REFERENCES

ARBUTHNOTT, G. W. & CROW, T. J. (1971). *Exp. Neurol.*, **30**, 484–491.

FONNUM, F., GROFOVA, L., RINVIK, E., STORM-MATHISEN, J. & WALBERG, F. (1974). *Brain Res.*, **71**, 77–92.

FOWLER, L. J. (1973). *J. Neurochem.*, **21**, 437–440.

FOWLER, L. J. & JOHN, R. A. (1972). *Biochem. J.*, **130**, 569–573.

KANAZAWA, I., MIYATA, Y., TOYOKURA, Y. & OTSUKA, M. (1973). *Brain Res.*, **51**, 363–365.

KÖNIG, J. F. R. & KLIPPEL, R. A. (1963). *The rat brain: a stereo taxic atlas of the forebrain and lower parts of the brain stem.* Williams & Wilkins: Baltimore.

LOWE, I. P., ROBINS, E. & EYERMAN, G. S. (1958). *J. Neurochem.*, **3**, 8–18.

TARSY, D., PYCOCK, C., MELDRUM, B. & MARSDEN, C. D. (1975). *Brain Res.*, **89**, 160–165.

UNDERSTEDT, U. (1971). *Acta physiol scand.*, **82**, suppl. 367, 69–93.

UNGERSTEDT, U. & ARBUTHNOTT, G. W. (1970). *Brain Res.*, **24**, 485–493.

Prevention of paracetamol-induced liver damage in mice with glutathione

Large doses of paracetamol can produce fatal liver necrosis in animals (Boyd & Bereczky, 1966) and in man (Prescott, Wright & others, 1971). Recent mechanistic studies suggest that the hepatotoxicity is mediated through the formation of an active metabolite which covalently binds to liver macromolecules (Mitchell, Jollow & others, 1973a; Jollow, Mitchell & others, 1973). Furthermore, the liver damage due to paracetamol is related to depletion of the hepatic glutathione (Mitchell, Jollow & others, 1973b). However, there appear to be no reports in the literature about the effect of glutathione administration on paracetamol hepatotoxicity, although precursors such as cysteine (Mitchell & others, 1973b) and the related compound cysteamine (Prescott, Swainson & others, 1974) are protective.

In an investigation on the use of liposomes as carriers of potential protective agents against drug-induced liver necrosis (Strolin Benedetti, Louis, Malnoë, Schneider, Smith, Lam & Kreber, unpublished results) it was found that glutathione injected intravenously could largely protect mice against the heptotoxic effect of large doses of paracetamol and these findings are now reported.

Paracetamol 18·9 mg ml in 0·9% saline was injected intraperitoneally into groups of at least 10 male mice of Swiss strain, 25 $\pm$ 3 g, which had been fasted overnight, at 500 mg kg^{-1}. This dose regularly produced liver damage, the extent of which varied considerably from animal to animal and from experiment to experiment. Glutathione, in 0·9% saline, was injected intravenously into the tail vein at doses from 32–800 mg kg^{-1} as follows: (a) as a single dose given at either 15, 45, 105 or 180 min after the dose of paracetamol; (b) as four equal divided doses given at 15, 45, 75 and 105 min before and (c) after the paracetamol.

Paracetamol induced liver damage was assessed by measurement of plasma glutamic - oxaloacetic transaminase (GOT) and glutamic-pyruvate transaminase (GPT) and by histopathological examination. Twenty-four hours after drug treatment dead mice were counted and the survivors killed by cutting the carotid artery under slight ether anaesthesia. Blood samples were taken from each animal and the serum transaminases estimated according to Reitman & Frankel (1957) and the results expressed in terms of international units $litre^{-1}$. The livers were removed and examined for

external damage and then prepared for histological examination in 10% neutral formaldehyde solution, followed by dehydration, clearing and staining with hemalum-eosin. Four degrees of severity of liver damage were distinguished, based upon histopathological examination; these were graded as follows: slight focal necrosis "1", clear focal necrosis "2", extensive focal or zonal necrosis "3" and massive necrosis "4". The mean of the scores for a group of ten or more mice gave the "necrosis index". In these experiments the transaminase concentrations were observed to be related to the index of necrosis and therefore the transaminase values were an indication of the extent of liver damage and in some experiments only these were measured.

