substances are also poorly extracted by the immature kidney[10,11] and in diseases of the nephron[6]. Thus, a variety of conditions exist which may be associated with errors in the calculation of extraction fraction.

As might be expected, variations in filtration fraction will affect the error in calculation of extraction fraction. Reduction of filtration fraction tends to reduce error of calculated extraction fraction because urine flow is a lower fraction of renal arterial flow.

Wolf[5] originally suggested that a correction for urine flow was necessary for calculation of renal plasma flow. His calculations show a systematic error ranging from 4 to 14% depending on urine flow. Somewhat similar errors (table) are expected for the calculation of extraction fraction, depending on urine flow and extent of solute extraction. The following calculation shows the error incurred when correction for urine flow is not made in the calculation of extraction fraction.

In the newborn dog, RPF = 1.0 ml/min/g kidney weight, GFR = 0.20 ml/min/g kidney weight, and E_x = 0.40[4]. As reported in the literature F.E. water may be 50% in certain experimental conditions in this species[9,12]. Thus, $0.20 \times 0.5 = 0.10$ ml/min/g kidney weight. Ru/Ra = 0.10/1.0 = 10%. From the table, E_x is actually 0.46 not 0.40, an error of 15%. This error will become greater as urine flow increases and solute extraction is reduced[13].

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Distribution of ^{14}C after topical application of ^{14}C-labeled 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) in mice

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Summary. Following topical application of ^{14}C-labeled 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine) to the skin of mice radioactivity was found in all viscera and tissues examined. Exclusive of the gut, highest values were recorded for the liver, kidney and lung.

Topically applied 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine) is an effective treatment for mycosis fungoides[2]. This paper reports the pattern of organ and tissue distribution of ^{14}C following the topical application of ^{14}C-labeled BCNU to mouse skin. Ethyl labeled ^{14}C-BCNU was supplied by Wm. H. Yanko, Monsanto Research Corp., at the request of Dr Vincent T. Oliverio, National Cancer Institute. The specific activity of the sample was 10.07 mCi/mmole (47.06 μCi/mg) with a radiochemical purity of 98.5%. The dorsal neck region of 7 female Swiss mice, weight 32–43 g, was shaved with an electric clippers 1 day prior to the application. 100 μl of ^{14}C-BCNU in methanol, 0.1062 mg (5 μCi), was applied to a 5 cm^2 shaved area and allowed to dry for 2 min. As a control for possible ingestion of the compound, 1 mouse was given 0.0212 mg (1 μCi) of ^{14}C-BCNU in propylene glycol s.c. and sacrificed after 6 h. The mice were placed in individual holding cages. Food and water were allowed ad libitum.

The mice were sacrificed at 1, 2, 3, 5, 6, 18 and 24 h by cervical dislocation. Carcass weights were determined, and the animals were carefully dissected. The lung, heart, liver, kidneys, spleen, brain, gut (entire gastro-intestinal tract) and pieces of muscle and fat were analyzed. After removal each tissue was immediately put in a separate, pre-weighed glass counting vial. Using a 7 ml Ten Broeck tissue homogenizer each sample was homogenized with 10–15 ml distilled water. For analysis of the ^{14}C content a published method was used[3]. This consisted of wet ashing a 2 ml sample of the homogenate with 2% potassium dichromate in concentrated sulfuric acid, and trapping the evolved $^{14}CO_2$ with ethanolamine. This was added to a scintillator fluid and counted with appropriate standards in a Beckman liquid scintillation counter. Results were expressed in μg of BCNU per g of organ (wet weight), utilizing a computer program. BCNU is rapidly degraded following i.v. or oral administration[5]. Hence the ^{14}C activity most likely represents fragments or metabolic products of the applied compound.

The table states the BCNU equivalents in mouse organs and tissues, expressed in μg/g wet weight, after a single application of 106.2 μg ^{14}C-BCNU to the shaved skin, and

Distribution of ^{14}C after application of ^{14}C-BCNU to mice[a]

	Topical application of 106.2 μg							s.c. (21.2 μg)
	1 h	2 h	3 h	5 h	6 h	18 h	24 h	6 h
Liver	3.72	2.74	2.39	1.50	0.27	0.22	0.20	0.30
Kidney	3.13	3.11	1.85	1.14	0.34	0.31	0.32	0.47
Lung	1.60	1.45	1.08	0.70	0.21	0.18	0.17	0.27
Heart	1.15	0.95	0.54	0.39	0.11	0.12	0.14	0.14
Spleen	1.08	1.43	0.94	0.70	0.03	0.19	0.19	0.05
Brain	0.53	0.37	0.36	0.31	0.11	0.11	0.10	0.13
Gut[b]	3.94	6.19[c]	4.33	1.42	0.37[c]	0.13	0.36	0.46
Feces	3.95	–	4.33	3.06	–	0.22	0.63	1.96
Muscle	0.70	0.56	0.34	0.21	0.06	0.05	0.07	0.22
Fat	0.41	0.16	0.27	0.17	0.14	0.03	0.08	0.17

[a] Expressed as μg BCNU-equivalent per g organ or tissue. [b] Entire gastro-intestinal tract. [c] Includes contents.

