In this paper we have shown that L-carnitine, administered by different routes, elicits different degrees of protection against lethal doses of ammonium acetate. Intraperitoneal injection of L-carnitine results in its preferential accumulation in brain, muscle and blood, while intravenous administration increases its location preferentially in liver. L-Carnitine has a clear stimulating effect on urea synthesis in mice challenged with non-lethal doses of ammonium acetate. This effect is seen for all routes tested. The results shown here support the postulation that the protection afforded by L-carnitine against acute hyperammonemia may have two components.

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REFERENCES

1. J Bremer, *Physiol. Rev.* **63**, 1420 (1983).
2. J. E. O'Connor, M. Costell and S. Grisolía, *FEBS Lett.* **166**, 331 (1984).
3. H. O. Conn and M. M. Lieberthal, *The Hepatic Coma Syndromes and Lactulose*. William & Wilkins, Baltimore (1979).
4. T. E. Duffy and F. Plum, in *The Liver: Biology and Pathobiology* (Eds. I. Arias, H. Popper, D. Schachter and D. A. Shafritz), p. 693. Raven Press, New York (1982).
5. E. Kvamme, *Prog. Neurobiol.* **20**, 109 (1983).
6. J. E. O'Connor, M. Costell and S. Grisolía, *Neurochem. Res.* **9**, 563 (1984).
7. R. A. Hawkins, A. L. Miller, R. C. Nielsen and R. L. Veech, *Biochem. J.* **134**, 1001 (1973).
8. B. Hindfelt, F. Plum and T. E. Duffy, *J. clin. Invest.* **59**, 386 (1977).
9. S. McCandless and S. Schenker, *Exp. Brain Res.* **44**, 325 (1981).
10. D. Hunninghake and S. Grisolía, *Analyt. Biochem.* **16**, 200 (1966).
11. M. Costell, J. E. O'Connor, M. P. Míguez and S. Grisolía, *Biochem. biophys. Res. Commun.* **120**, 726 (1984).

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γ-Acetylenic GABA antagonizes the decrease in synaptosomal GABA concentrations but not the seizures induced by 3-mercaptopropionic acid in rats

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γ-Aminobutyric acid (GABA), the mayor inhibitory neurotransmitter in mammalian brain, is thought to play a crucial role in the regulation of brain excitability. Thus, drugs which decrease synaptic GABA levels or act as antagonists at postsynaptic GABA receptors cause convulsions [1], whereas drugs which increase synaptic GABA concentrations or stimulate GABA receptors exert anticonvulsant effects [2]. Accordingly, evidence for impaired GABAergic activity has been found in several animal models of epilepsy, such as epileptic gerbils [3], epileptic dogs [4], and kindled rats [5], as well as in patients with certain types of epilepsy [6–8]. This strongly indicates that development of GABAmimetic drugs, i.e. compounds which selectively increase GABAmediated neurotransmission, may be a promising strategy in the search for new antiepileptic drugs. One category of GABAmimetic drugs, which has been extensively studied in this respect, are inhibitors of the GABA degrading enzyme GABA aminotransferase (GABA-T) [9, 10]. As shown by studies on GABA concentrations at the subcellular level, GABA-T inhibitors induce significant increases of GABA in nerve terminals (synaptosomes) of brain tissue [11–17], which obviously explain the anticonvulsant efficacy of such drugs in certain animal models of epilepsy [2, 10]. However, more recently we found that the time course of synaptosomal GABA increases induced by inhibition of GABA-T does not correlate with the time course of anticonvulsant effects obtained in this way [18]. In fact, at the time of maximum increase of GABA levels in synaptosomes, no effect on seizure thresholds could be determined [18]. This finding may relate to results of recent experiments of Abe and Matsuda [19], which suggested that two GABA pools exist in GABAergic nerve terminals, only one of which is preferentially associated with synaptic transmission. This pool is apparently not under the influence of GABA-T but is dependent on the activity of the GABA-synthesizing enzyme glutamate decarboxylase (GAD) [19]. Thus, inhibition of presynaptic GABA-T may be a relatively ineffective approach to enhance GABA-mediated transmission, which may explain the weak anticonvulsant efficacy of GABA-T inhibitors in most seizure models [20]. To investigate further the possibility that two pools of GABA with different functional meaning exist in nerve terminals, we examined the effects of the irreversible GABA-T inhibitor γ-acetylenic GABA (GAG) on seizures and the decrease in nerve terminal GABA levels induced by the convulsant 3-mercaptopropionic acid (3-MP) in rats. 3-MP is a selective inhibitor of GAD and is thought to cause seizures by decreasing synaptic GABA levels [21–25]. GAG was administered 4 hr prior to 3-MP, because after this time maximum effects of GAG on GABA concentrations in brain tissue and synaptosomes are reached [9, 12, 14]. To study the effects of GAG and 3-MP on GABA in nerve endings, a recently developed technique was used which allows the measurement of synaptosomal GABA levels in various discrete regions of one rat brain [23]. For comparison, GABA was also determined in whole tissue of the respective brain areas.

