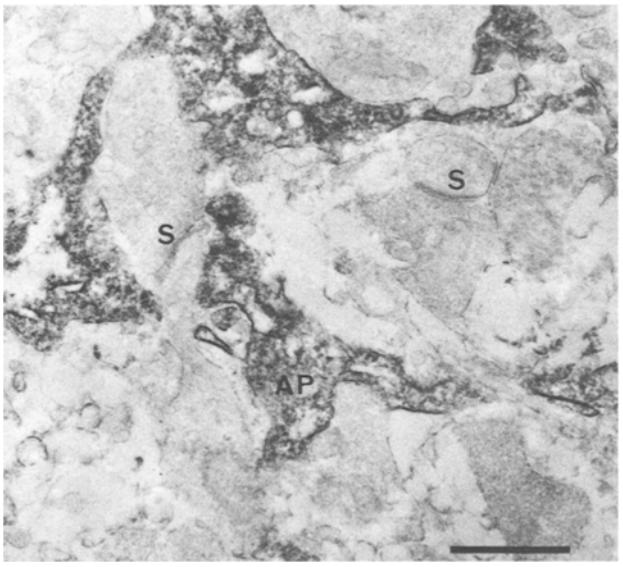

b Immunoelectron microscopically, no specific reaction precipitate is found in any part of neuron such as synaptic junctions (S). AP: Astrocytic processes. Bar = 1 µm.

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α-Particle track autoradiography for localization of a ^{211}At-astatinated drug

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Summary. A potential endoradiotherapeutic drug, 6-^{211}At-astato-2-methyl-1,4-naphthoquinol bis (diphosphate salt), incorporating the α-emitting radio-halogen astatine-211 of half-life 7.2 h, is shown to be valuable for localization studies by means of α-particle track autoradiography in malignant and normal cells and tissues in the mouse with transplanted adenocarcinoma of the rectum.

Attempts have been made since 1953 to develop radioactive anti-tumor drugs which target selectively to neoplastic cells and if possible to their stem cells, as an alternative approach to the treatment of patients with advanced and disseminated malignant tumors[3,4]. More effective isotopes providing high LET radiation are desirable. The compound incorporating radio-iodine-125, 6-^{125}I-iodo-2-methyl-1,4-naphthoquinol bis (diammonium phosphate) – abbreviated 6-^{125}-iodo-MNDP – has been synthesized[5] and appears promising in laboratory studies[6–8]. Much more precise information is required about the distribution of these compounds in the cells of malignant tumors and normal tissues[4,9–11].

This information can now be obtained by the use of the ^{211}At analogue, 6-^{211}At-astato-2-methyl-1,4-naphthoquinol bis (diphosphate salt), abbreviated 6-^{211}At-astato-MNDP (see fig. 1). Auto-radiography with ^{211}At was first used by Hamilton et al.[12] in studies of its uptake into the thyroid gland after i.v. injection of inorganic ^{211}At$^-$.

We now report studies of the distribution of the ^{211}At labeled compound, in malignant and normal cells and tissues of the mouse with transplanted adenocarcinoma of the rectum by means of α-particle track autoradiography on frozen sections.

Materials and methods. ^{211}At was prepared by the ^{209}Bi (α, 2n)^{211}At nuclear reaction using the Nuffield 1.52 m cyclotron at Birmingham University, by bombarding bismuth metal, melted on to supporting copper foils, with a 28 MeV α-particle beam.

The ranges of the α-particles of ^{211}At in unit density tissue are either 55 µm for energy 5.87 MeV for 42% of the disintegrations or 80 µm for energy 7.45 MeV for approximately 58% of the disintegrations[13–15].

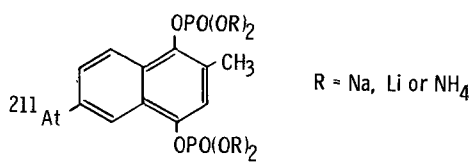


Figure 1. 6-^{211}At-astato-2-methyl-1,4-naphthoquinol bis (diphosphate salt).

