Cancer Chemother Pharmacol (1988) 22: 308–315

Cancer Chemotherapy and Pharmacology

Autoradiographic distribution of [^{14}C]-labelled pimonidazole in rhabdomyosarcoma-bearing rats and pigmented mice

François Laurent[1], Patrick Benard[2], Pierre Canal[3], and Georges Soula[1, 3]

[1] Laboratoire de Biochimie Appliquée de la Faculté des Sciences Pharmaceutiques – 35 chemin des Maraîchers, F-31062 Toulouse Cedex, France
[2] Laboratoire des Radioéléments et d'études métaboliques, Ecole Nationale Vétérinaire – I.N.R.A., 23 chemin des Capelles, F-31076 Toulouse, Cedex, France
[3] Groupe de Recherches Carcinologiques, Centre Claudius Regaud, 20, 24 rue du Pont Saint-Pierre, F-31052 Toulouse Cedex, France

Summary. The hypoxic cell radiosensitizer [2-^{14}C] pimonidazole (2-nitro-α-(piperidinomethyl)-1-imidazole ethanol) was injected i.p. into pigmented mice and rats bearing transplanted rhabdomyosarcoma. The injected dose level was 200 mg/kg, and the delivered activity was 96 μCi/kg. Whole-body autoradiography was carried out on all animals. We noted an extensive whole-body distribution of radioactivity. At short intervals, the autoradiograms were characterized by an accumulation of radioactivity in the metabolic and excretory organs (liver, kidney, urinary tract, and intestinal content) as well as in lymphomyeloid tissues (thyroid gland, suprarenal gland, and hypophysis) and salivary glands. In pigmented mice, the uveal and biliary tracts were the highest labelled. The liver and particularly the renal medulla were identified as sites of retention of radioactivity. In the tumor the radioactivity was detected only in peripheral regions, with higher uptake in viable zones than in necrotic islets.

Introduction

Over the past few years, a number of substituted 2-nitroimidazoles have been developed as radiosensitizers for the treatment of cancer [16]. Pimonidazole (Ro 03-8799) (2-nitro-α-(piperidinomethyl)-1-imidazole ethanol) is a potent, basic, hypoxic cell radiosensitizer [13] currently undergoing clinical trials [4]. It has shown a high distribution volume and accumulation in tissues including tumors [14]. When compared with misonidazole (MISO) – the first 2-nitro-imidazole studied as a radiosensitizer [14] – pimonidazole has been shown to be up to 2–3 times as effective as MISO [6].

This paper describes the distribution of [^{14}C] pimonidazole by whole-body autoradiography in rhabdomyosarcoma-bearing rats and pigmented mice. The results permit the localization of radioactivity within organs and the determination of the route of elimination; they are compared with those previously obtained with MISO [1].

Offprint requests to: G. Soula, Laboratoire de Biochimie, Centre Claudius Regaud 20, 24, rue du Pont Saint-Pierre, F-31052 Toulouse Cedex, France

Abbreviations used: MISO, misonidazole; [^{14}C] ou [^{14}C] Ro, [^{14}C] Ro 03-8799 plus Ro 03-8799-derived [^{14}C]-labelled metabolites

Materials and methods

Chemical. [2-^{14}C] Pimonidazole (specific activity, 0.48 μCi/mg) was obtained from Roche Laboratories (Welwyn Garden City, England). The drug was dissolved in a 0.9% NaCl solution to give a concentration of 10 mg/ml.

Before its use, the radiochemical purity of the labelled compound was determined by thin-layer chromatography in a chloroform/ethanol/20% ammonia solution mixture (85:15:1 v/v); it was found to be 98%.

Animals. All conventional animals were caged separately and fed ad lib. They were given [2-^{14}C] Ro 03-8799 i.p. at 0.200 g/g body weight, and the delivered activity was 96 μCi/kg.

Mice: The study was carried out on male C_{57} mice (supplied by C.E.R.J., France) weighing 20–30 g, which were sacrified 5, 15, and 30 min and 1, 2, 8, 12, and 24 h after treatment.

Rats: Male WAG rhabdomyosarcoma-bearing rats (supplied by Centre d'Elevage, C.N.R.S., Orléans, France) weighing 200–250 g were used. Solid tumors were produced by s.c. injection in the flank region of a cell suspension (0.3 ml at 10^7 cells/ml) obtained from a rhabdomyosarcoma induced by Ni_3S_2 [17].

Autoradiography. All animals were prepared for whole-body autoradiography [18]. First, they were frozen by rapid immersion in a freezing medium composed of methanol and dry ice. They were then embedded in a 10% aqueous solution of carboxymethylcellulose and frozen such that a film support formed all around the carcass. The frozen animals were cut sagittally until an appropriate section appeared. Whole-body sagittal sections were then obtained with either a Leitz 1300 k cryomicrotome or a PMV 450 semiautomatic microtome. Before each section was collected, adhesive tape was fixed to the surface of the animal to be sectioned. The microtome stage was then moved and a 20-μm-thick section was obtained on the adhesive tape. After the sections had been collected, they were dried by storage overnight at −20° C in a cryostat. The dried sections were then pressed directly against a single-coated X-ray film (Kodak Kodirex) in a light-tight bag and stored at −30° C. At the end of a 1-month exposure period, the section and the film were separated. The former was

stained when necessary with Mayer's hematoxylin for histological examination; the latter was developed, fixed, and rinsed. All of these operations were carried out in well-defined, identical conditions to produce autoradiograms that could be compared.