Materials and methods

For all experiments, female rats of the Wistar strain, weighing 200–220 g, were used. For the neurochemical determinations, groups of 10 rats were either injected with 3-MP, 50 mg/kg i.p., alone or after pretreatment with GAG, 100 mg/kg i.p. 4 hr before injection of 3-MP. The animals were killed by decapitation 3 min after injection of

the convulsant. Rats which already exhibited seizures at this time (mean seizure latency was 4 min with a range of 3–5 min) were not used in order to avoid unspecific effects of seizure activity on GABA levels. Five rats of each group were used for GABA determinations in whole tissue of brain regions and the other five for GABA analysis in synaptosomal fractions of the respective regions. Groups of five rats injected concomitantly with saline served as controls. Each rat brain was rapidly removed and dissected on a cold plate at −18° within 4 min after decapitation into 11 brain regions as described elsewhere [13]. For GABA determinations in whole tissue of brain regions, the individual regions were homogenized in 2 ml of 80% ethanol immediately after dissection, and GABA was measured by the enzymatic "GABAase" method [26] as described recently [13]. For measurement of GABA in synaptosomes, the individual regions were processed as shown in Fig. 1. Details of the subcellular fractionation technique used have been reported elsewhere [23], and it was shown that synaptosomal fractions obtained by this technique consist primarily of synaptosomes, whereas contamination with non-synaptosomal mitochondria and glia cells is very low [23]. 3-MP was included in the homogenizing medium of all samples at 1 mM in order to prevent *in vitro* increases of GABA during homogenisation and fractionation procedures [13, 23]. The GABA content of synaptosomal fractions was determined by a radioreceptor assay [27]. Protein was measured in whole tissue homogenates and synaptosomal fractions by the procedure of Lowry *et al.* [28] as modified by Markwell *et al.* [29].

The effects of GAG on seizures induced by 3-MP (50 mg/kg i.p.) was determined in further groups of 10 rats, which were observed for 30 min after injection of the convulsant. Seizures occurring in this time were rated as follows: 0, no seizure; 1, running seizures; 2, clonic seizures; 3, clonic seizures with loss of righting reflexes; 4, tonic forelimb extension; 5, tonic hindlimb extension. The maximum score for each rat was recorded and the mean of the 10 animals was used for comparison with rats which received 3-MP alone.

Results and discussion

As shown in Table 1, GAG exerted no significant effect on the percentage of animals convulsing or mean severity of the convulsions following injection of 3-MP. The only significant effect of GAG was an increase in the latency of the seizures. However, this effect was most marked 4 hr after administration of 50 mg/kg GAG, whereas 4 hr after 100 mg/kg, seizure latency was not different from values determined with 3-MP alone. Since GAG (100 mg/kg) had been reported to protect mice from seizures induced by 3-MP when injected 30 min prior to the convulsant [14], we did this experiment also in rats, but found no anticonvulsant effect of GAG except a moderate increase in seizure latency (Table 1).

