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THE DISTRIBUTION AND METABOLISM OF ACRYLAMIDE AND ITS NEUROTOXIC ANALOGUES IN RATS

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Abstract—Measurements of the concentrations of acrylamide and *N*-hydroxymethylacrylamide in blood of rats given a single intravenous dose indicated that both compounds distributed throughout total body water within a few minutes. The concentrations of both compounds decreased exponentially after this initial distribution, with a half-life of less than 2 hr. The reaction rates of the two compounds with glutathione *in vitro* were the same and the presence of rat liver supernatant increased the rates of both reactions. Both compounds caused a rapid decrease in liver glutathione *in vivo*, and the glutathione conjugates were excreted in the bile. The breakdown of *N*-hydroxymethylacrylamide to acrylamide in buffer was negligible and measurement of the specific radioactivity of the glutathione conjugate of *N*-hydroxy[^{14}C]methylacrylamide excreted in the bile of a dosed rat indicated that there is very little breakdown *in vivo*. Paper chromatography of blood extracts of rats dosed with *N*-methylacrylamide and *N*,*N*-diethylacrylamide did not give any evidence for breakdown of these compounds to acrylamide. The results indicated that neurotoxic analogues of acrylamide are not neurotoxic by virtue of formation of acrylamide. *N*-Hydroxymethylacrylamide and acrylamide are very similar in their distribution *in vivo* and in their reactivity with glutathione, an indication of their reactivity with thiol groups in general. The explanation of the fact that three times as much *N*-hydroxymethylacrylamide as acrylamide is required to produce neuropathy must lie in a specific difference in reactivity at the site which leads to the nerve damage.

Acrylamide produces peripheral neuropathy in a wide range of species including man (see reviews by Schaumburg and Spencer [1, 2]). In contrast to earlier findings, it has recently been shown [3] that three chemical analogues of acrylamide, namely *N*-hydroxymethylacrylamide, *N*-methylacrylamide and *N*,*N*-diethylacrylamide produce a similar neuropathy when given to rats in high doses over long periods. *N*-hydroxymethylacrylamide is very similar to acrylamide in its physical properties and the reactivity of the two compounds with thiols, compared by measuring their reactivity with glutathione in buffer, is the same [4]. However, *N*-hydroxymethylacrylamide is much less neurotoxic than acrylamide, about 3 times the total molar dose being required to produce the same degree of disability [3]. *N*-Methylacrylamide and *N*,*N*-diethylacrylamide are even less neurotoxic about 8 and 20 times the molar dose respectively being required.

The purpose of this paper is to determine the reasons for the differences in the neurotoxicity of these four acrylic amides. The previous findings on reactivity of acrylamide and *N*-hydroxymethylacrylamide with glutathione [4] have been confirmed and extended to studies *in vivo*. The distribution and removal of these two compounds have been studied by measuring blood concentrations after a single dose. The possibility that acrylamide itself is the only directly neurotoxic compound and that the analogues are converted to acrylamide *in vivo* has been examined.

MATERIALS AND METHODS

Animals. Male rats of the Porton strain (200 ± 20 g body wt) were used.

Special chemicals. *N*-Hydroxymethylacrylamide was kindly given by Vinyl Products Ltd (Carshalton); acrylamide was obtained from British Drug Houses Ltd. (Poole); reduced glutathione (Schwarz–Mann) from Micro-Bio Laboratories (London); ion exchange resin (AG 1-X8 200–400 mesh) from Biorad Laboratories Ltd. (St. Albans); Instagel from Packard Instrument Co. (Wembley); K^{14}CN and [^{14}C]paraformaldehyde from The Radiochemical Centre (Amersham). *N*-Methylacrylamide and *N*,*N*-diethylacrylamide were prepared by Dr. Mattocks and Mr. Jones in this laboratory. [1-^{14}C]acrylamide (mp 83–85°) was synthesized by reacting K^{14}CN with ethylene chlorohydrin to produce ethylene [^{14}C]cyanohydrin, which was then dehydrated by heating with sodium formate to produce [1-^{14}C]acrylonitrile. The [1-^{14}C]acrylonitrile was converted to acrylamide essentially as described by Weisgerber [5]. *N*-Hydroxy[^{14}C]methylacrylamide (mp 69–74°) was prepared by incubating acrylamide with [^{14}C]paraformaldehyde under alkaline conditions as described by Hashimoto and Aldridge [4] for the preparation of *N*-hydroxymethyl[1-^{14}C]acrylamide.

