FROM THE DEPARTMENT OF PHARMACOLOGY, ROYAL VETERINARY COLLEGE, STOCKHOLM, THE DIVISION OF RADIOBIOLOGY, RESEARCH INSTITUTE OF NATIONAL DEFENCE, SUNDBYBERG, AND THE DEPARTMENT OF ORAL HISTOPATHOLOGY, KAROLINSKA INSTITUTET, SCHOOL OF DENTISTRY, STOCKHOLM, SWEDEN.

RADIOPATHOLOGY OF AMERICIUM 241

I. Distribution of americium in adult mice

by

LARS HAMMARSTRÖM and AGNAR NILSSON

The increasing production and use of transuranic elements have enhanced the interest in their relative toxicities, metabolic behaviour and carcinogenic properties. At present the maximum permissible levels for transuranic elements are based on the assumption that their biologic risks are similar to those of plutonium; however, this does not always seem to be true. A comparison of the uptake of ^{241}Am and ^{239}Pu in the skeleton of rats indicated that the initial uptake of ^{239}Pu in the skeleton was about 1.4 times greater than that of ^{241}Am, while the rate of loss from the skeleton was almost the same for these nuclides. The rate of excretion from the body was higher for ^{241}Am than for ^{239}Pu and the retention of the latter in the liver was of a longer duration than that of ^{241}Am (TAYLOR et coll. 1961).

Differences also exist in terms of biologic effects. BENSTED et coll. (1965) have proved that 77 % (17/22) of rats given 3.0 μCi ^{239}Pu/kg body weight developed bone tumours. In addition, one case of renal carcinoma and one of myelogenous leukemia were observed. After the injection of 2.5 μCi ^{241}Am/kg body weight

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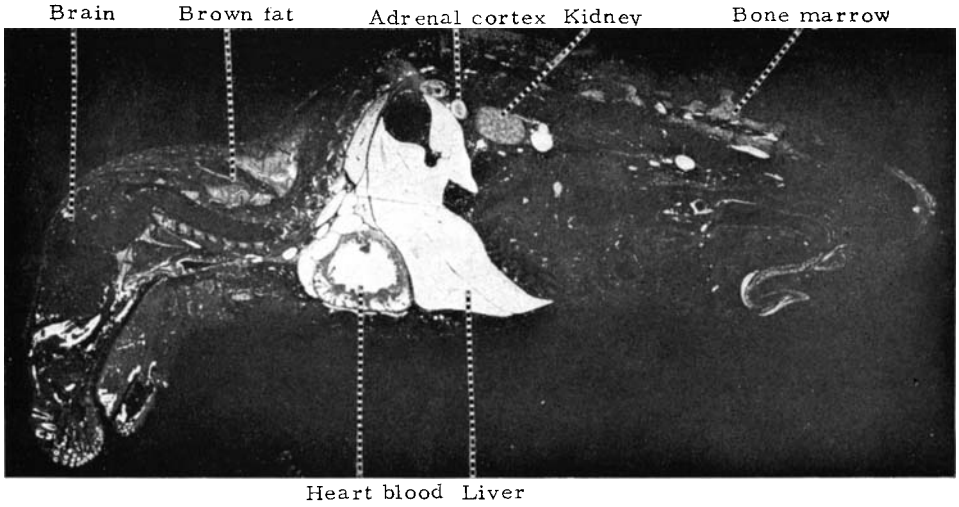

Fig. 1. Autoradiogram. Distribution of ^{241}Am in a male mouse 5 minutes after intravenous injection. High concentration (light areas) in the blood and liver as well as in the adrenal cortex; no demonstrable radioactivity in the bone, but the marrow displays a moderate concentration.

only four out of nineteen rats (21 %) developed osteosarcomas. Adrenal tumours were detected in two animals and one animal developed leukemia of an undetermined type.

As part of an investigation of the pathologic effects of ^{241}Am in mice the present study of the distribution of this nuclide was performed in order to obtain more information concerning the organs at risk.

Material and Methods

Labelled compound. Americium 241 with a concentration of 3.8 μCi/ml was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England. The radioactive compound was administered as a nitrate.

Animals. Nine adult male mice and four pregnant female mice of the CBA strain were used. Two of the female mice were injected on the 15th day of gestation and the other two female mice on the 18th day of gestation. The average weight of the male mice was 20 g and of the pregnant mice 30 to 35 g.