The effects of 3-MP alone and of combined treatment with GAG and 3-MP on GABA levels in whole tissue and synaptosomal fractions of various brain regions is shown in Fig. 2. As already reported recently [23], 3-MP injected alone decreased GABA levels in most brain regions; how-

RAT

| 30 sec

Dissection at -18°C (3 min)

Olf. bulb	Frontal cortex	Corpus striatum	Hippo-campus	Thalamus	Hypo-thalamus	Tectum	Subst. nigra	Pons	Medulla	Cerebellum

Homogenisation in 1 ml 0.32 M sucrose (4°C; buffered to pH 7.0 with TRIS).
In case of GABA determinations, the sucrose contains 1 mM 3-mercaptopropionic acid

Centrifugation at 1000 g for 10 min

Supernatant fluid (S_1) collected and layered onto 1 ml 1.2 M sucrose.
Gradient centrifugation at 220 000 g for 15 min

Gradient interface (synaptosomes, myelin, some microsomes) collected and layered onto 1 ml 0.8 M sucrose. Gradient centrifugation at 220 000 g for 15 min

Pellet (synaptosomes) resuspended in medium depending on further analysis

Measurement of protein content. Biochemical analysis

Fig. 1. Scheme for isolation of synaptosomal fractions from 11 regions of one rat brain. For details see Löscher *et al.* [23].

Table 1. Effect of GAG on seizures induced by 3-MP (50 mg/kg i.p.) in rats

Dose of GAG (mg/kg i.p.)	Time (hr)	No. of rats	% convulsing	Latency to convulsions (min)	Seizure score	Mortality (%)
—	—	10	90	4.0 ± 0.2	4.1 ± 0.5	50
50	4	10	100	7.7 ± 0.7†	4.0 ± 0.3	30
100	0.5	10	80	5.2 ± 0.4*	3.7 ± 0.7	10
100	4	10	80	3.9 ± 0.7	3.6 ± 0.6	50

GAG was injected prior to 3-MP at the times indicated. Seizure latency and score are given as means ± SE. Significance of differences to animals injected with 3-MP alone are marked by asterisks (*$P < 0.02$, †$P < 0.001$).

ever, differences were found between whole tissue and synaptosomal GABA alterations. Significant decreases in whole tissue GABA levels were determined in cortex, striatum, thalamus, tectum, pons and cerebellum, whereas GABA levels in olfactory bulb, hippocampus, hypothalamus, substantia nigra, and medulla were not significantly different from the saline-injected controls. As in whole tissue, GABA levels in synaptosomes were significantly reduced in thalamus, tectum, and cerebellum and, in contrast to whole tissue, also in olfactory bulb and hippocampus. However, in some regions, i.e. corpus striatum, pons and cerebellum, the relative decreases of GABA in whole tissue were more marked than those in synaptosomes. This might relate to the previous finding that in some regions, e.g. the cerebellum, 3-MP not only inhibits GAD but also increases GABA-T [24, 25], an enzyme which in contrast to GAD is localized to a significant extent in non-nerve terminal compartments.

Pretreatment of rats with GAG, 100 mg/kg 4 hr prior to injection of 3-MP, antagonized completely the decreases in GABA concentrations induced by 3-MP alone (Fig. 2). In fact, except in tectum and pons, GABA levels were increased significantly in both whole tissue and synaptosomes of the brain regions examined shortly before the seizures. The most marked increases were found in the forebrain areas and the cerebellum.