The glutathione conjugates of acrylamide and *N*-hydroxymethylacrylamide were prepared by reacting acrylamide or *N*-hydroxymethylacrylamide (0·32 mM) with equimolar glutathione at pH 8 in the presence of KCN (15 mM) to inhibit oxidation of glutathione.

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The product was separated by ion-exchange chromatography on an acetate resin. The eluate was concentrated to a small volume and the glutathione conjugate precipitated by addition of excess ethanol. The products migrated as a single ninhydrin-positive spot when subjected to paper chromatography in butanol–acetic acid–water (11:4:5 by vol.) and contained no unreacted acrylic compounds.

Diazomethane was prepared by the method of De Boer and Backer [6] and was re-distilled immediately before use.

Acetate resin was prepared from Bio-Rad AG1-X8 (200–400) resin (chloride form) by treatment with excess sodium acetate.

Dosing. Acrylamide (100 mg/kg) and *N*-hydroxymethylacrylamide (140 mg/kg) were given intravenously in 0·9% saline, *N*-methylacrylamide (120 mg/kg) and *N,N*-diethylacrylamide (176 mg/kg) were given intravenously in 60% *m*-dioxan-5-ol in water. The volume of each solution injected was 1 ml/kg.

Detection and assay of acrylic amides. Detection of acrylic amides on paper chromatograms and assays of acrylamide and *N*-hydroxymethylacrylamide were carried out by the method of Mattocks [7] for α-unsaturated carbonyl compounds using 4-dimethylcinnemaldehyde reagent.

Assay of free acrylamide and N-*hydroxymethylacrylamide in blood.* At various times after dosing with either compound, rats were anaesthetized with ether and 2 ml blood removed from the posterior vena cava into a heparinized syringe. Blood (1 ml) was mixed with 3 ml methanol containing Tris (hydroxymethyl)-aminomethane (0·1% w/v). The clear supernatant obtained after centrifugation of the extract in a bench centrifuge for 15 min was assayed by the method described above. Blood extracts from rats dosed with saline were used for blank values.

Paper chromatography of blood extracts. Blood extracts from rats dosed with acrylamide or its analogues were prepared as described above. Samples (100 μl) were applied to Whatman No. 1 chromatography paper. Papers were developed by descending chromatography for 16 hr at 25° using 60 ml butanol saturated with water.

Reaction of acrylamide and N-*hydroxymethylacrylamide with glutathione* in vitro. Liver supernatants were prepared by homogenizing livers in 5 vol. ice-cold 0·3 M sucrose and centrifuging at 27,000 *g* for 30 min. Denatured supernatants were obtained by maintaining the liver supernatant at 100° for 3 min. Reaction of acrylamide or *N*-hydroxymethylacrylamide with glutathione was carried out at 37° in 3 ml Na/K phosphate buffer (0·1 M) pH 7·3 EDTA (2 mM), glutathione (1–4 mM) and either sucrose (0·2 M) alone, or liver supernatant (equivalent to 0·13 *g* liver/ml) or denatured supernatant (equivalent to 0·13 *g* liver/ml). The reaction was started by the addition of acrylamide or *N*-hydroxymethylacrylamide (final concn 10 mM) and stopped by addition of 2 ml ice-cold perchloric acid (final concn 0·6 M). The supernatant obtained by centrifuging at 2500 *g* at 5° was used for assay of free glutathione using the Ellman [8] method as modified by Beutler *et al.* [9]. Estimations of the amount of glutathione remaining were made at 6 times during the 1st hr. Equations for the second order rate constants were calculated using an iterative method to determine the best fit. Second order rate constants were calculated from the slopes.

Assay of free glutathione in the liver. All rats were starved from the time of dosing until the livers were removed in order to avoid any effect of changes in food consumption on liver glutathione. The livers were homogenized in 11 vol. buffered ethanol as described by Johnson [10]. The homogenate was centrifuged at 2500 *g* for 20 min and the supernatant assayed for glutathione (the only non-protein sulphydryl compound present in significant quantities in the liver [11, 12]) as described above.

