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PROTECTION OF RAT EMBRYOS IN CULTURE AGAINST THE EMBRYOTOXICITY OF ACROLEIN USING EXOGENOUS GLUTATHIONE

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Abstract—The aldehyde acrolein is embryotoxic *in vivo* and *in vitro*. Since acrolein is reactive towards thiols, glutathione was evaluated for its protective effects against the *in vitro* embryotoxicity of acrolein. Day 10 rat embryos were cultured in the presence of acrolein and glutathione, either concurrently or sequentially, and evaluated for embryo deaths, malformations, growth retardation, and content of glutathione and protein. Acrolein, added alone at the initiation of the culture period, was embryolethal to 64 and 100% of the embryos at 120 and 160 μM respectively. At acrolein concentrations of 80 and 120 μM, 50 and 100%, respectively, of the surviving embryos were malformed. In addition, both of these concentrations of acrolein produced growth retardation manifested by significant decreases in the yolk sac diameter, crown-rump and head lengths, number of somites, and morphological score. Concurrent exposure to 100 or 500 μM glutathione markedly protected embryos against all of these effects. To study the mechanism of the protective effect of glutathione against the embryotoxicity of acrolein, the effects of sequential addition of acrolein and glutathione were determined. When rat embryos were cultured in the presence of acrolein for 2 hr prior to exposure to glutathione, even 500 μM glutathione could not offer any protection against the embryolethality, teratogenicity, and growth retardation induced by acrolein. However, a 6-hr preincubation with 500 μM glutathione, prior to exposure to acrolein (in the absence of exogenous glutathione), significantly decreased the incidence of embryo deaths at 160 μM acrolein and brought the number of deaths and malformations among embryos exposed to 120 μM acrolein down to a level not significantly different from control; unlike the embryos exposed concurrently to acrolein and glutathione, however, the sequential treatment with glutathione and acrolein did not protect against growth retardation. While there were some changes in the total glutathione and protein content of embryos and yolk sacs with acrolein exposure, none of the treatments had any overall effect on the glutathione concentration per mg protein. Thus, exogenous glutathione can protect against the *in vitro* embryotoxicity of acrolein. We propose that this protection is mediated in part by a direct interaction between glutathione and acrolein, added concurrently to the serum medium, and in part by an indirect effect on the embryo of glutathione added prior to acrolein.

The toxic aldehyde acrolein is present in the environment as a component of cigarette smoke [1] and automobile exhaust [2], and as a by-product of and reactant in many industrial processes [3]. Acrolein is also a metabolite of the widely used anticancer and immunosuppressant drug and known teratogen, cyclophosphamide [4]. Acrolein is embryolethal and teratogenic *in vivo* in several species after intraamniotic injection [5–8], and *in vitro* in rat whole embryo culture [9].

Acrolein is known to be reactive towards thiols, forming adducts with the tripeptide, glutathione, *in vivo* and *in vitro*; such conjugates can be formed both nonenzymatically and enzymatically with the glutathione-*S*-transferases [10–12]. Acrolein can deplete hepatic glutathione levels in intact rats [13] as well as in cultured hepatocytes [14]. It seems likely that thiols such as glutathione protect against toxic effects of acrolein, either directly by reacting with the aldehyde or indirectly by enhancing tissue levels of sulfhydryls.

Thiols can protect against the teratogenicity of certain drugs. Pretreatment of pregnant rats with cysteine, glutathione or sodium 2-mercaptoethane sulfonate (MESNA) can decrease significantly the *in vivo* teratogenicity of cyclophosphamide [15–17]. *In vitro*, Kitchin *et al.* [18] demonstrated that the addition of glutathione to the culture medium can protect against the embryolethality and growth retardation, but not the teratogenicity, of mercuric chloride in cultured rat embryos. However, these investigators [18] also found that a glutathione concentration of 100 μM causes a 100% incidence of telencephalic abnormalities in the exposed embryos.

The effects of glutathione on cultured rat embryos and on the *in vitro* embryotoxicity of acrolein were assessed in this study by culturing day 10 rat embryos in the presence of acrolein and glutathione, either concurrently or sequentially.

METHODS

Chemicals. Acrolein (99% pure) was purchased from the Aldrich Chemical Co. (Montreal, Quebec).

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Reduced glutathione was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tyrode's saline, Hanks' Balanced Salt Solution, Waymouth's MB 752/1 medium with L-glutamine, and penicillin/streptomycin (10,000 units/ml and 10,000 μg/ml respectively) were purchased from Gibco Laboratories (Burlington, Ontario). Glutathione reductase for the assay of glutathione was purchased from Boehringer Mannheim (Laval, Quebec).