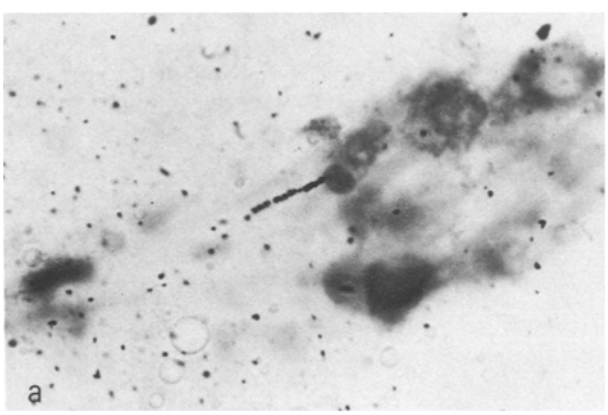
Distribution of origins of α-particle tracks in cells for mice with transplanted induced adenocarcinoma of the rectum after 30 min and 60 min after i.p. injection of 6-^{211}At-astato-MNDP

Cells	Site of origin of α-particle	30 min No.	(%)	60 min No.	(%)
Tumor	Nuclear membrane	16	(44)	11	(30)
	Nucleolus	2	(6)	0	(0)
	Nucleus (within nuclear membrane & not clearly nucleolus)	6	(17)	16	(43)
	Total	24	(67)	27	(73)
	Cytoplasm near nuclear membrane	1	(3)	0	
	Cytoplasm – other parts	0		3	(11)
	Cytoplasm near plasma membrane			1	
	Plasma membrane	11	(30)	4	(16)
	Just outside fixed plasma membrane			2	
	Total	36	(100)	37	(100)
Lung: alveolar cells	Nuclear membrane	1		5	
	Nucleus			3	
	Cytoplasm	1		1	
	Plasma membrane			3	
	Just outside fixed plasma membrane			2	
	Total	2		14	
Spleen	Nuclear membrane	2			
	Cytoplasm near nuclear membrane	1			
	Just outside fixed plasma membrane	1			
	Total	4			
Colon	Nucleus	0		1	
Bone marrow (from femur)		No tracks seen			

The compound, 6-^{211}At-astato-MNDP, has been prepared both by a 5-step procedure similar to that for the 6-^{125}I-iodo-analogue[3] and recently by a rapid heterogeneous thermal isotopic exchange process[16].

The animal experiments were carried out on male C57Bl mice of weight 31–34 g with a rectal adenocarcinoma, originally chemically induced[17], transplanted s.c. in the flank. The weights of the tumors were about 0.5–0.8 g. The thyroid function was blocked with potassium perchlorate given s.c. 30 min before the i.p. injection of 2.4–5 µCi of 6-^{211}At-astato-MNDP in 1 mg of compound. The mice were killed with ether at 30 min or 60 min after injection of the compound.

The autoradiographs of frozen sections of thickness about 3 µm were prepared in a cryostat with freeze-dehydration to minimize diffusion[18] and then coated with 1:1 diluted liquid Ilford Nuclear Emulsion K2 or K0 to give final thickness of emulsion layer approximately 2.5 or 5 µm. Exposures were from 15 to 24 h at 2 °C. The sections were fixed histologically in 1:3 v/v acetic-alcohol for 10 min. After photographic development and the use of a 1% acetic acid stop bath, the slides were dipped in 0.5% gelatin solution and dried in air. This was followed by photographic fixation and washing. Finally, the slides were transferred to acetate buffer at pH 4.2 and stained with methyl-green and pyronine for 30 min at room temperature[19].