Breakdown of N-*hydroxymethylacrylamide* in vitro. Stoppered flasks containing solutions of *N*-hydroxymethylacrylamide (7·53 mg/ml) in buffers at different pH values were incubated at 37°. Blank flasks and flasks containing standards of formaldehyde (2–500 μg/ml) at the appropriate pH values were incubated alongside sample flasks. At various times, 5 ml aliquots were removed and added to 1 ml Schiff's reagent for aldehydes [13]. The absorbance was measured at 560 nm after 30 min. Phosphate buffers (0·1 M) were used for pH 3·5, 5·5 and 7·0. Although 3·5 is outside the buffering range for phosphate, the pH did not alter during the experiment. Tris–HCl buffers (0·1 M) were used for pH 8·0 and 9·0.

Identification of the glutathione conjugates of acrylamide and N-*hydroxymethylacrylamide in extracts of liver and bile.* Ethanolic extracts of liver were prepared as described for the assay of liver glutathione. The acidic amino acids were separated on a carbonate ion-exchange column as described by Gaitonde [14], and applied to Whatman No. 1 chromatography paper after concentration by rotary evaporation. Bile was collected by cannulation of the bile duct close to the duodenum. The rats were maintained under Nembutal anaesthesia and body temperature was maintained at 37–39° by means of a lamp. The bile (10 μl) was applied directly to Whatman No. 1 chromatography paper. Eluates of acetate columns (see below) were concentrated by rotary evaporation before application. Amino acids and peptides in all samples were separated by descending chromatography in butanol–acetic acid–water (11:4:5 by vol.) at 25° for 16 hr using 60 ml solvent. Amino acid and peptides were detected by ninhydrin spray and sulphur compounds by the platinic iodide spray of Toennies and Kolbe [15].

Separation of the glutathione conjugates of acrylamide and N-*hydroxymethylacrylamide from bile by ion-exchange chromatography.* Bile (1·5 ml) was applied directly to ion-exchange columns (15·5 × 0·9 cm) of acetate resin and washed on with 1 ml water. The column was eluted with 120 ml 0·1 N acetic acid followed by 0·22 N acetic acid. The glutathione conjugates of acrylamide and *N*-hydroxymethylacrylamide eluted in the same volume, between 120 and 190 ml. The concentration of glutathione conjugates in the eluate was assayed with ninhydrin [16]. Absorbance was linear for standards of both conjugates and the extinction coefficients were the same. A standard was included in each series assayed. The purity of all fractions was checked by paper chromatography as described above.

Estimation of specific radioactivity of glutathione conjugates. The glutathione conjugates excreted in the bile of rats dosed with either [1-^{14}C]acrylamide or

N-hydroxy[^{14}C]methylacrylamide were isolated and measured in the way described above. The radioactivity was measured by scintillation counting in Instagel. Efficiency of counting was estimated by internal standardization. The chemical and radiochemical purity of all fractions was checked by paper chromatography as described above. The chromatograms were examined for ninhydrin-positive material and scanned for counts using a Packard Radiochromatogram Scanner (Model 7200). The specific radioactivity was compared with that of preparations *in vitro* made by reacting 0·3 ml of the solution used for dosing with glutathione as described for preparation of the unlabelled conjugates except that the concentration of reactants was 84 mM. The solution containing the conjugates was diluted in bile from saline-dosed rats then processed by ion exchange chromatography in the same way as the bile from animals dosed with the labelled compounds.

RESULTS

Concentration of acrylamide of N-*hydroxymethylacrylamide in blood after intravenous dosing.* The blood concentrations of both compounds fell exponentially after intravenous dosing (Fig. 1). The half-lives for acrylamide and *N*-hydroxymethylacrylamide are 1·90 and 1·55 hr respectively. Extrapolation of the decay curve back to zero time gives a concentration very close to the theoretical value for dilution in total body water, indicated by the arrow on Fig. 1. Removal of acrylamide from the blood is predominantly via the kidneys, since Hashimoto and Aldridge have shown that 60 per cent of the dose is excreted in the urine.