The present experiments demonstrate that although GAG at the pretreatment time used for the neurochemical determinations did not exert any anticonvulsant effect on seizures induced by 3-MP, it prevented the decrease in nerve terminal GABA brought about by 3-MP. This apparent paradox may be explained by the already mentioned recent findings of Abe and Matsuda [19] suggesting the existence of 2 different GABA pools in nerve terminals. The latter authors concluded from their subcellular experiments with [^{14}C]glutamic acid and [^{3}H]GABA that one pool consists of newly synthesized GABA and waits in cytoplasm (probably near the presynaptic membrane) to be released into the synaptic cleft. This pool is associated with GAD and is not under the influence of GABA-T. Inhibition of GAD by 3-MP would thus directly decrease the amount of GABA which is available for synaptic transmission. The second pool consists of newly taken up GABA from the synaptic cleft and stays in synaptic vesicles (possibly near synaptic mitochondria) to be degraded. Stimulated release of GABA from this pool, which is closely under the influence of GABA-T, is significantly lower than that from the newly synthesized GABA pool. By inhibition of GABA-T, GAG would thus only increase the level of newly taken up GABA without enhancing the level of newly synthesized GABA. Actually, Abe and Matsuda [19] have shown that treatment of mice with another GABA-T inhibitor, namely aminooxyacetic acid, enhances the level of newly taken up GABA about 3-fold without any effect on the level of newly synthesized GABA. In experiments with synaptosomes as in the present study, the marked effect of GABA-T inhibition of newly taken up GABA may thus mask alterations in the functional more relevant pool of GABA in nerve terminals. In other words, 3-MP could decrease the amount of GABA to be released despite synaptosomal GABA levels appear to be elevated by GAG. In this respect, it should be noted, however, that GAG not only inhibits GABA-T but also causes (less marked) inhibition of GAD [12], which may be relevant for experiments with 3-MP. Nevertheless, other inhibitors of GABA-T, such as γ-vinyl GABA (GVG) and ethanolamine-O-sulfate, which even at very high doses do not inhibit GAD, have also been reported as being ineffective against seizures induced by 3-MP [20, 30]. In contrast, aminooxyacetic acid at doses which do not inhibit GAD *in vivo* [12] has recently been shown to attenuate 3-MP induced seizures in rats, but this effect may be unrelated to the GABA elevating action of this GABA-T inhibitor [18]. At toxic doses, both AOAA and GAG themselves cause seizures, which seem to be the consequence of marked inhibition of GAD [9, 31, 32].

Although further experiments with other inhibitors of GABA-T are necessary before definite conclusions can be drawn, the present data on GAG using 3-MP as a tool to induce GABA impairment may indicate that GABA-T inhibitors are no candidate drugs for diseases which involve impairment of GABA-mediated neurotransmission caused by reduction of GAD activity. In fact, GAG and GVG both were found to be ineffective in patients with Huntington's disease, which is known to be associated with decreased GAD activity in certain brain regions [30]. On the other hand, however, recent clinical reports indicate that high doses of GVG may be an efficacious treatment for certain types of epilepsy [30]. In any event, with respect to the different pharmacological possibilities to enhance GABA mediated neurotransmission, one may assume that GABA-mimetic drugs which directly increase synaptic GABA concentrations by inhibition of GABA uptake from the synaptic cleft, or act as GABA agonists at GABA receptors are more effective than GABA-T inhibitors in restoring normal GABA function in diseases in which a deficiency in GABA has been implicated [33, 34].