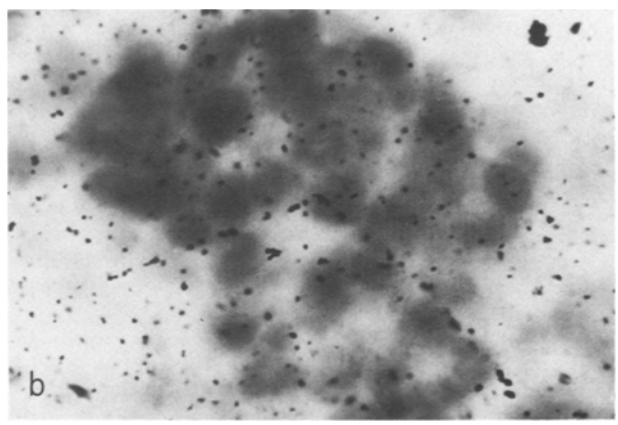


Figure 2. *a* The autoradiograph of a section shows the commonest finding of a single α-particle track arising in the nuclear membrane of a cell of a malignant acinus of the tumor. × 1060. *b* The autoradiograph of a section shows the right hand α-particle track arising from the nucleus and the left hand track arising from the cytoplasm of a tumor cell. The origin of the right-hand track is associated with Auger electrons and that of the left-hand track with a high energy electron. The occurrence of 2 tracks with adjacent origins is rare. × 1060.

Control experiments were carried out without radioactivity and after the radioactivity had decayed, and also with $^{211}At^{-}$. No α-particles were seen in the control experiments without radioactivity. Some chemical artefact was observed.

Results. Examples of α-particle track autoradiographs of sections of the murine rectal adenocarcinoma at 30 min after i.p. injection of 6-^{211}At-astato-MNDP are shown in figure 2,a and b.

The distribution of the origins of α-particle tracks in cells of the tumors and certain normal tissues are shown in the table for unambiguous photomicrographs. At least about two-thirds of the tumor cells show localization of the compound in the nucleus, including the nuclear membrane and in a few cases unmistakeably in the nucleolus. There is early accumulation of the compound in the plasma membrane and nuclear membrane, greater at 30 min than 60 min. Activity observed just outside the plasma membrane in the fixed preparations could be due to shrinkage of the cytoplasm on fixation but may be due to outward diffusion of labeled metabolites formed in the plasma membrane.

It was found that 4.2% of the 1567 tumor cells were labeled at 30 min after i.p. injection of approximately 5 µCi 6-^{211}At-astato-MNDP and 2.2% of 3554 tumor cells were labeled at 60 min after i.p. injection of 2.4 µCi of the compound. The labeled tumor cells are proliferating cells in the growing areas of tumor with rather dense DNA staining and relatively little cytoplasm which contains RNA and are not inconsistent with the characteristics assumed for stem cells.

The many fewer tracks observed in the lungs appear to arise in alveolar cells (type II pneumocytes). Parallel biodistribution studies showed significantly lower uptake ($p < 0.001$) of 6-^{211}At-astato-MNDP into both lung and spleen in comparison with that for ^{211}At-astatide anion[20].

For practical purposes, no tracks were observed in the normal colon. No tracks were seen in the rather limited bone marrow material examined.

iscussion. α-Particle track autoradiography provides a unique opportunity for identifying the intra-cellular localization of the compound.

Further studies of the distribution of the compound are necessary. However, its possible human therapeutic application is as yet uncertain because the radiations from the electron-capture decay of ^{207}Bi of half-life 38 y in 42% of the disintegrations of ^{211}At may be associated with a significant late carcinogenic hazard if the ^{207}Bi cannot be removed from the body. Moreover, the dose of radiation to the lungs must be accurately evaluated. These aspects of the problem require further biological and microdosimetric evidence.

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Multi-electrode recording system for the study of spatio-temporal activity patterns of neurons in the central nervous system[1]

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Summary. A new type of recording microelectrode with mechanical and electrical properties suitable for use in microelectrode assemblies for neurophysiological studies in the central nervous system was developed by adaptation of principles from optical fiber technology. A microdrive for independent positioning of up to 7 electrodes for the recording of electrical activity from individual neurons was constructed. It operates by the combined action of a stepping motor and a system of independently controllable piezoelectric clutches and brakes for each electrode.

Beginning in the early 1960's, innovative techniques for the quantitative analysis of patterns of electrical impulses of individual neurons, recorded with a single microelectrode, have been developed. The results raised the expectations that suitable technical and conceptual refinements would lead to a more comprehensive clarification of the percep-