Concentration of glutathione in the liver after dosing with acrylamide or N-*hydroxymethylacrylamide.* After dosing rats with either compound, the liver glutathione fell rapidly, reaching a minimum of about 36 per cent normal values between 2 and 4 hr, then returned to normal, or slightly higher than normal

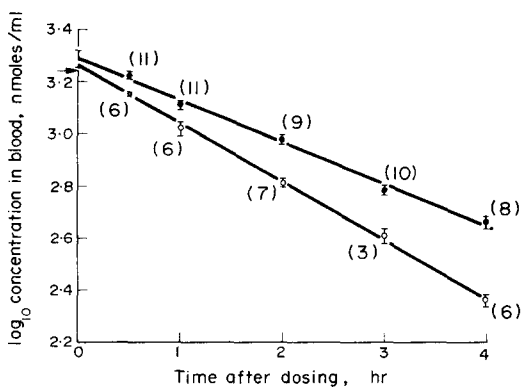

Fig. 1. Semi-log plot of the blood concentrations of acrylamide and *N*-hydroxymethylacrylamide after intravenous dosing. The concentration of acrylamide (●) or *N*-hydroxymethylacrylamide (○) at different times after an i.v. dose of 100 mg/kg or 140 mg/kg respectively was measured as described in Methods. The bars represent the standard errors and the number of rats is given in brackets. The theoretical concentration of either compound diluted in total body water (assumed to be 70% of total body wt [20]) is indicated by the arrow. The best straight lines were calculated by an iterative method.

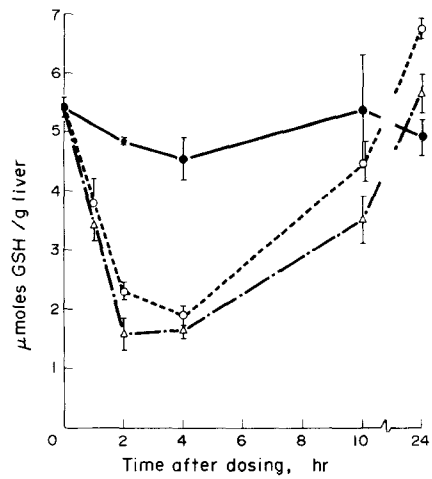

Fig. 2. Concentration of glutathione in the liver after dosing with acrylamide or *N*-hydroxymethylacrylamide. The concentration of glutathione in the liver at different times after dosing with either saline (●), 100 mg/kg acrylamide (○) or 140 mg/kg *N*-hydroxymethylacrylamide (△) was measured as described in the text. The bars represent the standard errors. Each point is the mean of the values from three rats except for the zero time measurements, which is the mean of six values. All rats were dosed between 9.00 and 10.00 a.m.

concentration by 24 hr (see Fig. 2). There is no gross difference between the effects of the two compounds. The control value did not vary greatly over the 24-hr period. Jaeger *et al.* [17] have reported that depriving rats of food greatly reduced the diurnal variation in liver glutathione. However, in our rats, unlike those of the above authors, there was little diurnal variation even in fed rats (Edwards, unpublished data).

Reaction of acrylamide and N-*hydroxymethylacrylamide with glutathione* in vitro. The second order rate-constants for the reaction of acrylamide and *N*-hydroxymethylacrylamide with glutathione at pH 7·3 and 37° were both about 1·0 (see Table 1), slightly higher than those obtained by Hashimoto and Aldridge under similar conditions [4]. The rate of reaction was faster (K = 1·65–2·46) in the presence of liver supernatant. Denaturing the supernatant abolished the stimulation of the rate of reaction, indicating that the stimulation of the reaction rate is due to an enzyme or enzymes in the liver supernatant. Boyland and Chasseaud [18] have described the presence of glutathione *S*-alkenetransferases in the liver which catalyse the reaction of glutathione with a number of $\alpha\beta$-unsaturated carbonyl compounds.

The breakdown of N-*hydroxymethylacrylamide* in vitro. The breakdown of *N*-hydroxymethylacrylamide at different pH's was monitored by measuring formaldehyde release. Breakdown at 37° was negligible at pH < 7 and slow even at pH 9 as shown in Table 2. These results are slightly higher than those obtained by Rostovski and Novichkova [19] at 30°.