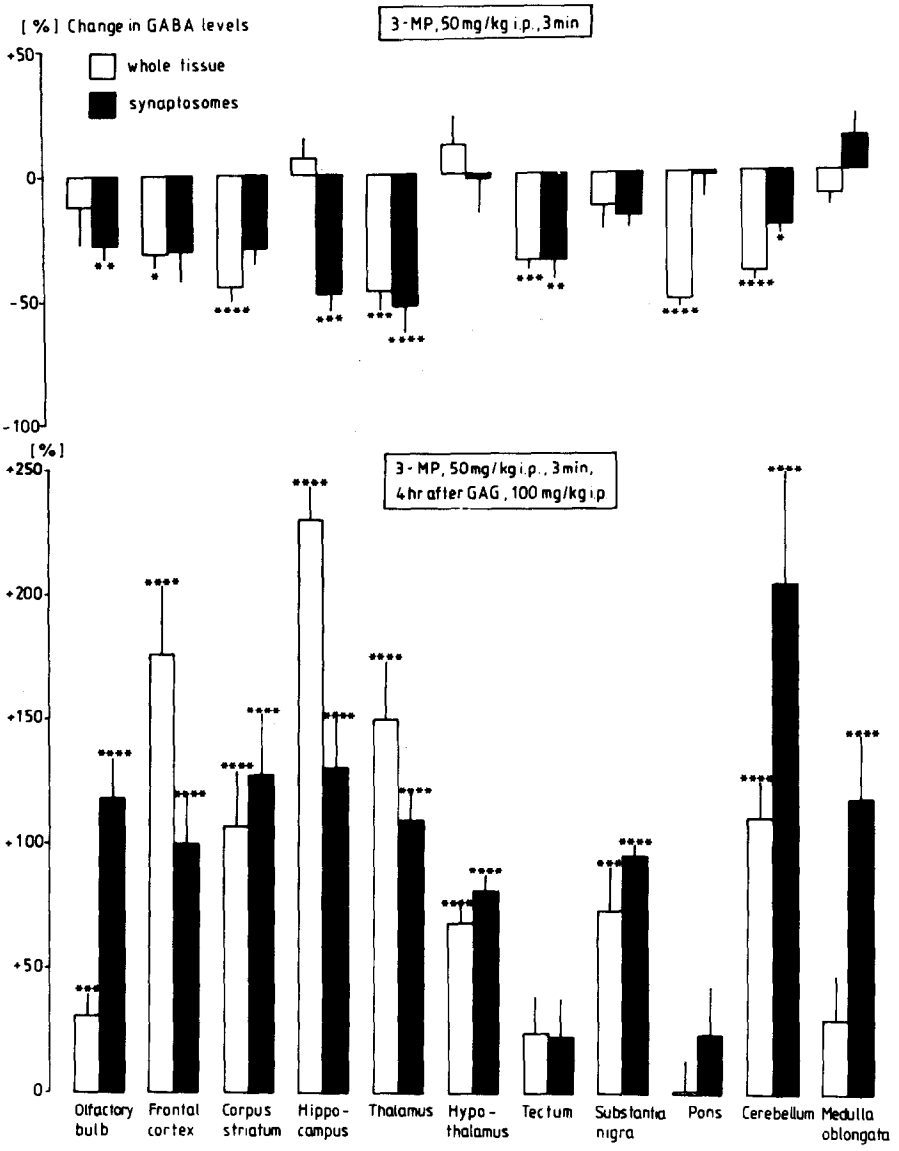

Fig. 2. Effect of 3-MP (50 mg/kg i.p. 3 min before decapitation) alone and after pretreatment with GAG (100 mg/kg i.p., 4 hr before 3-MP) on GABA levels in whole tissue and synaptosomal fractions of 11 rat brain regions. Results are expressed as percentage changes (mean ± SE) from concurrent control values; 5 animals were used per group. The significance of differences from the individual control groups is indicated by asterisks *P < 0.05; **P < 0.02; ***P < 0.01; ****P < 0.001). Control values for GABA in whole tissue and synaptosomal fractions (in brackets) of the respective regions in nmol/mg of protein (means ± SE of 10 rats) were: olfactory bulb, 23.7 ± 2.5 (16.2 ± 1.3); frontal cortex, 10.8 ± 1.3 (12.2 ± 2.8); corpus striatum, 17.6 ± 1.8 (17.9 ± 3.2); hippocampus, 11.3 ± 1.6 (13.0 ± 1.8); thalamus, 17.3 ± 3.1 (18.7 ± 2.6); hypothalamus, 20.0 ± 1.9 (20.7 ± 2.8); tectum, 20.1 ± 1.3 (20.7 ± 2.8); substantia nigra, 16.8 ± 2.5 (23.3 ± 3.1); pons, 12.9 ± 1.5 (17.6 ± 2.0); cerebellum, 9.4 ± 1.1 (18.0 ± 2.3); and medulla oblongata, 8.7 ± 0.9 (22.5 ± 2.9).