Animals. Timed-gestation pregnant Sprague–Dawley rats (180–200 g) were purchased from Charles River Canada, Inc. (St. Constant, Quebec). The day on which spermatozoa were found in the vaginal smear was considered day zero of pregnancy. Rats were housed in the McIntyre Animal Centre (McGill University, Montreal, Quebec) and given Purina rat chow and water *ad lib.*

Embryo culture procedure. The embryo culture procedure used in this study was based on the system described by New [19], modified as previously described [9]. Pregnant rats were etherized on the morning of day 10 of gestation, the uterine horns were removed, and the embryos were dissected out and cultured in sterile 60-ml roller bottles. The culture medium consisted of 80% heat-inactivated filtered rat serum, 20% Tyrode's saline and penicillin/streptomycin (final concentrations were 50 units/ml and 50 μg/ml respectively). The bottles were gassed [9], and the embryos were cultured for a total of 45 hr at 37°.

At the end of the culture period all embryos were removed and examined for viability. Only those embryos with a yolk sac circulatory system score of one or greater by the scoring system of Brown and Fabro [20] and with a heart beat were evaluated further. The embryos were classified as normal or abnormal, the abnormalities were documented, and representative photographs were taken. The yolk sac diameter, crown-rump length and head length were measured and the number of somites counted. The embryo scoring system of Brown and Fabro [20] was used to determine a morphological score for each embryo. Embryos and their yolk sacs were individually frozen at −80° for subsequent assay of glutathione and protein content. Total glutathione of homogenized samples was measured by the enzymatic cycling method of Tietze [21] (using optical density at 412 nm as the end point), as modified by Brehe and Burch [22], and expressed as nmoles glutathione equivalents/mg protein. Protein content was measured by the method of Lowry *et al.* [23].

This study was subdivided into three different sets of experiments. In the first set, both acrolein (80, 120 or 160 μM) and glutathione (100 or 500 μM) were added concurrently to the embryos in the serum medium at the initiation of the culture period. In the next two sets of experiments, the embryos were exposed to the two drugs sequentially. In the second set, the embryos were first exposed to acrolein (5, 10 or 20 μM) in Waymouth's medium for 2 hr prior to culture in rat serum medium containing glutathione (100 or 500 μM) but not acrolein. (Previous experiments have demonstrated that exposure of embryos to acrolein in a serum-free medium produces embryotoxic effects at lower concentrations than in serum medium [9].) In the third set, embryos were exposed to 500 μM glutathione in rat serum medium for 6 hr prior to exposure to acrolein (80, 120 or 160 μM) in fresh rat serum medium with no added glutathione. The glutathione content of embryos and yolk sacs for embryos cultured for 6 hr with no drug or 500 μM glutathione and for 2 hr with no drug, 80 μM or 120 μM acrolein was determined.

Statistics. All data on embryo deaths and malformations were analyzed by the Fisher Exact Test [24]. Comparisons of the yolk sac diameter, crown-rump length, head length, number of somites, morphological score, and glutathione and protein contents were done by the Kruskal–Wallis test [25] with the Mann–Whitney U-Test [26] and the Bonferroni correction [25] when necessary to isolate the differences when three treatment groups were compared (experiments using two concentrations of glutathione). The level of significance used throughout was $P \leq 0.05$.

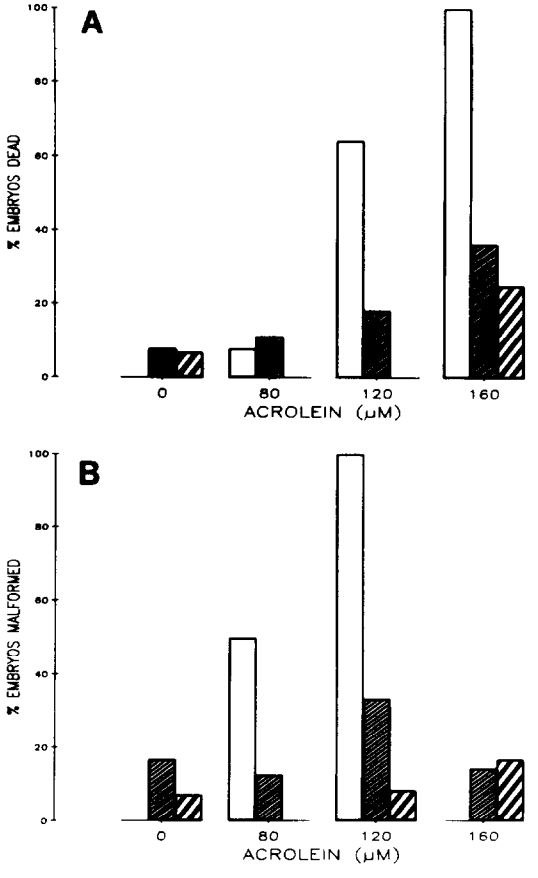

Fig. 1. Effects of glutathione on the embryolethality (A) and teratogenicity (B) of acrolein toward cultured rat embryos. Embryos were concurrently exposed to acrolein (0, 80, 120 or 160 μM) and glutathione (0, 100 or 500 μM) in rat serum medium. Key: (□) embryos exposed to acrolein only; (▨) embryos exposed to acrolein plus 100 μM glutathione; and (▨) embryos exposed to acrolein plus 500 μM glutathione. One hundred sixty-eight embryos were cultured with a mean of 11 embryos per group. The proportions of dead and malformed embryos in each group were compared using the Fisher Exact test [24].