Identification of acrylamide in the blood of rats dosed with acrylamide analogues. *N*-Hydroxymethylacrylamide could not be separated from acrylamide by any of a considerable number of chromatographic methods attempted. However, it was possible to separate *N*-methylacrylamide from acrylamide by descending chromatography in water-saturated

Table 1. The rate of reaction of acrylamide and *N*-hydroxymethylacrylamide with glutathione *in vitro*

Addition of glutathione	Rate constant (l./mole/min)
Acrylamide	0·94 ± 0·09, 1·04 ± 0·02, 0·99 ± 0·03
N-Hydroxymethylacrylamide	1·17 ± 0·03, 1·04 ± 0·05
Acrylamide + liver supernatant	1·65 ± 0·15, 2·06 ± 0·2
Acrylamide + denatured supernatant	0·87 ± 0·04
N-Hydroxymethylacrylamide + supernatant	1·82 ± 0·05, 2·46 ± 0·07

The rate constants were calculated from second order rate plots as described in the methods. Values of the rate constant ± standard errors obtained from individual experiments are given. The concentrations of glutathione were 1–4 mM, and of acrylamide and *N*-hydroxymethylacrylamide 10 mM. The concentration of glutathione in the absence of acrylic amides was constant both in the presence and absence of liver supernatant.

Table 2. Breakdown *in vitro* of *N*-hydroxymethylacrylamide

pH	% Molar conversion in 24 hr
3·5	<0·5
5·5	<0·5
7·0	2·7
8·0	3·7
9·0	6·3

The % molar breakdown of *N*-hydroxymethylacrylamide to acrylamide and at different formaldehyde pH values was estimated by measuring formaldehyde release as described in methods.

butanol. If *N*,*N*-diethylacrylamide was chromatographed in this system no diazomethane-positive spot could be detected on the paper. *N*,*N*-Diethylacrylamide applied to chromatography paper but not eluted gave a strong colour, so presumably the compound had evaporated during chromatography. Therefore it was possible to identify acrylamide (or *N*-hydroxymethylacrylamide since these co-chromatograph in this system) in the presence of *N*-methylacrylamide or *N*,*N*-diethylacrylamide. Chromatography of methanolic extracts of blood of rats 0·5 and 1 hr after dosing rats with either *N*-methylacrylamide or *N*,*N*-diethylacrylamide indicated that neither acrylamide nor *N*-hydroxymethylacrylamide had been formed (Table 3). A 5 per cent molar conversion to acrylamide or *N*-hydroxymethylacrylamide could have been detected.

Identification of conjugation products of acrylamide and N*-hydroxymethylacrylamide in liver and bile.* Since it has been found impossible to separate acrylamide from *N*-hydroxymethylacrylamide in blood samples, *N*-hydroxy[^{14}C]methylacrylamide was therefore prepared so that breakdown to acrylamide would result in a loss of label and could be detected by a fall in the ratio of ^{14}C-label to acrylic amide (specific radioactivity).

Radioactive *N*-hydroxymethylacrylamide polymerizes very readily and isolation of the monomer free from contamination with polymerized forms (which no longer contain the acrylic bond) was very difficult. This made measurements of the ratio of ^{14}C-label to acrylic amides (specific radioactivity) by studying the compounds circulating in the blood, subject to error. The possibility of carrying out analogous measurements on the conjugation products formed by reaction with glutathione *in vivo* was therefore investigated. This approach depends on the fact that estimation of ^{14}C-label measures only the conjugate formed with *N*-hydroxy[^{14}C]methylacrylamide, whereas chemical assay with ninhydrin measures the sum of the concentrations of conjugate formed from *N*-hydroxy[^{14}C]methylacrylamide and that formed from any acrylamide liberated. It is essential that the two compounds behave identically in all the systems used, (see below).