Sections were also obtained from animals injected with unlabelled pimonidazole. These control sections were prepared by the same procedure and pressed against Kodak Kodirex X-ray film to verify that no positive or negative chemography occurred with this film under the conditions of our experiments.

Histological determinations of different tissues and of viable or necrotic tumor areas were done under an optic microscope by an examination of the section stained with Mayer's hematoxylin.

Results

The tissue distribution patterns of radioactivity were similar in both animal species. Unless otherwise stated, the distribution pattern is described for the rat.

[^{14}C] was rapidly absorbed from the peritoneal cavity and widely distributed throughout the body. A degree of tissue localization, probably reflecting the metabolic and excretory pathways, was observed in all animals. In addition, radioactivity was registered in tissues belonging to the lymphomyeloid and endocrinal or reproductive systems as well as in bone, the central nervous system, pigmented tissues, and the rhabdomyosarcoma.

Blood

The radioactivity appeared in the bloodstream after 5 min (Fig. 1 A) but was relatively weak at all times.

Metabolic excretory organs

The concentration of the radioactivity in the liver reached a high level immediately after the injection (Fig. 1 A). Radioactivity in the liver remained high for up to 4 h (Fig. 1 E) and could still be detected 2 days after injection (Fig. 1 H). After 2 h (Fig. 1 D), particularly in the liver, [^{14}C] appeared in a mottled pattern, probably reflecting a hepatic parenchymal distribution, as the conjunctive tissue was not labelled.

In mice (Fig. 2), the bile duct was strongly labelled after 5 min (Fig. 2 A) and the radioactive intensity was still increasing 8 h after injection (Fig. 2 F).

Similarly, the content of the gastrointestinal tract was very strongly labelled (Fig. 1), and even after 48 h (Fig. 1 H) the radioactivity was high. In the intestinal mucosa (Fig. 1), the activity was almost of the same order of magnitude as in the liver. A short time after the injection, a very high level of radioactivity also appeared in the kidney and urinary tract. A high concentration was registered up to at least 2 h (Fig. 1 D) and remained in the renal medulla up to 24 h (Fig. 1 G).

Lymphoid system

The thymus accumulated a relatively high amount of radioactivity at short intervals (5 min to 1 h) (Fig. 3 E). The spleen was also identified as an organ that accumulated a relatively high level of isotope at the same intervals (Fig. 3 C). No preferential localization was noticed within these tissues.

Endocrinal and reproductive tissue

A rather high uptake of radioactivity was observed in endocrinal tissues. The most accentuated uptake was noticed in the thyroid gland (Fig. 3 A), and the radioactivity was still visible up to 16 h after injection (Fig. 1 F). In the hypophysis (Fig. 3 B) and adrenal cortex (Fig. 3 B), the radioactivity was slightly weaker than in the thyroid. Although the activity in the adrenal medulla was weaker than in the cortex 30 min after injection (Fig. 3 G), after 4 h the radioactivity was similar in both parts of the gland (Fig. 4 E).

The level of uptake in the reproductive tract was high, particularly in the epididymis, at short intervals (30 min to 1 h) (Figs. 1 B, C). The testicular interstitial tissue was radiolabelled but the level was lower than in the epididymis. In contrast, the radioactivity was more persistent in the testis (Fig. 1 F).

Central nervous system (CNS)

The labelled isotope rapidly penetrated the blood-brain barrier. The radioactivity was greater in the brain than in the blood, and the [^{14}C] accumulation was higher in the gray matter (Fig. 1 B), particularly within the cerebellum (Fig. 3 B 2).

Pigmented tissues

Whereas no accumulation was found in the eyes of albinos, in pigmented mice the uptake in the uveal tract was very high, exceeding that of any other tissue at any time (Fig. 4), except the biliary tract. This radioactivity did not seem to persist after 12 h. Similar but lower accumulation was also seen in Harder's glands. On the other hand, the isotope was present in the hair follicles of pigmented mice.

Other tissues

A weak retention of radioactivity in the lung, muscle, and bone marrow was seen, but this had almost disappeared after 24 h. Nevertheless, this activity was always higher than that in the blood. On the other hand, the broncheal tissue (Fig. 3 D) and the salivary gland were strongly labelled (Fig. 3 B).

Rhabdomyosarcoma tissue

The radioactivity penetrated very quickly into the tumors, but the distribution was heterogenous (Fig. 5). The regional distribution depended on the histology.

In the core region, the radioactivity was detected only after 4 h (Fig. 5 D). This area was necrotic and sometimes cystic. In the presence of cysts no radioactivity was detected.

In the peripheral region, the distribution of the radioactivity was related to cellular heterogeneity. Histological examination showed viable cells surrounding necrotic islets. The radioactivity was higher in viable areas and more concentrated than in muscle. In necrotic regions the level of radioactivity was lower than in the viable regions.