RESULTS

Concurrent exposure to acrolein and glutathione. The effects of concurrent exposure to glutathione and acrolein on the cultured rat embryos are shown in Fig. 1A. A total of 168 embryos were cultured, with a mean of 11 per group. There were no deaths among the control embryos, and only one embryo death at each concentration of glutathione alone or at 80 μM acrolein. At 120 μM acrolein, 64% of the embryos did not survive, and 160 μM acrolein was 100% embryolethal. Concurrent exposure to acrolein plus either 100 or 500 μM glutathione significantly reduced the incidence of embryo deaths compared to acrolein alone, at both 120 and 160 μM acrolein. In the presence of 500 μM glutathione, the incidence of embryo deaths at any concentration of acrolein was not significantly ($P > 0.05$) different from control. Thus, glutathione offered complete protection against the embryolethality of acrolein toward cultured rat embryos.

The effect of glutathione on the teratogenicity of acrolein was as marked as that on embryolethality (Fig. 1B). Glutathione alone did not produce a significant number of malformed embryos compared to control. At 80 μM acrolein, 50% of the surviving embryos were malformed, while at 120 μM acrolein all of the surviving embryos were malformed. Both concentrations of glutathione significantly reduced the incidence of malformations induced by 80 and 120 μM acrolein. In the presence of either 100 or 500 μM glutathione, the incidence of embryo malformations with even 160 μM acrolein was not significantly different from control. Figure 2 illustrates the extent of this protection. The embryo on the left was exposed to 120 μM acrolein alone, whereas the embryo on the right was exposed concurrently to 120 μM acrolein and 500 μM glutathione.

Exposure of the embryos to 80 or 120 μM acrolein resulted in significant growth retardation as measured by the yolk sac diameter, crown-rump length, number of somites and the morphological score (Fig. 3). Concurrent exposure to 500 μM glutathione significantly protected against the decrease in the yolk sac diameter at 80 μM acrolein. Both 100 and 500 μM glutathione significantly protected against the decrease in the yolk sac diameter, number of somites and morphological score caused by 120 μM acrolein. In the presence of either concentration of glutathione, the crown-rump lengths (and the head lengths, data not shown) of embryos exposed to 120 μM acrolein were increased to values not significantly different from control.

The total glutathione (reduced plus oxidized) contents and the protein contents of the embryos and their yolk sacs are shown in Table 1. Exposure to 120 μM acrolein, the highest concentration compatible with survival, resulted in a significant decrease in the embryonic protein content and in the total glutathione of the yolk sacs. In the 120 μM acrolein-treated groups co-cultured with either concentration of glutathione, these values were increased to levels not significantly different from control. Culture with acrolein and glutathione, either

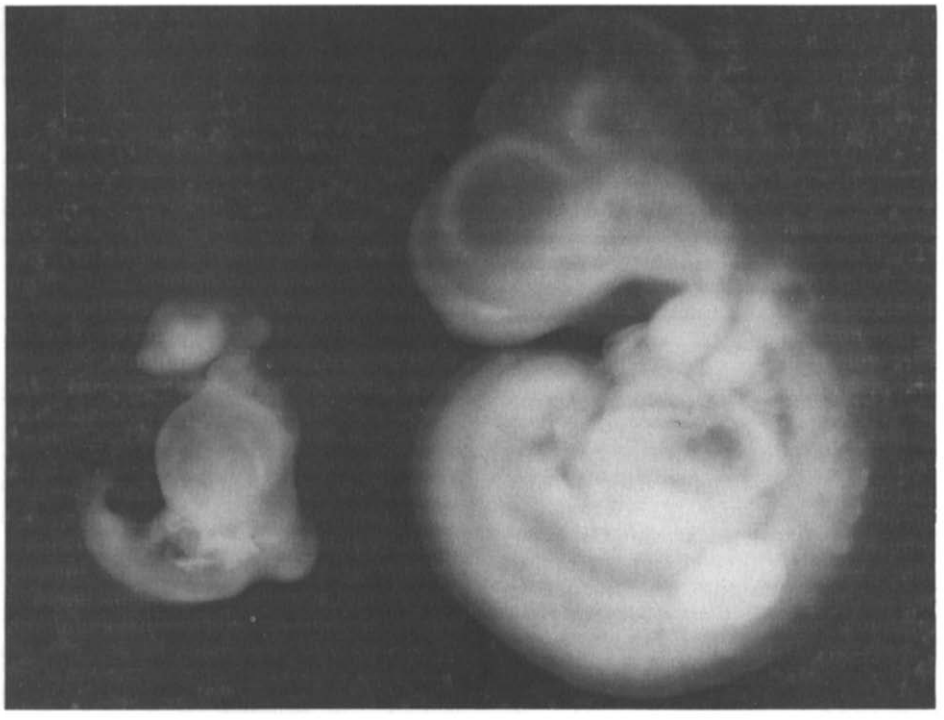

Fig. 2. Effects of glutathione on the teratogenicity of acrolein to rat embryos *in vitro*. The embryo on the left was exposed to 120 μM acrolein alone; it is growth retarded and has a small head, maxillary protrusions, and an enlarged pericardium. The embryo on the right was exposed to 120 μM acrolein plus 500 μM glutathione and is indistinguishable from control.