Preliminary experiments indicated that glutathione administered intravenously to mice was taken up by the liver. Thus, at 15 and 60 min after the intravenous injection of [^{14}C]glutathione (320 mg kg^{-1}) mouse livers contained 11 and 9% respectively, of the injected radioactivity. According to Mitchell & others (1973b) extensive covalent binding of paracetamol and the associated liver damage does not occur until the endogenous liver glutathione content has been reduced by 70% or more. The glutathione content of mouse liver is 1·5–1·9 mg and to attain this amount by dosing, assuming a 10% uptake and that it is unchanged, would require a dose of glutathione of about 800 mg kg^{-1}. This was the reason for the use of the 800 mg kg^{-1} dose. In experiments 1–4 in Table 1, the glutathione was given intravenously in four divided doses in an attempt to replace the liver glutathione as it was being depleted by the paracetamol over a period of time. Glutathione so administered protected mice against the hepatotoxic effects of a large dose of paracetamol (500 mg kg^{-1}). Thus, plasma concentrations of transaminases and necrosis indices were lower in animals that had received glutathione (4 × 80 or 4 × 128 mg kg^{-1}) and paracetamol compared to animals that had received paracetamol alone.

Individual variations in transaminase concentrations were wide and there were also marked differences in the values between one experiment and another. Thus, the

Table 1. *Effect of glutathione on paracetamol toxicity in mice.* Paracetamol (500 mg kg^{-1}) was injected intraperitoneally into groups of ten mice. The dose of glutathione was injected intravenously in four equal doses at 15, 45, 75 and 105 min after (expts 1, 2, 3, 4) or at the same times before (expt 5) the administration of the paracetamol. Mortalities, serum transaminases and necrosis indices were determined 24 h after the paracetamol administration. Transaminases are means ± s.e.m., n.d. = not determined. The *P* values represent the probability compared to the paracetamol treated group in each experiment.

	Dose (mg kg^{-1}) of:		Serum transaminases at 24 h I.U. litre^{-1} ± s.e.m.				Necrosis index	Mortality
Expt No.	Paracetamol	Glutathione	GOT	*P*	GPT	*P*	(0–4)	(%)
—	—	—	77 ± 14	—	14 ± 2	—	0	0
1	500	—	444 ± 195	—	178 ± 77	—	2·0	0
	500	4 × 80	106 ± 24	<0·6	35 ± 5	<0·1	0·5	0
2	500	—	2064 ± 104	—	2187 ± 372	—	3·0	60
	500	4 × 128	390 ± 87	<0·001	405 ± 127	<0·001	1·5	0
3	500	—	904 ± 140	—	372 ± 124	—	2·0	0
	500	4 × 128	185 ± 9	<0·001	35 ± 2	<0·05	0·5	0
4	500	—	n.d.	—	n.d.	—	n.d.	40
	500	4 × 8	n.d.	—	n.d.	—	n.d.	40
5	500	—	833 ± 340	—	275 ± 91	—	n.d.	0
	500	4 × 124	358 ± 102	<0·3	164 ± 48	<0·4	n.d.	0

transaminase values at 24 h after paracetamol in experiment 1 were much lower than those in experiment 2. The reasons for the differences and the individual variations in response are not clear. Analysis of the results using the Student's *t*-test showed a statistically significant difference for the transaminase concentrations in those animals that had received paracetamol alone, and those that received glutathione as well. Thus, in experiments 2 and 3 where glutathione was given as 4×128 mg kg^{-1}, the difference from the paracetamol alone treated group was highly significant. Glutathione at 4×80 mg kg^{-1} (expt 1) was protective but the Student's *t*-test showed no statistical significance. There was no protection at 4×8 mg kg^{-1} (expt 4) and 40% of the animals died—the same percentage as those receiving paracetamol alone. This showed that the protective effect of glutathione is dose dependent. In experiment 5 (Table 1) glutathione given in four divided doses *before* the paracetamol did not protect the animals significantly. Table 2 shows the results of various single doses of glutathione at different times after paracetamol. Experiments 6, 7, 8 were with single doses of glutathione varying from 32 to 800 mg kg^{-1}, administered 15 min after paracetamol. At 800 mg kg^{-1} protection appeared to be virtually complete as the transaminase concentrations were similar to those of the controls and there were no overt signs of liver damage. At 200 and 500 mg kg^{-1} the protection was less complete as shown by the transaminase values but the results are statistically highly significant by the Student's *t*-test. At 100 mg kg^{-1} the protective action has almost disappeared and at 32 mg kg^{-1} there was no protection. The 800 mg kg^{-1} dose was well tolerated (separate experiments showed that the LD50 in mice of glutathione given intravenously was 6000 mg kg^{-1}).

Table 2 shows the results of giving glutathione as a single dose of 500 mg kg^{-1} at 45, 105, 180 min (expts 9–11) after paracetamol. Protection was significant at 15 and 45 min, but it was weaker when the glutathione was injected at 105 min and there was no protection when glutathione was given 180 min after the dose of paracetamol.