Discussion

The results described above show the rapid and widespread distribution of the radioactivity originating from [^{14}C] pimonidazole. As early as 5 min after the injection,

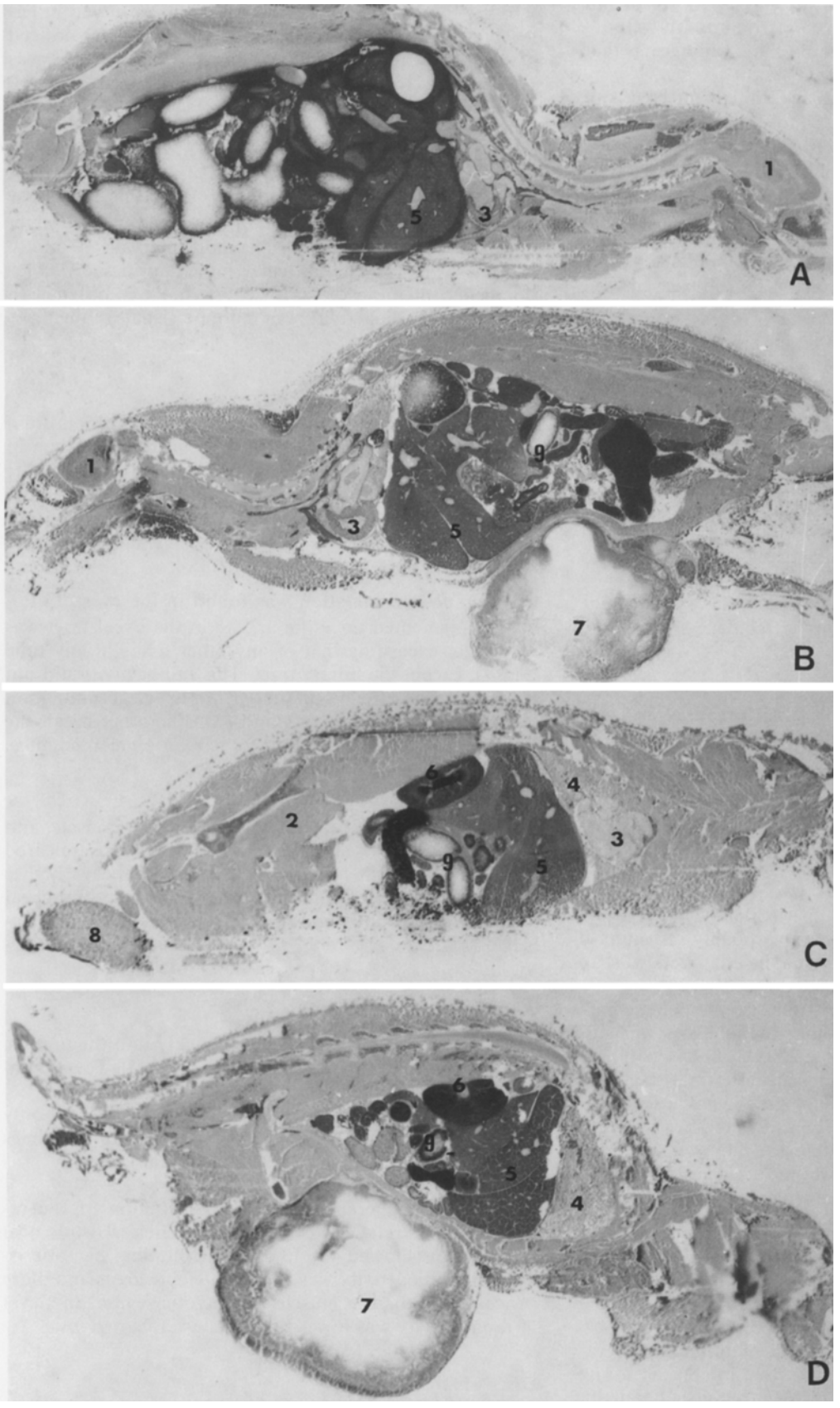
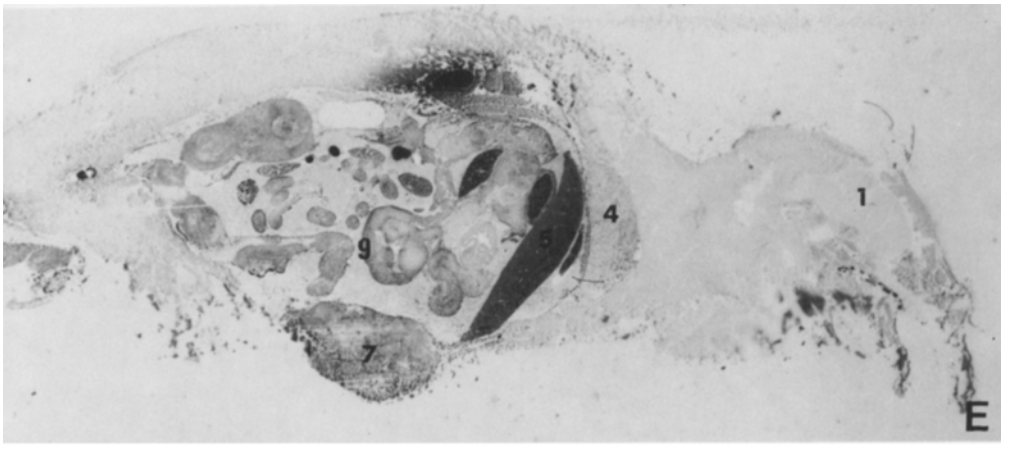
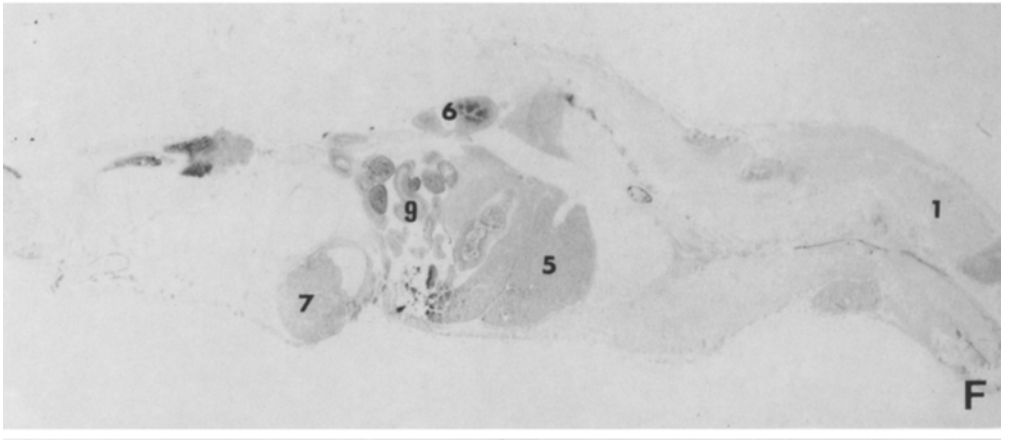
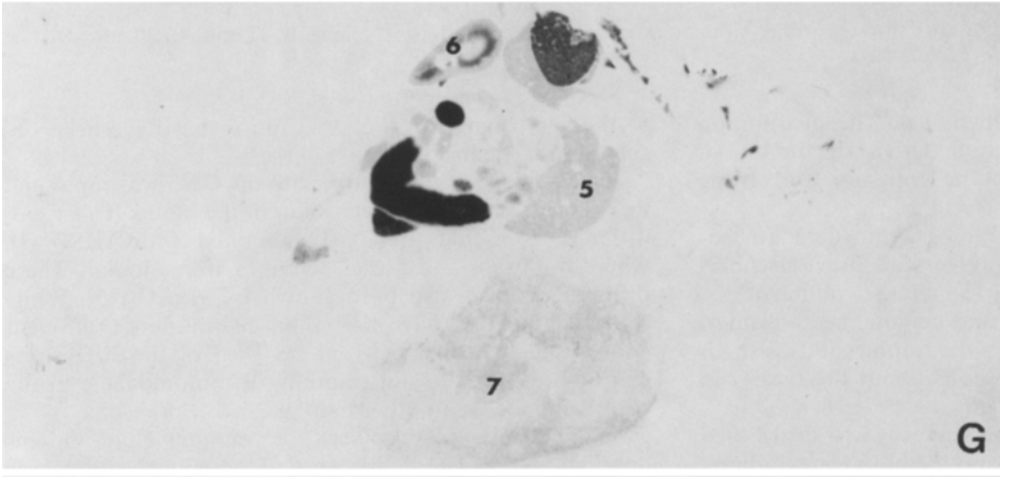
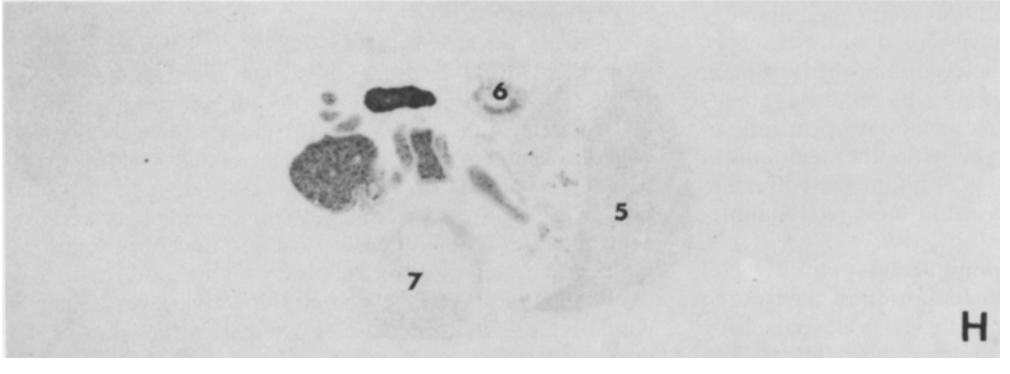