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REFERENCES

1. B. Meldrum, in *GABA-Neurotransmitters* (Eds. P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod), pp. 390–405. Munksgaard, Copenhagen (1979).
2. W. Löscher, in *Epilepsy and GABA Receptor Agonists. Basic and Therapeutic Research* (Eds. G. Bartholini, L. Bossi, K. G. Lloyd and P. L. Morselli), pp. 109–119. Raven Press, New York (1985).
3. R. W. Olsen, A. M. Snowman, R. Lee, P. Lomax and J. K. Wamsley, *Ann. Neurol.* **16** (suppl.), S90 (1984).

4. W. Löscher and D. Schwartz-Porsche, *J. Neurochem.* **46**, 1322 (1986).
5. W. Löscher and W. S. Schwark, *Brain Res.* **339**, 146 (1985).
6. K. G. Lloyd, C. Munari, L. Bossi, C. Stoeffels, J. Talairach and P. L. Morselli, in *Neurotransmitters, Seizures, and Epilepsy* (Eds. P. L. Morselli, K. G. Lloyd, W. Löscher, B. Meldrum and E. H. Reynolds), pp. 325–334. Raven Press, New York (1981).
7. W. Löscher and H. Siemes, *Epilepsia* **26**, 314 (1985).
8. J. H. Wood, T. A. Hare, B. S. Glaeser, J. C. Ballenger and R. M. Post, *Neurology* **29**, 1203 (1979).
9. W. Löscher, in *Neurotransmitters, Seizures, and Epilepsy* (Eds. P. L. Morselli, K. G. Lloyd, W. Löscher, B. Meldrum and E. H. Reynolds), pp. 93–102. Raven Press, New York (1981).
10. M. G. Palfreyman, P. J. Schechter, W. R. Buckett, G. P. Tell and J. Koch-Weser, *Biochem. Pharmac.* **30**, 817 (1981).
11. M. Abe, *J. Nutr. Sci. Vitaminol.* **24**, 419 (1978).
12. W. Löscher, *J. Neurochem.* **36**, 1521 (1981).
13. W. Löscher, M. Vetter, F. Müller, G. Böhme and G. Stoltenburg-Didinger, *Neurochem. Int.* **6**, 441 (1984).
14. S. Sarhan and N. Seiler, *J. Neurosci. Res.* **4**, 399 (1979).
15. J. D. Wood, E. Kurylo and J. D. Newstead, *Can. J. Biochem.* **56**, 667 (1978).
16. J. D. Wood, M. P. Russell and E. Kurylo, *J. Neurochem.* **35**, 125 (1980)
17. M. J. Iadarola and K. Gale, *Molec. Cell. Biochem.* **39**, 305 (1981).
18. W. Löscher and M. Vetter, *Biochem. Pharmac.* **34**, 1747 (1985).
19. M. Abe and M. Matsuda, *Neurochem. Res.* **8**, 563 (1983).
20. W. Löscher, W., *Naunyn-Schmiedeberg's Archs Pharmac.* **315**, 119 (1980).
21. A. Karlson, F. Fonnum, D. Malthe-Sørenssen and J. Storm-Mathisen, *Biochem. Pharmac.* **23**, 3035 (1974).
22. W. Löscher, *Biochem. Pharmac.* **28**, 1397 (1979).
23. W. Löscher, G. Böhme, F. Müller and S. Pagliusi, *J. Neurochem.* **45**, 879 (1985).
24. G. Rodriguez de Lores Arnaiz, M. Alberici di Canal and E. De Robertis, *J. Neurochem.* **19**, 1379 (1972).
25. G. Rodriguez de Lores Arnaiz, M. Alberici di Canal, M. Robiolo and M. Mistrorigo De Pacheco, *J. Neurochem.* **21**, 615 (1973).
26. C. F. Baxter, in *Methods of Neurochemistry*, Vol. 3 (Ed. R. Fried), pp. 1–73. Marcel Dekker, New York (1972).
27. W. Löscher, *J. Neurochem.* **32**, 1587 (1979).
28. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
29. M. A. Markwell, S. M. Haas, L. L. Bieber and N. E. Tolbert, *Analyt. Biochem.* **87**, 206 (1978).
30. E. J. Hammond and B. J. Wilder, *Gen. Pharmac.* **16**, 441 (1985).
31. R. Tapia, H. Pasantes, M. P. de la Mora, B. G. Ortega and G. H. Massieu, *Biochem. Pharmac.* **16**, 483 (1967).
32. J. D. Wood and S. J. Peesker, *J. Neurochem.* **20**, 379 (1973).
33. G. Bartholini, *Med. Res. Rev.* **5**, 55 (1985).
34. W. Löscher, *Drugs Fut.* **11**, 39 (1986).