Dosing with either acrylamide or *N*-hydroxymethylacrylamide causes a loss of glutathione from the liver (see Fig. 2). Paper chromatograms of bile and of liver extracts of rats dosed with acrylamide were identical

Table 3. Separation of acrylamide, *N*-hydroxymethylacrylamide, N-methylacrylamide and *N*,*N*-diethylacrylamide by paper chromatography and their identification in the blood of dosed rats

Sample	R_f of spots			
Acrylamide			0·78	
N-Hydroxymethylacrylamide			0·78	
N-Methylacrylamide				0·95
N,*N*-Diethylacrylamide	No spot			
Control blood	0·051	0·34		
Blood of rats dosed with acrylamide	0·051	0·34	0·78	
Blood of rats dosed with *N*-methylacrylamide	0·051	0·34		0·95
Blood of rats dosed with *N*,*N*-diethylacrylamide	0·051	0·34		

Blood extracts were prepared and chromatograms run and developed as described in the text. The detection of acrylic compounds was sensitive down to 10 μg acrylamide and *N*-hydroxymethylacrylamide and 50 μg, *N*,*N*-diethylacrylamide and *N*-methylacrylamide. The spots at R_f 0·051 and 0·34, present in all blood samples, were very pale. *N*,*N*-diethylacrylamide probably evaporates during chromatography (see Results).

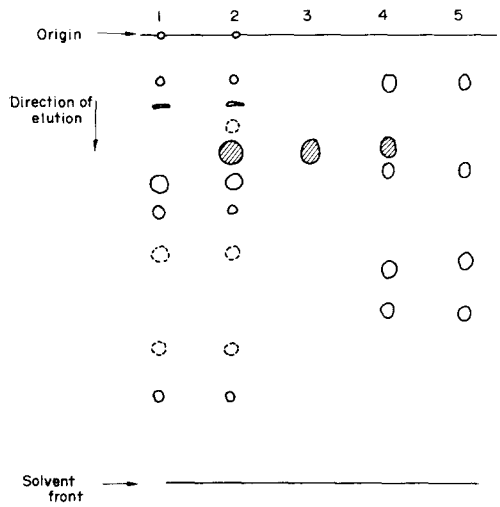

Fig. 3. Diagram of paper chromatography of liver extracts and bile of rats dosed with acrylamide or *N*-hydroxymethylacrylamide. Chromatography of extracts was carried out as described in the text. The spots indicated are those which were ninhydrin-positive. A dotted outline indicates that the spot was very pale and not always detectable. The hatched spot is the position to which purified glutathione conjugates of acrylamide or *N*-hydroxymethylacrylamide run. This spot gives a strong positive reaction with the platinic iodide spray for sulphur compounds. Key to Samples—(1) Control bile. (2) Bile collected from rat 0–$2\frac{1}{2}$ hr after dosing with acrylamide or *N*-hydroxymethylacrylamide. (3) Standard glutathione conjugate of acrylamide or *N*-hydroxymethylacrylamide. (4) Liver extract from rat 15 or 30 min after dosing with acrylamide or *N*-hydroxymethylacrylamide. (5) Control liver extract.

with those of rats dosed with *N*-hydroxymethylacrylamide (Fig. 3). The glutathione conjugates of both compounds could be identified as a single spot (absent from control samples) which gave colour with ninhydrin, contained sulphur as shown by the platinic iodide reagent, and had the same R_f as the standard conjugates. There were only traces of the conjugates in the liver extract but the concentration in bile was relatively high, so bile was used for further experiments.

Measurement of glutathione conjugates of acrylamide and N-hydroxymethylacrylamide in bile of dosed rats. Bile was collected from rats from 0–2·5 hr after dosing with saline, acrylamide (100 mg/kg) or *N*-hydroxymethylacrylamide (140 mg/kg) and the glutathione conjugates isolated by ion-exchange chromatography. The elution profile of bile from a rat dosed with acrylamide was indistinguishable from that of bile from a rat dosed with *N*-hydroxymethylacrylamide. The fraction of the eluate which contained the glutathione conjugates of acrylamide or *N*-hydroxymethylacrylamide was assayed for ninhydrin-positive material. The amount of ninhydrin positive material in this fraction of bile from saline-dosed rats is very small (see Table 4). After equimolar doses of acrylamide or *N*-hydroxymethylacrylamide, very similar quantities of the glutathione conjugates were excreted in the bile.