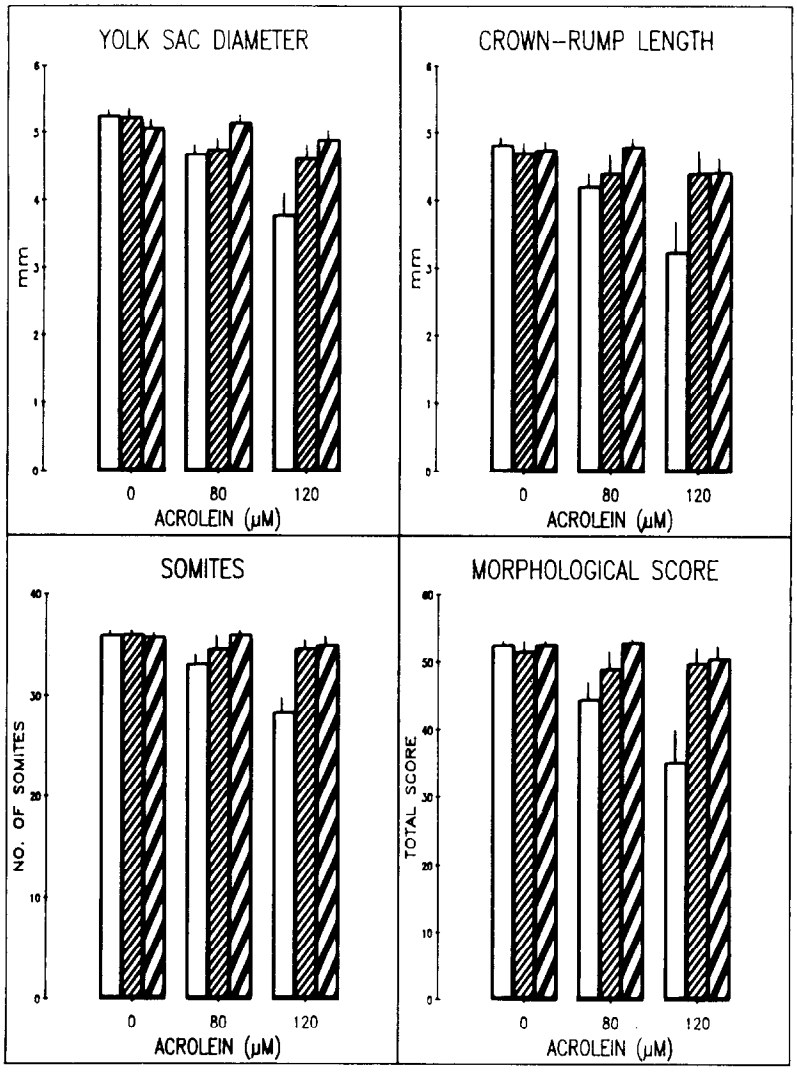

Fig. 3. Effects of acrolein plus glutathione on embryo growth *in vitro*. Embryos were concurrently exposed to acrolein (0, 80 or 120 μM) and glutathione (0, 100 or 500 μM) in rat serum medium. Key: (□) embryos exposed to acrolein only; (▨) embryos exposed to acrolein plus 100 μM glutathione; and (▩) embryos exposed to acrolein plus 500 μM glutathione. Values represent the means ± SEM. One hundred sixty-eight embryos were cultured with a mean of 11 embryos per group.

individually or in combination, did not alter the glutathione concentration per mg protein in either the embryo or yolk sac.

Thus, concurrent exposure to glutathione and acrolein protects against the embryolethality, teratogenicity and growth retardation of acrolein toward cultured rat embryos without affecting the glutathione concentration of either the embryos or their yolk sacs. Moreover, exposure to glutathione concentrations of 100 or 500 μM does not cause any increase in embryo malformations.

Since there was no effect of acrolein on the glutathione content per mg protein of either the embryos or their yolk sacs at the end of the culture period, the glutathione and protein contents of these tissues were assessed after a short time (2 hr) of exposure to 80 or 120 μM acrolein. Two hours was chosen as this length of exposure to acrolein is likely to be sufficient to produce the embryotoxic effects observed (see the second set of experiments). The results are shown in Table 2. There was no effect of either concentration of acrolein on the total glutathione content of the embryos or yolk sacs, but a significant increase in the protein content of both embryos and yolk sacs exposed to 120 μM acrolein occurred. This protein increase was great enough to result in a significant decrease in the glutathione concentration per mg protein among the embryos but not among the yolk sacs.