Table 2. *Effect of glutathione on paracetamol hepatotoxicity in mice.* Paracetamol (500 mg kg^{-1}) was injected intraperitoneally into groups of ten mice. The glutathione was injected intravenously at 15 min (expts 6, 7 and 8), 45 min (expt 9), 105 min (expt 10) and 180 min (expt 11) after the paracetamol. Mortalities, necrosis indices and transaminases were determined 24 h after paracetamol administration. Transaminase values are means ± s.e.m., n.d. = not determined. The *P* values represent the probability compared with the paracetamol treated group in each experiment.

Expt No.	Dose (mg kg^{-1}) of: Paracetamol	Glutathione	Serum transaminases at 24 h I.U. litre^{-1} ± s.e.m. GOT	*P*	GPT	*P*	Necrosis index (0–4)	Mortality (%)
—	—	—	77 ± 14	—	14 ± 2	—	0	0
6	500	—	1380 ± 131	—	1092 ± 267	—	n.d.	30
	500	32	1614 ± 253	—	1537 ± 278	—	n.d.	30
	500	100	773 ± 230	<0·1	783 ± 310	<0·5	n.d.	30
	500	500	238 ± 77	<0·001	278 ± 146	<0·02	n.d.	10
7	500	—	904 ± 140	—	372 ± 124	—	2·0	0
	500	520	169 ± 28	<0·01	22 ± 7	<0·2	0·6	0
8	500	—	2340 ± 411	—	1328 ± 216	—	n.d.	0
	500	200	471 ± 228	<0·001	360 ± 180	<0·02	n.d.	0
	500	500	197 ± 49	<0·01	177 ± 54	<0·001	n.d.	0
	500	800	87 ± 14	<0·001	29 ± 5	<0·001	n.d.	0
9	500	—	1380 ± 131	—	1092 ± 267	—	n.d.	30
	500	500	361 ± 162	<0·001	343 ± 195	<0·05	n.d.	0
10*	500	500	726 ± 153	<0·01	882 ± 250	<0·6	n.d.	0
11*	500	500	1623 ± 181	—	1123 ± 257	—	n.d.	0

* For control paracetamol values see Expt. 9.

If a comparison is made between experiment 1 (320 mg kg^{-1} of glutathione in four 80 mg kg^{-1} doses at 15, 45, 75, 105 min after paracetamol) and experiment 8 (200 mg kg^{-1} glutathione as a single dose 15 min after paracetamol) it can be seen that the latter treatment is the more effective. This can be explained by the fact that, as endogenous glutathione depletion by paracetamol is rapid, the dose of glutathione at 75 and 105 min in experiment 1 are too late to be beneficial. This is confirmed by the results in experiments 10 and 11 (Table 2).

These findings indicate that intravenously administered glutathione when given in an adequate amount and at an appropriate time either as a single or divided dose can largely protect mice against the hepatotoxic effects of a large dose of paracetamol. This finding is surprising in view of the belief expressed in the literature (Prescott & others, 1974) that glutathione does not readily enter cells. These results could also be seen to support the view of Mitchell & others (1973b) of the importance of glutathione depletion in paracetamol-induced liver damage.

Battelle Research Centre,
7, route de Drize,
1227 - Carouge,
Geneva, Switzerland.

St. Mary's Hospital Medical School,
University of London, London, U.K.

M. STROLIN BENEDETTI
A. LOUIS
A. MALNOË
M. SCHNEIDER
R. LAM
L. KREBER
R. L. SMITH

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REFERENCES

BOYD, F. M. & BERECZKY, C. M. (1966). *Br. J. Pharmac. Chemother.*, **26**, 606–614.

JOLLOW, D. J., MITCHELL, J. R., POTTER, W. Z., DAVIS, D. C., GILLETTE, J. R. & BRODIE, B. B. (1973). *J. Pharmac. exp. Ther.*, **187**, 195–202.

MITCHELL, J. R., JOLLOW, D. J., POTTER, W. Z., DAVIS, D. C., GILLETTE, J. R. & BRODIE, B. B. (1973a). *Ibid.*, **187**, 185–124.

MITCHELL, J. R., JOLLOW, D. J., POTTER, W. Z., GILLETTE, J. R. & BRODIE, B. B. (1973b). *Ibid.*, **187**, 211–217.

PRESCOTT, L. F., SWAINSON, C. P., FORREST, A. R. W., NEWTON, R. W., WRIGHT, H. & MATTHEW, H. (1974). *Lancet*, **1**, 588–592.

PRESCOTT, L. F., WRIGHT, N., ROSCOE, P. & BROWN, S. S. (1971). *Ibid.*, **1**, 519–522.

REITMAN, S. & FRANKEL, S. (1957). *Am. J. clin. Path.*, **28**, 56–63.