Fig. 1. Whole-body autoradiograms of rats bearing rhabdomyosarcoma tumor injected with [^{14}C] pimonidazole and sacrificed at: **A**, 5 min; **B**, 30 min; **C**, 1 h; **D**, 2 h; **E**, 4 h; **F**, 16 h; **G**, 24 h; **H**, 48 h. *1*, brain; *2*, muscle; *3*, heart; *4*, lung; *5*, liver; *6*, kidney; *7*, tumor; *8*, testis; *9*, intestinal mucosa


1
4
9
7
E


6
9
1
5
7
F


6
5
7
G


6
5
7
H

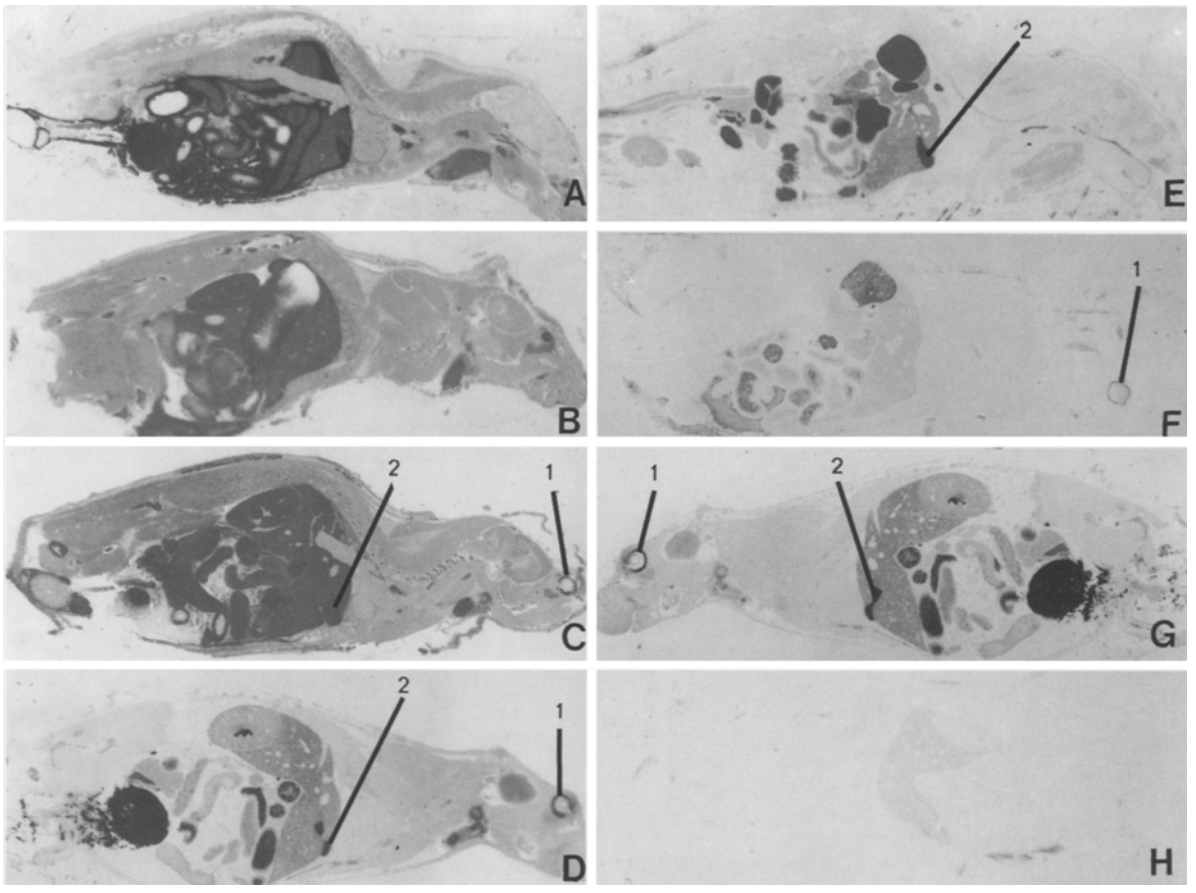


Fig. 2. Whole-body autoradiograms of mice injected with [^{14}C] pimonidazole and sacrificed at: **A,** 5 min; **B,** 15 min; **C,** 30 min; **D,** 1 h; **E,** 2 h; **F,** 8 h; **G,** 12 h; **H,** 24 h. *1*, eyes; *2*, bile duct

radioactivity was detected in all tissues. The distribution pattern showed a low level of radioactivity in the blood, extensive distribution of the isotope in tissues, and strong activity in the renal and biliary tracts.

Although whole-body autoradiographic data are entirely qualitative, these results agree with previous pharmacokinetic observations [2, 10, 15]: a large volume of distribution, high renal clearance, and hepatic metabolism to the N-oxide. However, the autoradiographic study also allows us to draw further conclusions about the tissues in which the radioisotope is retained.

In the rat kidney, the radioactivity was first distributed uniformly between the cortical and medulla zones, whereas marked radioactivity remained only in the medulla after 12 h. The same pattern has been observed with MISO [1], but only until 12 h. This probable fixation of the isotope in the medulla might be a sign of a possible nephrotoxic effect of Pimonidazole or one of its metabolites. Indeed, an identical pattern has been observed with other xenobiotics known to be nephrotoxic, such as ethionin [7]. Nevertheless, no durable radioactivity stayed in the mice renal medulla.

The basic character of pimonidazole (pka 8.71) [16] may perhaps explain the high concentration of the isotope in the stomach by ion trapping, as has been described for other basic compounds [8]. On the other hand, this basicity may also be related to a possible association with melanin [9]. We noticed an accumulation of radioactivity in the uveal tract and hair follicles. This finding might be related to the very high tumor/plasma ratios that have been observed in some melanomas [5, 11].

The passage of the isotope into the CNS was rapid and its concentration was greater than in the blood. This result differed from that previously obtained with MISO [1], where the brain and blood activities were similar. These differences have also been shown by HPLC [13]. While these compounds have a similar distribution coefficient (0.40 and 0.43, respectively, at pH 7.4) [16], the differences can be attributed to ion trapping of pimonidazole in the cerebral tissue, as in other tissues.

High uptake was also seen in lymphomyeloid and endocrinal tissues. The significance of these localizations was difficult to explain.