Autoradiographic procedure. Each animal was given 0.26 ml of the solution of ^{241}Am, corresponding to 1 μCi, intravenously in a tail vein. After predetermined survival periods the animals were anaesthetized with ether and killed by

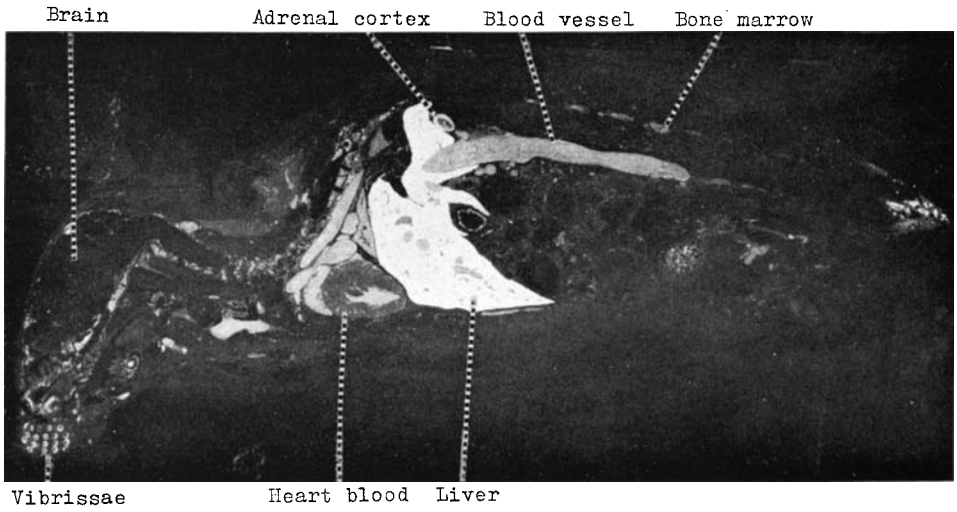

Fig. 2. Autoradiogram. Distribution of ^{241}Am in a male mouse 30 minutes after intravenous injection. High concentration in the liver and adrenal cortex; the concentration in the blood is lower than in the liver; no demonstrable radioactivity in the bone but a moderate concentration in the marrow.

freezing in hexane cooled with solid CO_2 (— 70° C). One male mouse was killed at each of the time intervals of 5 minutes, 30 minutes, 24 hours, 4 days and 15 days after injection. Two mice were killed 30 days after injection and two after 60 days. The female mice that were injected on the 15th day of gestation were killed 24 hours and 4 days after injection and the two that were injected on the 18th day of pregnancy were killed 4 hours and 24 hours after injection.

The animals immediately before freezing were placed in an aqueous solution of carboxy-methylcellulose applied on a large microtome stage. The specimens, after freezing in hexane-CO_2, were thus ready for sectioning, which was performed in a freezebox (—15° C). To obtain sections through the whole animals; adhesive tape (No. 810, Minnesota Mining and Manufacturing Co) was attached to the exposed surface of the frozen specimen before cutting; the sections then came off adhering to the tape. Sections, 20 μ thick, were taken and freeze-dried in the box for 2 days, after which they were brought to room temperature in an air-tight box. The sections were then pressed against a roentgen film (Structurix D7, Gevaert) and exposed for four weeks. After the sections had been removed, the films were developed in G-230 and fixed in D-305 (Gevaert); the sections were stained with hematoxylin and eosin. The autoradiographic procedure has been described in detail by Ullberg (1954, 1958).