Preincubation with acrolein in a serum-free medium. Embryos incubated in Waymouth's medium for 2 hr prior to the continuation of culture in rat serum medium showed no significant differences in incidence of deaths or malformations or

Table 1. Effect of acrolein plus concurrent glutathione (GSH) on embryo and yolk sac glutathione and protein content

	Embryos				Yolk sacs			
	N	Total GSH (nmol)	Protein (μg)	GSH/Protein (nmol/mg)	N	Total GSH (nmol)	Protein (μg)	GSH/Protein (nmol/mg)
Control	13	8.0 ± 0.8	770.6 ± 66.3	10.8 ± 1.1	14	6.8 ± 0.5	475.2 ± 35.2	14.8 ± 1.4
100 μM GSH	9	7.8 ± 1.2	803.4 ± 83.2	10.2 ± 1.2	8	7.5 ± 0.4	604.1 ± 81.6	14.4 ± 2.0
500 μM GSH	11	8.3 ± 0.8	836.6 ± 60.9	10.1 ± 0.8	11	7.6 ± 0.5	503.5 ± 43.6	15.8 ± 1.3
80 μM Acrolein	8	7.6 ± 1.1	647.3 ± 78.6	12.0 ± 1.6	8	6.8 ± 0.5	475.7 ± 67.8	16.1 ± 1.7
80 μM Acrolein + 100 μM GSH	6	8.4 ± 1.6	548.0 ± 81.6	14.9 ± 1.1	7	6.6 ± 0.7	520.6 ± 65.4	13.4 ± 0.9
80 μM Acrolein + 500 μM GSH	8	10.0 ± 0.6	737.0 ± 45.1	14.2 ± 1.3	8	8.0 ± 0.4	533.3 ± 41.9	15.7 ± 1.4
120 μM Acrolein	5	4.4 ± 1.6	335.1 ± 90.1*	14.3 ± 3.1	5	4.1 ± 0.7*	283.9 ± 43.5	14.9 ± 1.5
120 μM Acrolein + 100 μM GSH	7	6.9 ± 1.4	704.5 ± 112.8	9.9 ± 1.5	8	6.4 ± 0.9	426.2 ± 53.6	15.3 ± 2.1
120 μM Acrolein + 500 μM GSH	10	5.4 ± 0.9	730.8 ± 67.9	8.1 ± 1.5	9	6.3 ± 0.8	346.1 ± 24.0	18.1 ± 2.2
160 μM Acrolein + 100 μM GSH	3	7.4 ± 1.3	658.9 ± 124.3	11.3 ± 0.5	5	8.3 ± 1.2	562.0 ± 100.7	15.5 ± 0.9
160 μM Acrolein + 500 μM GSH	3	4.3 ± 0.9	456.0 ± 156.4	10.9 ± 1.6	3	5.6 ± 0.7	346.8 ± 49.1	16.4 ± 1.0

Values are the means ± SEM.
* Significantly lower for acrolein-exposed embryos compared to control ($P \leq 0.05$).

in the measured parameters of yolk sac diameter, crown-rump length, head length, number of somites, morphological score, or total glutathione content compared to control (data not shown). Exposure to 5, 10 or 20 μM acrolein for 2 hr in Waymouth's medium prior to culture in control rat serum medium resulted in a 10, 70 and 100% incidence of embryo deaths respectively. This was significantly different from control at 10 and 20 μM acrolein. Neither 100 nor 500 μM glutathione, added to the serum medium, could protect against this embryolethality of acrolein. Among embryos exposed to 5 or 10 μM acrolein, 67 and 100% of the surviving embryos were malformed. As for embryolethality, glutathione added subsequently to the serum medium could not protect against the teratogenicity of acrolein.

Exposure of embryos to 5 or 10 μM acrolein in Waymouth's medium also significantly decreased yolk sac diameter, crown-rump length, head length, number of somites and morphological score compared to control embryos (data not shown). Neither 100 nor 500 μM glutathione significantly protected against any of these effects.

The addition of glutathione after preincubation with acrolein had no effect on either the total glutathione or protein content or on the glutathione concentration per mg protein compared to acrolein alone (data not shown).

Thus, exposure to glutathione subsequent to acrolein exposure does not protect against the toxic effects of acrolein in cultured rat embryos.

Preincubation with glutathione. Exposure to acrolein alone in this third set of experiments resulted in significant embryolethality at 120 and 160 μM acrolein and significant teratogenicity at 120 μM (Fig. 4). A total of 123 embryos were cultured with a mean of 15.4 per group. The 6-hr preincubation with glutathione (500 μM) had no effect on the incidence of embryo deaths and malformations. However, glutathione pretreatment significantly reduced the incidence of dead embryos produced by 160 μM acrolein and brought the incidence of embryo deaths and malformations caused by 120 μM acrolein down to levels that were not significantly different from control.