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Effect of dietary ascorbate on covalent binding of benzene to bone marrow and hepatic tissue *in vivo**

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Benzene produces aplastic anemia in humans and is a human leukemogen [1, 2]. The biochemical mechanics whereby benzene produces these effects are presently unknown. However, several reports have demonstrated a relationship between covalent binding in the bone marrow, the target site, and benzene-induced blood dyscrasia in laboratory animals [3, 4]. Toxicity appears to be the result of the metabolic activation of benzene [5, 6]. Ascorbate has been shown to be effective in preventing the *in vitro* covalent binding of the metabolites of benzene and phenol, the major metabolite of benzene [7–9]. We have demonstrated previously that, when phenol and hydrogen peroxide are incubated with bone marrow preparations isolated from guinea pigs with low ascorbate tissue concentration, there is a 4-fold increase in *in vitro* covalent binding of phenol equivalents to bone marrow tissue when compared to guinea pigs on a high ascorbate intake [9]. Ascorbate cannot be synthesized in the guinea pig or human and must be procured through dietary intake, and the concentrations of ascorbate in tissue can vary widely from individual to individual due to their respective dietary intake [10]. In the present communication, we have investigated the effect of ascorbate on covalent binding of [^{14}C]benzene metabolites *in vivo*. To evaluate the effect of ascorbate on covalent binding *in vivo*, guinea pigs were placed on different dietary intakes of ascorbate followed by i.p. administration of [^{14}C]benzene.

Covalent binding was inversely related to dietary ascorbate intake and to the concentration of ascorbate in both liver and bone marrow. Covalent binding was altered by 2- and 1.4-fold in the liver and bone marrow respectively. The concentration of ascorbate in bone marrow and hepatic tissue ranged from 0.12 to 2.63 μmoles/g tissue depending upon the dietary intake.

Three groups of four male Hartley guinea pigs (200–250 g) were placed on the following dietary intakes of ascorbate: 2.0, 0.35 and 0.05 mg ascorbate/ml drinking water for 4 weeks prior to the i.p. administration of benzene. All guinea pigs received ascorbate-deficient guinea pig chow *ad lib.* (ICN Nutritional Biochemicals, Cleveland, OH). The growth rate for each group of guinea pigs was not significantly different. The guinea pigs on the lowest intake showed no signs of a scorbutic condition. After 4 weeks on the diets, each guinea pig was injected with 660 mg/kg [^{14}C]benzene intraperitoneally twice, 12 and 6 hr before termination. The specific activity of [^{14}C]benzene was either 52.7 or 15.2 μCi/μol. [^{14}C]Benzene was injected in 0.5 ml corn oil. Six hours after the second injection the guinea pigs were terminated by decapitation. Each liver was removed, and a 20% homogenate was made with 100 mM sodium phosphate buffer, pH 7.4. Femurs were removed from each guinea pig, and the bone marrow was scraped out of the femoral cavity. Each marrow sample was weighed and a 10% homogenate was prepared. Hepatic homogenate (250 μl) and bone marrow homogenate (1.2 ml) were extracted, and the covalently bound benzene equivalents in these tissues were determined by the method

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