A wide intratumoral variation in [^{14}C] concentration was noted. In the core region, which corresponded to necrotic tissue with cyst fluid, limited or not radioactivity at all was detectable. Rich et al. [12] have shown that MISO concentration in the tumor was correlated inversely with the degree of necrosis. Blasberg et al. [3] have reported high MISO concentrations in the tumor periphery and low central values. The limited distribution in central tumor regions might be a result of relatively poor blood flow.

In the tumor periphery, the radioactivity was higher in viable regions than in muscle, whereas in the islets of necrotic cells the rate of radioactivity was similar to that in muscle. With MISO, the concentrations in the viable areas and in muscular tissue were of the same order, whereas the concentration in necrotic islets was much lower.

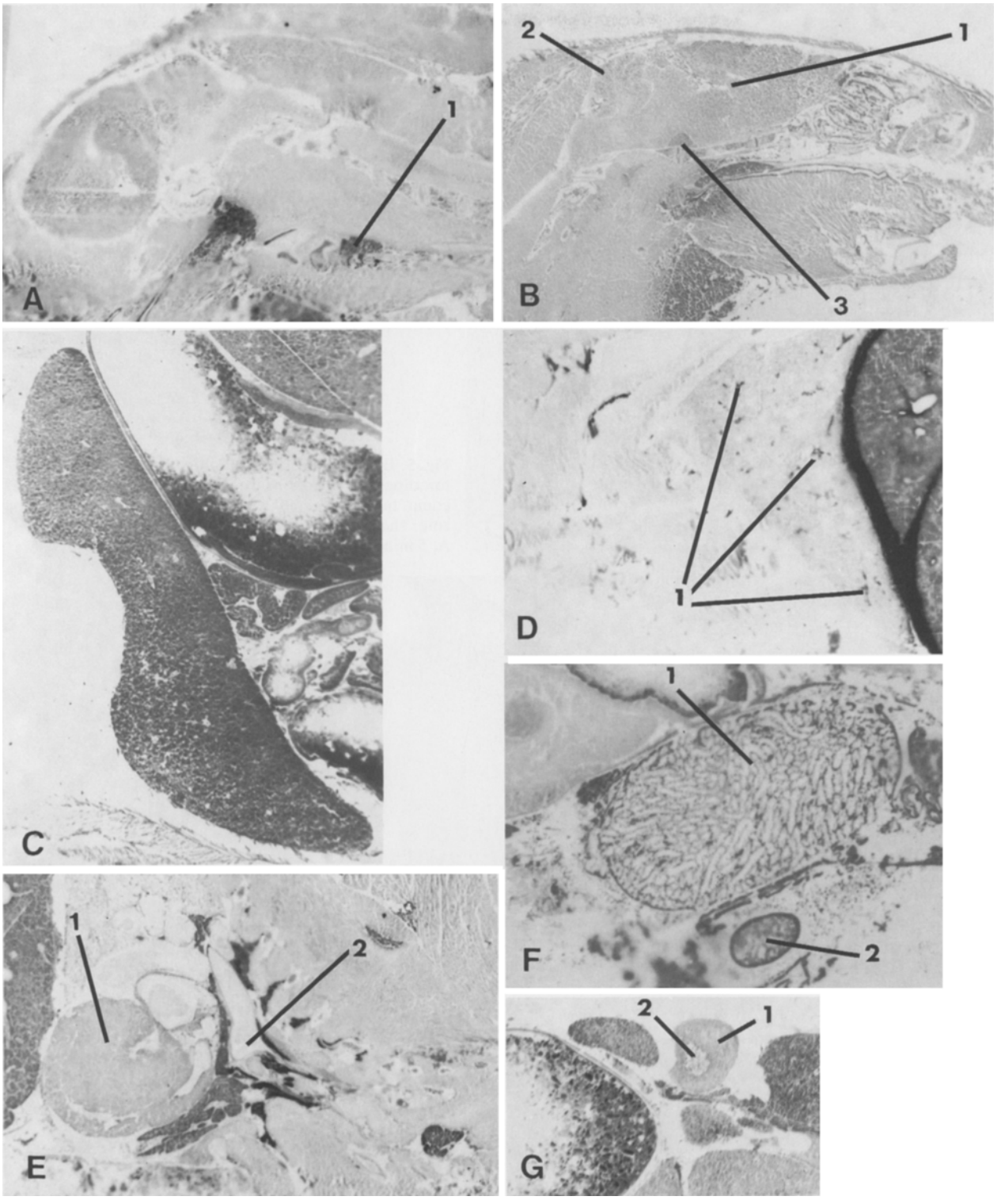


Fig. 3. Enlargement (× 3) of whole-body autoradiograms of rats bearing rhabdomyosarcoma tumor injected with [^{14}C] pimonidazole. **A,** thyroid gland (*1*) (30 min); **B,** brain (*1*), cerebellum (*2*), and hypophysis (*3*) (30 min); **C,** spleen (30 min); **D,** bronchi (*1*) (5 min); **E,** heart (*1*) and vessel (*2*) (30 min); **F,** testis (*1*) and epididymis (*2*) (30 min); **G,** adrenal gland: cortex (*1*) and medulla (*2*) (30 min)