As in the first set of experiments, acrolein itself produced growth retardation (Fig. 5). In contrast to the results obtained with simultaneous exposure to glutathione and acrolein, preincubation with glutathione (500 μM) did not protect against the growth-retarding effects of acrolein.

The glutathione and protein levels of both the embryos and their yolk sacs at the end of the culture period were measured. Neither acrolein alone nor glutathione alone had a significant effect on the total glutathione content, protein content or the glutathione concentration per mg protein of the embryos or their yolk sacs (data not shown). In addition, preincubation with glutathione did not alter any of these variables at the end of the culture period compared to acrolein alone.

An explanation for the lack of effect of glutathione on embryonic and yolk sac glutathione levels could be that, if an alteration in glutathione levels occurred, it did so transiently and earlier in the culture period. Therefore, glutathione and protein

Table 2. Glutathione (GSH) and protein contents of day 10 rat embryos cultured for 2 hr in the presence of 80 or 120 μM acrolein

	Total GSH (nmol)	Protein (μg)	GSH/Protein (nmol/mg)
Embryos:			
Control	0.59 ± 0.09	57.57 ± 4.16	9.82 ± 1.16
80 μM Acrolein	0.53 ± 0.06	72.78 ± 8.80	8.38 ± 1.16
120 μM Acrolein	0.41 ± 0.05	98.22 ± 9.17*	4.59 ± 0.64*
Yolk sacs:			
Control	1.68 ± 0.15	124.78 ± 9.52	13.79 ± 1.36
80 μM Acrolein	1.86 ± 0.19	142.83 ± 7.19	13.17 ± 1.35
120 μM Acrolein	1.89 ± 0.07	170.94 ± 7.69*	11.24 ± 0.57

Values are means ± SEM; N = 12 for all groups.
* Significantly different from control ($P \leq 0.05$).

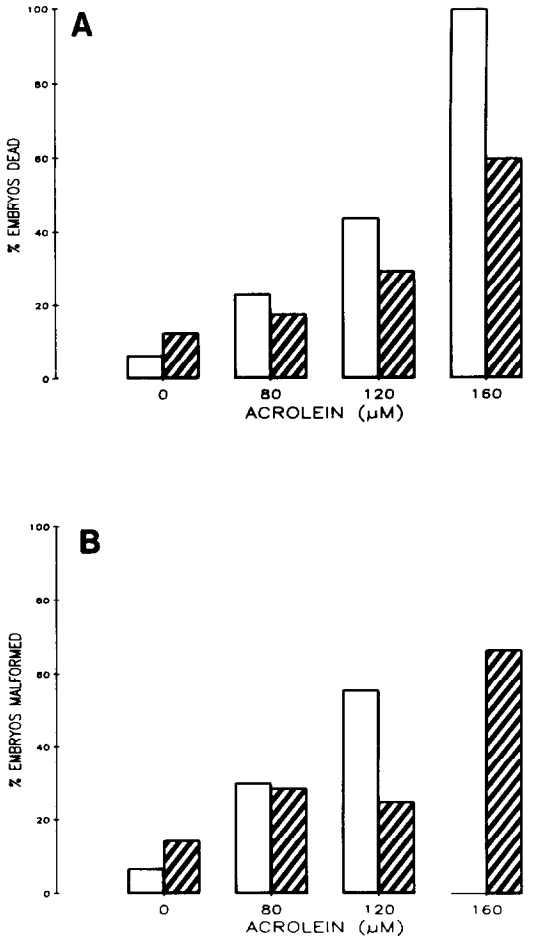

Fig. 4. Effect of a 6-hr preincubation with glutathione on the embryolethality (A) and teratogenicity (B) of acrolein to cultured rat embryos. Embryos were cultured in rat serum medium containing either no added glutathione (□) or 500 μM glutathione (▨) for 6 hr, and then were transferred to fresh rat serum medium, containing no added glutathione, and acrolein (0, 80, 120 or 160 μM) One hundred twenty-three embryos were cultured, with a mean of 15.4 embryos per group. The proportions of dead and malformed embryos in each treatment group were compared with the Fisher Exact Test [24].

levels were measured 6 hr after the initiation of culture in control embryos and yolk sacs and in those exposed to 500 μM glutathione. Preincubation with glutathione did not increase significantly the total glutathione content, the protein content or the glutathione concentration per mg protein of the embryos or of the yolk sacs (data not shown).

Thus, preincubation with 500 μM glutathione for 6 hr prior to acrolein exposure protected against both embryo deaths and malformations without altering the concentration of total glutathione in the embryo or yolk sac.

DISCUSSION

An ultimate goal in the study of teratology is the prevention of congenital malformations. Glutathione may have a role in the protection of the embryo and fetus against drugs and reactive intermediates. Within the human fetal liver, acid-soluble sulfhydryl groups are in the same concentration range as in the human adult liver. As well, glutathione synthetic and degradative enzymes are already present in the first trimester of gestation; the levels of these enzymes are higher in the fetal than the adult liver [27].