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after a s.c. injection of 21.2 μg ^{14}C-BCNU. The relative organ distribution following s.c. injection was in accord with that following topical application, suggesting that oral ingestion was not a factor in the results.

Activities were found in all tissues examined indicating facile percutaneous penetration. Interpretation of data for the gut is difficult due to the likelihood of fecal contamination. Exclusive of the gut highest values were found in the liver, kidney and lung. High values for liver and kidney in mice were also found by Wheeler et al.[4] and De Vita et al.[5] following i.p. injection. We noted a sharp drop in values for all viscera between 2 and 6 h after topical application as did Wheeler et al. following i.p. injection[4]. Activity in the brain again demonstrates the ability of BCNU or its products to cross the blood-brain barrier[5].

Mouse skin is thinner and more hirsute than human skin. Using in vitro chambers, Marzuli et al.[6] found mouse skin to be many fold more permeable than human skin. Extrapolation of the present data to man must take this enhanced permeability of mouse skin into consideration. Percutaneous penetration of BCNU in man is the subject of another study in progress by the authors.

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Analgesic effects of 3-carboxysalsolinol alone and in combination with morphine

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Summary. A biphasic dose-response pattern is generated by the isoquinoline, 3-carboxysalsolinol, in analgesia tests conducted in mice. Carbidopa pretreatment enhances this effect, as well as the morphine-induced analgesic increase by 3-carboxysalsolinol. Naloxone blockade of all of these responses suggests an interaction of the alcohol-based isoquinoline with central opiate receptors.

Ross and his colleagues have shown that morphine (M), ethanol (ETOH) and salsolinol (SAL) can diminish calcium levels in brain regions. This action can be suppressed by the narcotic antagonist naloxone (N)[2]. SAL is a tetrahydroisoquinoline (TIQ) derived from condensation of the primary metabolite of ETOH, acetaldehyde, with dopamine[3–5]. We have reported that SAL and the related 3-carboxysalsolinol (3cSAL), derived from acetaldehyde and L-DOPA prolong ETOH-induced narcosis in mice, with 3cSAL being more potent by far[5]. The suggested opiate-related action of SAL in the calcium experiments together with the enhanced ETOH-based narcosis observations have led us to examine 3cSAL for in vivo effects associated with opiates. Accordingly, 3cSAL was given to mice, alone and with M, and analgesia was assessed. Parallel studies were conducted with equimolar quantities of the 3cSAL precursor, L-DOPA, for comparison.

Materials and methods. Analgesia was measured by a modification of Haffner's tailclip method[6,7]. Male, Swiss-Webster mice (20–25 g) received i.p. injections (15 μl/g) of 3cSAL, L-DOPA or M (hydrochloride) or combinations of 3cSAL or L-DOPA and M. All drug solutions were prepared in acidic saline (1 drop of concentrated HCl per 5 ml of saline). The use of acidic saline assisted dissolution of the amino-acid compounds. Studies were performed on mice that also received carbidopa (CD), given orally (120 μM/kg) 1 h prior to injections of other substances. The actions of N (1 mg/kg) on drug-induced analgesia was determined by administering the narcotic antagonist i.p. just prior to injecting the other drugs. Analgesia tests were conducted 30 min after injections of M and/or the amino-acid compounds, or acidic saline. The results were analyzed statistically by determinations of differences between sample proportions[8].

Results and discussion. The levels of analgesia produced by 3cSAL, by 3cSAL after administration of CD and by L-DOPA are shown in figure 1. It is evident that 3cSAL produces a dose-related biphasic pattern of analgesia with the optimal level (54%) occurring in mice receiving a dose of 200 μM/kg. A similar pattern of analgesia was produced when 3cSAL was administered to animals pretreated with the peripheral inhibitor of L-amino-acid decarboxylase, carbidopa[9]. Inhibition of this enzyme potentiated the 3cSAL effect about 50fold. In the presence of CD, optimal analgesia (40%) was obtained with a 4 μM/kg dose of 3cSAL. CD on its own produced no evidence of analgesic activity.

L-DOPA afforded a low but increasing level of analgesic activity. A dose of 200 μM/kg of this amino acid gave a 30% level of analgesia which is significantly lower ($p < 0.03$) than that produced by the equimolar quantity of its isoquinoline analogue. Although a possible biphasic pattern of L-DOPA-induced analgesia was not pursued in this study it may exist as Major and Pleuvry have shown that a much higher dose of L-DOPA attenuates analgesia[10].

Morphine-induced analgesia is illustrated in figure 2. As expected, increased levels of analgesia occurred with higher doses. Combined treatments of M and 3cSAL

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