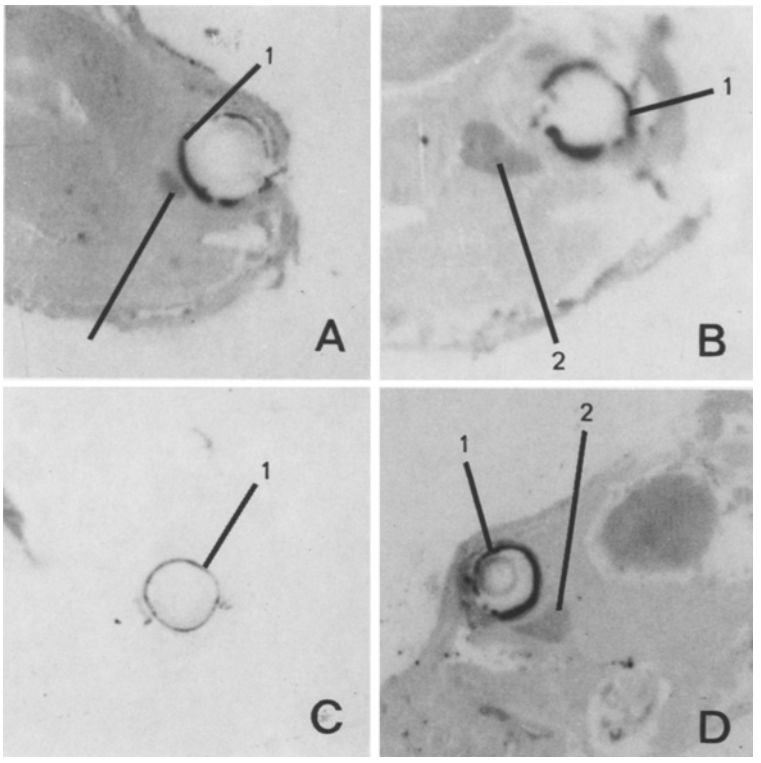
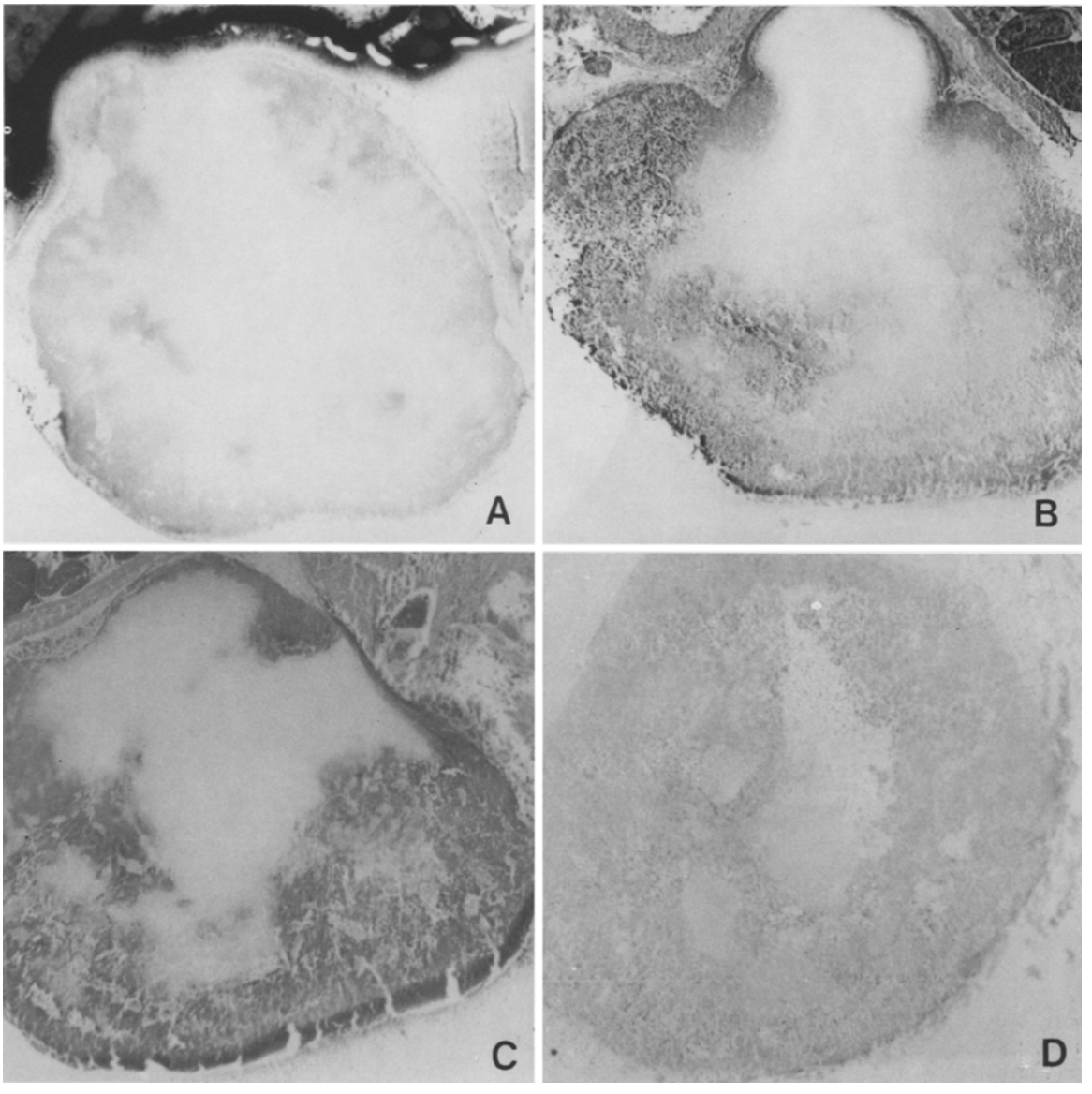