In this study we have shown that reduced glutathione has no apparent toxic effects and can protect cultured rat embryos against the embryotoxicity of acrolein. Despite the protection against the embryolethality, teratogenicity and the growth retardation of acrolein provided by glutathione, there was no increase in the glutathione levels in either the yolk sac or the embryo.

To determine whether the observed protective effect of exogenous glutathione was mediated by a direct effect, such as conjugation of acrolein with glutathione, or by an indirect effect on the embryo, such as an enhancement of embryonic and/or yolk sac glutathione levels, embryos were exposed to the two drugs sequentially. It was found that glutathione added subsequent to exposure to acrolein for 2 hr in Waymouth's medium could not protect against any of the toxic effects of this drug; glutathione cannot protect against or undo cell damage that has already occurred. In contrast, pre-exposure to glutathione prior to acrolein exposure did protect against the

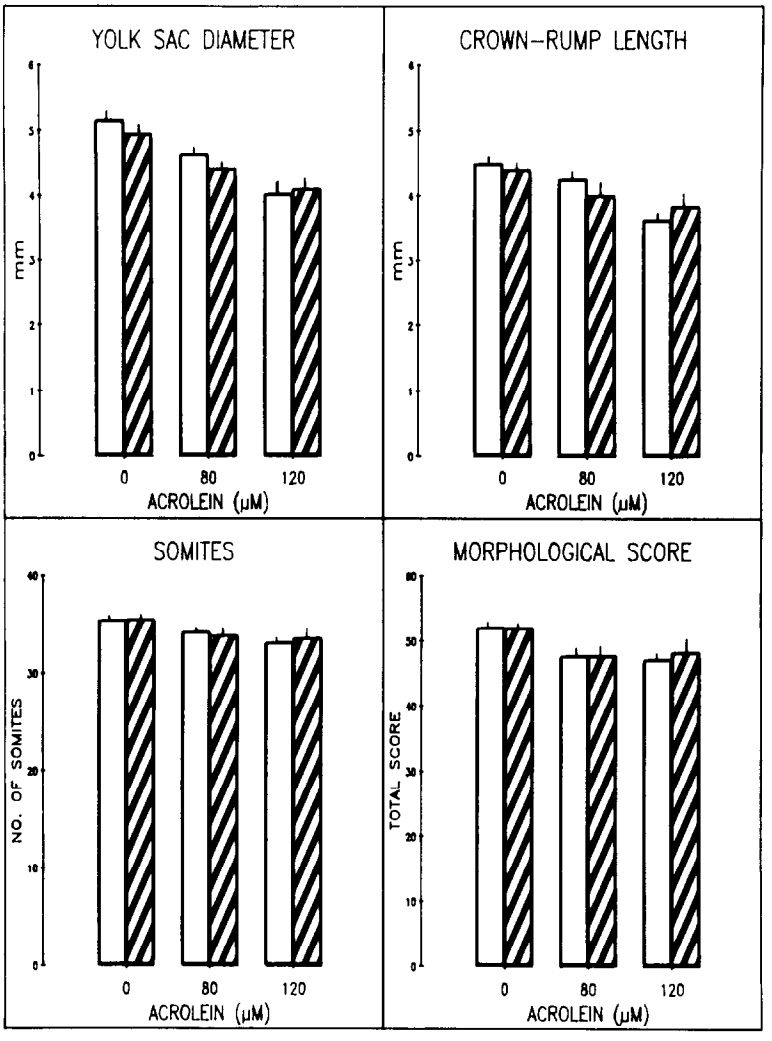

Fig. 5. Effects of preincubation with glutathione on the effects of acrolein on embryo growth *in vitro*. Embryos were cultured for 6 hr in rat serum medium without (0) or with (500 μM) glutathione. The medium was removed and acrolein (0, 80 or 120 μM) was added to fresh serum medium. Key: (□) embryos exposed to acrolein only; and (▨) embryos exposed to 500 μM glutathione prior to exposure to acrolein. Values represent the means ± SEM. One hundred twenty-three embryos were cultured, with a mean of 15.4 embryos per group.

embryolethality and teratogenicity of acrolein. Thus, the role of glutathione in protecting the embryo against acrolein involves more than simply direct binding of the two drugs.

Exogenous glutathione has been reported to protect rat small intestinal epithelial cells against injury by oxidants [28]; these cells can take up intact glutathione by a sodium-dependent transport system [28]. In other systems, it appears that glutathione cannot readily cross cell membranes to enter cells [29–31], and glutathione would probably need to be broken down to its constituent amino acids and resynthesized within the cell. The ability of the yolk sac and embryo to take up exogenous glutathione has not, to the best of our knowledge, been investigated. However, when embryos and yolk sacs were assayed for glutathione at the end of the culture period, there was no effect of added exogenous glutathione on either the glutathione content or glutathione concentration per mg protein. It is possible that it is not the total glutathione content, but rather the ratio of reduced to oxidized glutathione or the reduction of protein sulfhydryls, that is important in protecting the embryo from acrolein toxicity. Another possibility is that, while glutathione levels are not actually being increased by the addition of glutathione, the amino acid cysteine, formed from degradation of the added glutathione, is responsible for the observed effects. Cysteine is known to be protective against toxicity due to irradiation and a variety of chemicals [32, 33] and against cyclophosphamide teratogenesis [15, 16].