The specific radioactivity of glutathione conjugates excreted in bile after dosing with [1-^{14}C]acrylamide or N-hydroxy[^{14}C]methylacrylamide. The specific radioactivity of *N*-hydroxymethylacrylamide conjugate excreted from dosed rats is marginally lower than that of material prepared *in vitro* (Table 5). No lowering of specific radioactivity is seen in the excretion products of rats dosed with [1-^{14}C]acrylamide suggesting that the change is not an artifact induced by the method. When fractions were subjected to paper chromatography, all the radioactivity was contained in a single spot coincident with the only ninhydrin-positive spot.

DISCUSSION

It has been shown [3] that about 3-times as much *N*-hydroxymethylacrylamide as acrylamide is required to produce the same severity of neuropathy in rats. The behaviour of acrylamide and *N*-hydroxymethylacrylamide *in vivo* as determined in this paper does not provide an explanation for this difference. Hashimoto and Aldridge [4] have shown that after dosing with [1-^{14}C]acrylamide or *N*-hydroxymethyl [1-^{14}C]acrylamide, similar amounts of label were bound to proteins in different subcellular fractions of brain and in different tissues. They concluded that the distribution and reactivity of the two compounds was very similar. The results in this paper provide further evidence that this is in fact the case. Both compounds distribute very rapidly throughout the total body water and are then removed with a half-life of under 2 hr. The slightly more rapid removal of *N*-hydroxymethylacrylamide is not sufficient to account for the difference in toxicity. The second order rate constants for reaction of the two compounds with glutathione in buffer are the same for the two compounds as shown previously by Hashimoto and Aldridge [4], although the actual values obtained by these workers (0·91) was slightly lower than in this study (see Table 1.). There is no great difference between the reaction rates of the two compounds with glutathione *in vitro* in the presence of

Table 4. Ninhydrin colour in 120–190 ml eluate from acetate column separations of bile collected from rats from 0–2·5 hr after dosing

Dose	Ninhydrin values		
Saline	0·029,	0·022, 0·027	0·020,
N-Hydroxymethylacrylamide (140 mg/kg)	0·592,	0·502	
Acrylamide (100 mg/kg)	0·540,	0·582	

The fraction containing the glutathione conjugates (120–190 ml) was prepared as described in the methods and 1-ml portions assayed for ninhydrin-positive materials. The values given are the optical densities of the fractions from different rats.

Table 5. Specific radioactivity of glutathione conjugates excreted in bile after dosing with *N*-Hydroxy[^{14}C]methylacrylamide or [1-^{14}C]acrylamide

Dose	Time (hr)	Sample	Specific radioactivity (dis/min per nmole)	% Of control	Significance
N-Hydroxy[^{14}C]methylacrylamide	0–1½	Dosed rat	67·8, 74·2	100	
		in vitro prepn	68·2, 73·4		
	0–2	Dosed rat	65·7 ± 0·6 (3)	91	P = 0·02
		in vitro prepn	72·0 ± 1·8 (3)		
	0–2½	Dosed rat	68·1 ± 1·4 (4)	96	P = 0·15
		in vitro prepn	70·6 ± 0·7 (3)		
	All times combined	Dosed rat	67·9 ± 1·0 (9)	95	P = 0·03
		in vitro prepn	71·2 ± 0·8 (8)		
[1-^{14}C]Acrylamide	0–2½	Dosed rat	142 ± 3·4 (4)	100	
		in vitro prepn	135, 147		

Animals were dosed and the glutathione conjugates isolated from the bile as described in Methods. *In vitro* preparations were made by reacting some of the solution used for dosing with glutathione and isolating the product after mixing with control bile. Values are given as the means ± standard error with the number of rats in brackets, or as individual values. Significance of the difference between the specific activity of the glutathione conjugate isolated from dosed rats and that of the *in vitro* preparation was measured by Student's *t*-test.

liver supernatant, although the reaction of *N*-hydroxymethylacrylamide with glutathione may be marginally faster.

The concentration of glutathione in the liver after equimolar doses of acrylamide or *N*-hydroxymethylacrylamide falls rapidly in the first 2 hr after dosing. The liver glutathione concentrations returned to approximately normal values within 24 hr after dosing.