Fig. 4. Enlargement of whole-body autoradiograms of mice injected with [^{14}C] pimonidazole, demonstrating the uptake of the isotope in the uveal tract: **A,** 15 min; **B,** 30 min; **C,** 8 h; **D,** 12 h. *1,* uveal tract; *2,* Harder's glands

Fig. 5. Enlargement ($\times 3$) of whole-body autoradiograms of rats bearing rhabdomyosarcoma tumor injected with [^{14}C] pimonidazole, showing the uptake within the tumor. **A,** 5 min; **B,** 30 min; **C,** 2 h; **D,** 4 h



Acknowledgements. The rhabdomyosarcoma cell suspension was kindly provided by Prof. I. Chouroulinkov. The secretarial aid of Jeanine Leon is also appreciated.

References

1. Akel G, Benard P, Canal P, Soula G (1986) Distribution and tumor penetration properties of radiosensitizer [2-^{14}C] misonidazole (Ro 07-0582) in mice and rats as studied by whole-body autoradiography. Cancer Chemother Pharmacol 17: 121
2. Allen JG, Dische S, Lenox-Smith I, Malcolm SL, Saunders MI (1984) The pharmacokinetics of a new radiosensitizer, Ro 03-8799, in humans. Eur J Clin Pharmacol 27: 483
3. Blasberg R, Horowitz M, Strong J, Molmar P, Patlak C, Owens E, Fenstermacher J (1985) Regional measurements of [^{14}C] misonidazole distribution and blood flow in subcutaneous RT-9 experimental tumors. Cancer Res 45: 1692
4. Dische S, Saunders MI, Lee ME, Adams GE, Flockhart IR (1977) Clinical testing of the radiosensitizer Ro 07-0582: experience with multiple doses. Br J Cancer 35: 567
5. Dische S, Saunders MI, Dunphy EP, Bennett MH, Desrochers C, Stratford MRL, Minchinton AI, Orchard RA (1986a) Concentrations achieved in human tumors after administration of misonidazole, SR-2508 and Ro 03-8799. Int J Radiat Oncol Biol Phys 12: 1109
6. Dische S, Saunders MI, Bennett MH, Dunphy EP, Des Rochers C, Stratford MRL, Minchinton AI, Wardman P (1986b) A comparison of the tumour concentrations obtainable with misonidazole and Ro 03-8799. Br J Radiol 59: 911
7. Durand S, Benard P, Braun JP (1980) Ethionine and methionine distribution and toxicity in the rat kidney. Toxicol Lett 5: 257
8. Gessner T, Trudnowski R, Rico R, Rempel J (1976) Passage of intravenously administered pethidine into gastric juice in man. J Pharm Pharmacol 28: 79
9. Lindquist NG (1973) Accumulation of drugs on melanin. Acta Radiol 325 (Suppl): 1
10. Malcolm SL, Lee A, Groves JK (1983) HPLC analysis of Ro 03-8799 in biological samples. J Chromatogr 273: 327
11. Newman HFV, Bleehen NM, Workman P (1986) A phase I study of the combination of two hypoxic cell radiosensitizers, Ro 03-8799 and SR-2508: toxicity and pharmacokinetics. Int J Radiat Oncol Biol Phys 12: 1113
12. Rich TA, Dische S, Saunders MI, Stratford MRL, Minchinton A (1981) A serial study of the concentration of misonidazole in human tumors correlated with histologic structure. Int J Radiat Oncol Biol Phys 7: 197
13. Roberts JT, Bleehen NM, Walton MI, Workman P (1986) A clinical phase I toxicity study of Ro 03-8799: plasma, urine, tumour and normal brain pharmacokinetics. Br J Radiol 59: 107
14. Saunders MI, Dische S, Fermont D, Bishop A, Lenox-Smith I, Allen JG, Malcolm SL (1982) The radiosensitizer Ro 03-8799 and the concentrations which may be achieved in human tumours: a preliminary study. Br J Cancer 46: 706
15. Saunders MI, Anderson PJ, Bennett MH, Dische S, Minchinton A, Stratford MRL, Tothill M (1984) The clinical testing of Ro 03-8799 pharmacokinetics, toxicology, tissue and tumor concentrations. Int J Radiat Oncol Biol Phys 10: 1759
16. Smithen CE, Clarke ED, Dale JA, Jacobs RS, Wardman P, Watts ME, Woodcock M (1980) Novel (nitro-1-imidazolyl) alkanolamines as potential radiosensitizers with improved therapeutic properties. In: Brady LH (ed) Radiation sensitizers: their use in the clinical management of cancer. Masson, New York pp 22
17. Sweeney FL, Pot-Derpum J, Poupon MF, Chouroulinkov I (1982) Heterogeneity of the growth and metastatic behavior of cloned cell lines derived from a primary rhabdomyosarcoma. Cancer Res 42: 3776
18. Ullberg S (1977) The technique of whole-body autoradiographic study. Sci Tools 2

Received December 23, 1986/Accepted April 13, 1988