Unlike Kitchin *et al.* [18], we found that glutathione concentrations as high as 500 μM did not cause abnormalities. A few embryos with slightly smaller telencephalons were seen among embryos

exposed to 500 μM but not 100 μM glutathione. However, these telencephalons appeared normally developed, and the size decrease was not significant.

In summary, the results presented here demonstrate that the embryotoxic effects of acrolein *in vitro* can be lessened or prevented by glutathione, either when the embryos are exposed to the two compounds concurrently or when embryos are exposed to glutathione prior to acrolein. That a sulfhydryl compound like glutathione can protect against drug-induced teratogenicity has potential clinical applications. Administration during pregnancy of a glutathione precursor or a glutathione monoester (which is more easily taken up by cells [34]) may be a useful treatment in protecting against maternal drug exposure.

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REFERENCES

1. R. A. Johnstone and J. R. Plimmer, *Chem. Rev.* **59**, 885 (1959).
2. M. Tanimoto and H. Uehara, *Environ. Sci. Technol.* **9**, 153 (1975).
3. C. Izard and C. Libermann, *Mutation Res.* **47**, 115 (1978).
4. R. A. Alarcon and J. Meienhofer, *Nature, Lond.* **233**, 250 (1971).
5. U. Claussen, W. Hellman and G. Pache, *Arzneimittel-Forsch/Drug Res.* **30**, 2080 (1980).
6. B. F. Hales, *Cancer Res.* **42**, 3016 (1982).
7. A. Korhonen, K. Hemminki and H. Vainio, *Acta pharmac. tox.* **52**, 95 (1983).
8. V. L. Slott and B. F. Hales, *Teratology* **32**, 65 (1985).
9. V. L. Slott and B. F. Hales, *Teratology* **34**, 155 (1986).
10. C. M. Kaye, *Biochem. J.* **134**, 1093 (1973).
11. P. M. Giles, *Xenobiotica* **9**, 745 (1979).
12. J. M. Patel, J. C. Wood and K. C. Liebman, *Drug Metab. Dispos.* **8**, 305 (1980).
13. H. L. Gurtoo, J. H. Hipkens and S. D. Sharma, *Cancer Res.* **41**, 3584 (1981).
14. A. Zitting and T. Heinonen, *Toxicology* **17**, 333 (1980).
15. R. Ashby, L. Davis, B. B. Dewhurst, R. Espinal, R. N. Penn and D. G. Upshall, *Cancer Treat. Rep.* **60**, 477 (1976).
16. B. F. Hales, *Teratology* **23**, 373 (1981).
17. V. L. Slott and B. F. Hales, *Toxic. appl. Pharmac.* **82**, 80 (1986).
18. K. T. Kitchin, M. T. Ebron and D. Svensgaard, *Fd chem. Toxic.* **22**, 31 (1984).
19. D. A. T. New, *Biol. Rev.* **53**, 81 (1978).
20. N. A. Brown and S. Fabro, *Teratology* **24**, 65 (1981).
21. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
22. J. E. Brehe and H. B. Burch, *Analyt. Biochem.* **74**, 189 (1976).
23. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
24. J. H. Zar, *Biostatistical Analysis*, p. 281. Prentice Hall, Englewood Cliffs, NJ (1974).
25. S. A. Glantz, *Primer of Biostatistics*, p. 292. McGraw-Hill, New York (1981).
26. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th Edn. Iowa State University Press, Ames, IA (1967).
27. D. Rollins, A. Larsson, B. Steen, K. Krishnaswamy, L. Hagenfeldt, P. Moldeus and A. Rane, *J. Pharmac. exp. Ther.* **217**, 697 (1981).
28. L. H. Lash, T. M. Hagen and D. P. Jones, *Proc. natn. Acad. Sci. U.S.A.* **83**, 4641 (1986).
29. K. Yoshimura, Y. Iwauchi, S. Sugiyama, T. Kuwamura, Y. Odaka, T. Sotoh and H. Kitagawa, *Res. Commun. Chem. Path. Pharmac.* **37**, 171 (1972).
30. M. E. Anderson, R. J. Bridges and A. Meister, *Biochem. biophys. Res. Commun.* **96**, 848 (1980).
31. G. L. Jensen and A. Meister, *Proc. natn. Acad. Sci. U.S.A.* **80**, 4714 (1983).
32. T. A. Connors, *Eur. J. Cancer* **2**, 293 (1966).
33. H. Thor, P. Moldeus and S. Orrenius, *Archs Biochem. Biophys.* **192**, 405 (1979).
34. R. N. Puri and A. Meister, *Proc. natn. Acad. Sci. U.S.A.* **80**, 5258 (1983).