N-hydroxymethylacrylamide is not broken down to acrylamide *in vitro* to any extent. The rapid removal of free *N*-hydroxymethylacrylamide from the body water means that only a rapid breakdown would produce sufficient acrylamide to exert any toxic affects.

The reaction products of acrylamide and *N*-hydroxymethylacrylamide with liver glutathione are very rapidly excreted in the bile and the proportion of the dose excreted by this route is the same following dosing rats with either compound. The specific radioactivity of the glutathione conjugate excreted after dosing with *N*-hydroxy[^{14}C]methylacrylamide is marginally lower than that of the injected material. This indicates a conversion of less than 9 per cent. Because the glutathione conjugates of the two compounds behave identically in all the systems used for their isolation and assay, estimation of the ratio of counts to moles of glutathione conjugate gives a measure of the proportion of a dose of *N*-hydroxy[^{14}C]methylacrylamide which has broken down to acrylamide.

These results indicate that the neurotoxicity of *N*-hydroxymethylacrylamide, is due to toxicity of the compound itself and not the result of conversion to acrylamide *in vivo*. Similarly, no evidence for the conversion of *N*-methylacrylamide or *N*,*N*-diethylacrylamide could be found by paper chromatography of blood extracts from dosed rats.

Acrylamide covalently binds to protein in nervous tissue, predominantly by reaction with the sulphydryl groups in cysteine [4]. The reactivity of *N*-hydroxymethylacrylamide with sulphydryl groups measured by its reactivity with glutathione, a simple thiol, is the same as that of acrylamide. Since three times the dose of this compound, compared with acrylamide, is required to produce neuropathy, it may be presumed that either certain sulphydryl groups show differential reactivity towards the two compounds, or the binding site which is related to the development of neuropathy is not a sulphydryl group. A quantitative comparison between the effects of acrylamide and *N*-hydroxymethylacrylamide (and possibly the other two analogues of acrylamide) should give an indication of the relevance of a particular binding site to the development of neuropathy.

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REFERENCES

1. P. S. Spencer and H. H. Schaumburg, *Can. J. Neurol. Sci.* **1**, 143 (1974).
2. P. S. Spencer and H. H. Schaumburg, *Can. J. Neurol. Sci.* **1**, 152 (1974).
3. P. M. Edwards, *Br. J. Indust. Med.* **32**, 31 (1975).
4. K. Hashimoto and W. N. Aldridge, *Biochem. Pharmac.* **19**, 2591 (1970).
5. C. A. Weisgerber, *Chem. Abs* **49**, 11004 (b) (1955) U.S. 2,683,173 6th July, 1954.
6. Th. J. De Boer and H. J. Backer, *Org. Synth.* **36**, 16 (1965).
7. A. R. Mattocks, *Analyt. Chem.* **40**, 1347 (1968).
8. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
9. E. Beutler, O. Duron and B. M. Kelly, *J. Lab. clin. Med.* **61**, 882 (1963).
10. M. K. Johnson, *J. Chromat.* **23**, 474 (1966).
11. C. W. Owens and R. V. Belcher, *Biochem. J.* **94**, 705 (1965).
12. M. K. Johnson, *Biochem. J.* **98**, 38 (1966).
13. F. D. Snell and C. T. Snell, *Colorimetric Methods of Analysis*, 3rd Edn, Vol. III, p. 255. D. Van Nostrand, New York (1953).
14. M. K. Gaitonde, *J. Neurochem.* **8**, 234 (1961).
15. G. Toennies and J. J. Kolbe, *Analyt. Chem.* **23**, 823 (1951).
16. E. W. Yemm and E. C. Cocking, *Analyst* **80**, 209 (1955).
17. R. J. Jaeger, R. B. Conolly and S. D. Murphy, *Res. Commun. Chem. Path. Pharm.* **6**, 465 (1973).
18. E. Boyland and L. F. Chasseaud, *Biochem. J.* **109**, 651 (1968).
19. E. N. Rostovski and L. M. Novichkova, *Zh. Prikl. Khim., Mosk.* **41**, 346 (1966).
20. D. S. Dittmer (Ed.) *Biological Handbooks: Blood and Other Body Fluids*, p. 253. Federated American Society for Experimental Biology, Washington D.C. (1961).