table 2) it was decided to examine briefly the effect of some other sulphur-containing compounds, including GSH itself, on the GSH content of rat liver.

A pair of male rats was injected intraperitoneally with a fine suspension of each compound in arachis oil or with an aqueous solution of GSH neutralised with sodium bicarbonate. The compounds were given in doses corresponding on a mole for mole basis with a standard dose of 25 mg of L-cysteine per 100 g b.w. L-Cystine and DL-homocystine were applied at half this dose. In the case of DL-lanthionine the full dose and half this dose were examined. L-Ethionine was studied at half the standard dose. The results are shown in Table 6.

It was found that of the substances examined only L-ethionine caused an increase in rat liver GSH content.

Effect of adenine on the ability of DL-*ethionine and of* 3′-MeDAB *to increase male rat liver* GSH

It is known that treatment of rats with DL-ethionine results in a decline in the adenosine triphosphate (ATP) content of rat liver.[19] Other hepatocarcinogens such as 4-dimethylaminoazobenzene and 2-AAF have the same effect[20] Since ATP is involved at one stage in the enzymatic synthesis of GSH *in vitro* it appeared to us that lack of

liver ATP (if it occurs under our experimental conditions) ought to hinder rather than favour GSH synthesis.

Treatment with adenine has been found to prevent the decline in liver ATP due to DL-ethionine.[19] Therefore it seemed of interest to determine what the effect of simultaneously administered adenine would be on the GSH content of the livers of male rats which have been injected with DL-ethionine, 3′-MeDAB or arachis oil only.

TABLE 6. EFFECT OF VARIOUS SULPHUR-CONTAINING COMPOUNDS ON THE GSH CONTENT OF MALE RAT LIVER 24 HR AFTER INJECTION

Compound injected	Dose (mg/100 g b.w.)	Liver GSH (mg%)
L-Ethionine	16·9	228
L-Cysteine	25·0	154
L-Cystine	25·0	182
DL-Homocystine	27·7	168
DL-Lanthionine	43·0	156
DL-Lanthionine	21·5	177
GSH	63·4	168
nil	—	189

DL-ethionine was injected into pairs of male rats at the usual dose level (33·7 mg/100 g b.w.) with or without 27·9 mg of adenine/100 g b.w. incorporated in the arachis oil vehicle. The same dose of adenine was given to rats which also received 8·25 mg of 3′-MeDAB per 100 g b.w. One pair of rats was used for each value reported in Table 7.

TABLE 7. EFFECT OF ADENINE INJECTION ON GSH CONTENT OF LIVERS OF MALE RATS INJECTED WITH DL-ETHIONINE, 3′-MeDAB OR WITH ARACHIS OIL ONLY

Compound(s) injected (mg/100 g b.w.)	Liver GSH (mg%)
DL-Ethionine (33·7)	255
DL-Ethionine (33·7) + adenine (27·9)	248
3′-MeDAB (8·25)	254
3′-MeDAB (8·25) + adenine (27·9)	252
Arachis oil only	168
Arachis oil + adenine (27·9)	140

Evidently simultaneous injection of adenine + carcinogen had practically no effect on the liver GSH increase due to the carcinogenic agent alone. Adenine by itself however caused some decrease in the GSH content of male rat liver. It would appear that increase in rat liver GSH content due to DL-ethionine or to 3′- MeDAB is probably independent of changes in liver ATP level.

DISCUSSION

Hepatocarcinogens of various chemical types have now been found to share with aminoazo dyes the property of bringing about an increase in rat liver GSH and a parallel has been observed between this effect and the known sex differences in response of rats to the carcinogenic action of 2-AAF. On the other hand no sex difference was found after injections of DL-ethionine and Farber[15] detected no sex difference on

the part of rats to the hepatocarcinogenic action of this compound. Shull[19] cited several references which show that very pronounced biochemical changes often occur in female rat liver but not in male rat liver due to the action of DL-ethionine. These include inhibition of protein synthesis, development of fatty liver and depression of liver glycogen. It would appear that the phenomenon of liver GSH increase may be more closely linked with the hepatocarcinogenic activity of DL-ethionine than any of these other changes.

DL-Ethionine has profound effects on the metabolism of L-methionine[15] and Wase *et al.*[21] have shown that there is a greater uptake and utilization of ^{35}S-methionine by livers of rats fed 2-AAF than by normal rat liver. Miller and Miller[22] have expressed the view that S-adenosylethionine might be the proximate carcinogen of ethionine, and Scribner *et al.*[16] proposed that an azo dye-methionine compound might act as a carcinogenic methylating agent for nucleic acids and proteins. There appears to be a fair amount of evidence implicating derangements of sulphur-containing amino acids especially methionine in hepatocarcinogenesis but it is not yet clear how increased GSH synthesis in liver fits into this general picture.

Work on changes in the concentrations of the polyamines SPD and SP in rat liver has so far failed to reveal any obvious relations between these changes and the carcinogenicity or GSH-stimulating ability of the injected substances. Thus the SPD content was often increased by injections of hepatocarcinogens. However injections of the non-carcinogenic aminoazo dyes 2-MeDAB and 2-MeODAB increased rat liver SPD as much as did injections of the carcinogenic substances, 2-AAF and DL-ethionine. Furthermore the SPD increase due to 2-MeDAB on the one hand and to 2-MeODAB on the other was accompanied in the case of the former compound by a drop in liver GSH content and in the latter by an increase in liver GSH.

Raina *et al.*[23] found that intraperitoneal injections of saline solutions of DL- ethionine (32·6 or 97·8 mg/100 g b.w.) caused a drop of 30 per cent in rat liver SPD content in 24 hr and 50 per cent in SP content in 3 days. Later the SPD content increased considerably while the SP content continued to drop. Our experiments (Table 2) confirm the drop in SP at 24 hr but show an increase in SPD at this time. Raina *et al.*[23] also noted that carbon tetrachloride (a substance said to be non-hepatocarcinogenic for rats) caused an increase in SPD and a decrease in rat liver SP.

That there may well be a relationship between GSH and polyamine metabolism in mammalian tissues is indicated by the following observations with regenerating rat liver. A marked increase in SPD with practically no change in SP occurred at 48–72 hr after partial hepatectomy.[24] Smith[25] showed that rat liver non-protein thiol (probably main GSH) increased to a maximum at 36 hr after partial hepatectomy. Thus in regenerating rat liver a situation is found similar in general to that obtaining in the livers of hepatocarcinogen-treated rats.

Other work suggests that GSH and polyamines may be intimately concerned in cellular control mechanisms. For example, SPD and SP are known to exert an antimutagenic action on bacteria.[26] Polyamines have growth promoting action on mammalian cells[27] and it has been suspected for many years that GSH plays a vital role in cell division. It would seem worthwhile to investigate in more detail possible relations between GSH, SP and SPD in normal, precancerous and tumour tissue.

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