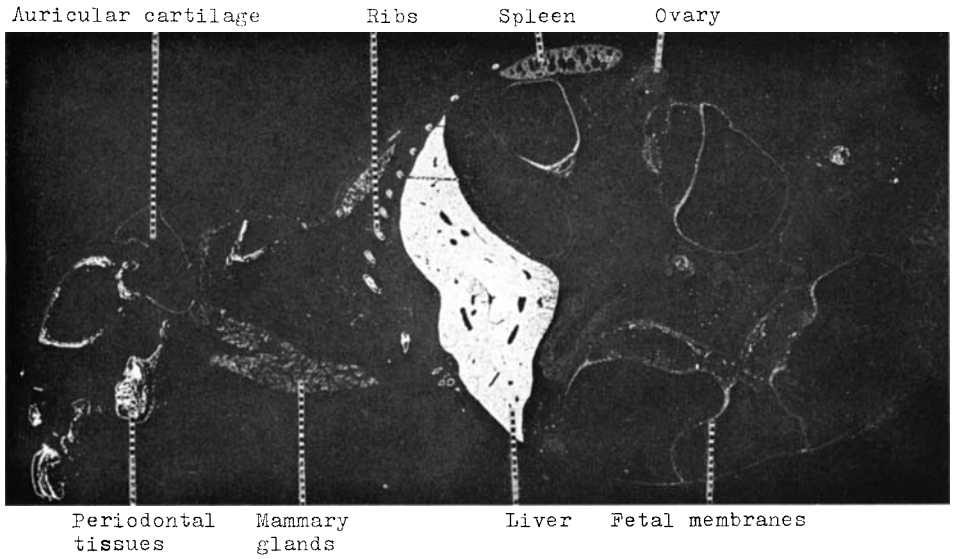

Fig. 3. Autoradiogram. Distribution of ^{241}Am in a pregnant mouse (19th day of gestation) 24 hours after intravenous injection. Uptake in the liver, endosteal and periosteal parts of the bone, periodontal tissues, auricular cartilage, fetal membranes, mammary glands and red pulp of the spleen.

Results

Shortly after the intravenous injection of americium the highest concentration appeared in the blood and the liver. Some other richly vascularized tissues, such as the bone marrow, spleen, kidney, adrenal cortex, lungs, brown fat and nasal mucosa had a moderate concentration. No radioactivity was noted in the mineralized tissues, shortly after injection (Fig. 1).

The concentration in the blood decreased during the first few hours and after 4 hours radioactivity was no longer discernible. The liver and the skeletal tissues seemed to be the major sites of deposition. A high and persistent uptake of radioactivity was also seen in the adrenal cortex, a few ovarian follicles, the marginal sinuses of the spleen, and the dental pulp. The distribution pattern remained fairly unchanged during the whole investigation period.

The distribution in the different tissues will be described more in detail below.

The excretion of the injected americium appeared to occur slowly; only a slight decrease of radioactivity in the organism was autoradiographically observable 30 to 60 days after injection.

Blood. The concentration of americium was high shortly after injection but after 4 hours no radioactivity was seen.

Bone. There was a latency in the uptake of americium in the bone. After 4 hours, a deposition at the endosteal and periosteal surfaces of bone was observable and after 24 hours there was a high concentration at these sites. The concentration at the endosteal surfaces was usually higher than at the periosteal surfaces. The concentration as well as the localization seemed to be unchanged during the remaining investigation period. The bone marrow had a moderate concentration of ^{241}Am at all the intervals studied.

Cartilage. Americium was taken up in the tracheal and auricular cartilage. As in bone, there was a latency of some hours in the uptake which was limited to the surface. The intervertebral discs had no detectable amounts of the injected ^{241}Am.

Teeth. A marked accumulation was observed in the dental pulp and in the periodontal membrane (cf. Fig. 5). There seemed to be some radioactivity at the surface of the developing enamel of the incisors.

Gastro-intestinal tract. Some radioactivity was seen in the contents of the stomach close to the secretory mucosa in all the animals studied and a small amount of radioactivity was also present in the intestinal lumen after long survival periods. No radioactivity appeared in the gastric or intestinal mucosa, however. Five minutes after injection, the concentration of ^{241}Am in the liver was the same as in the circulating blood. It then seemed to increase slightly and at all the intervals studied was the highest in the body. The distribution was fairly even shortly after injection but with time a redistribution towards a higher concentration around the central veins occurred. Americium was never observed in the gall bladder (cf. Fig. 6). The salivary glands and the pancreas never had any observable amounts of ^{241}Am.

Respiratory tract. The concentration in the lungs followed that of the circulating blood. A high concentration was recorded in the bronchial cartilage, however.

Urinary tract. The whole kidney exhibited a moderate concentration of radioactivity, and in addition a higher accumulation was observed in small spots in the renal cortex. No accumulation was present in the renal pelvis but radioactivity over the mucosa of the ureter and urethra persisted up to 60 days after the injection.

Endocrine organs. The adrenal cortex had a fairly high concentration, which persisted 60 days after the injection; at 5 minutes it had become evenly distributed. Twenty-four hours and more after the injection the concentration in an outer zone, presumably the zona glomerulosa, exceeded that of the other zones of the cortex. This higher concentration in the outer zone was not present in the female mice. No radioactivity was observed in the adrenal medulla. A low concentration was present in the pituitary, thyroid and pancreatic islets. Some radio-

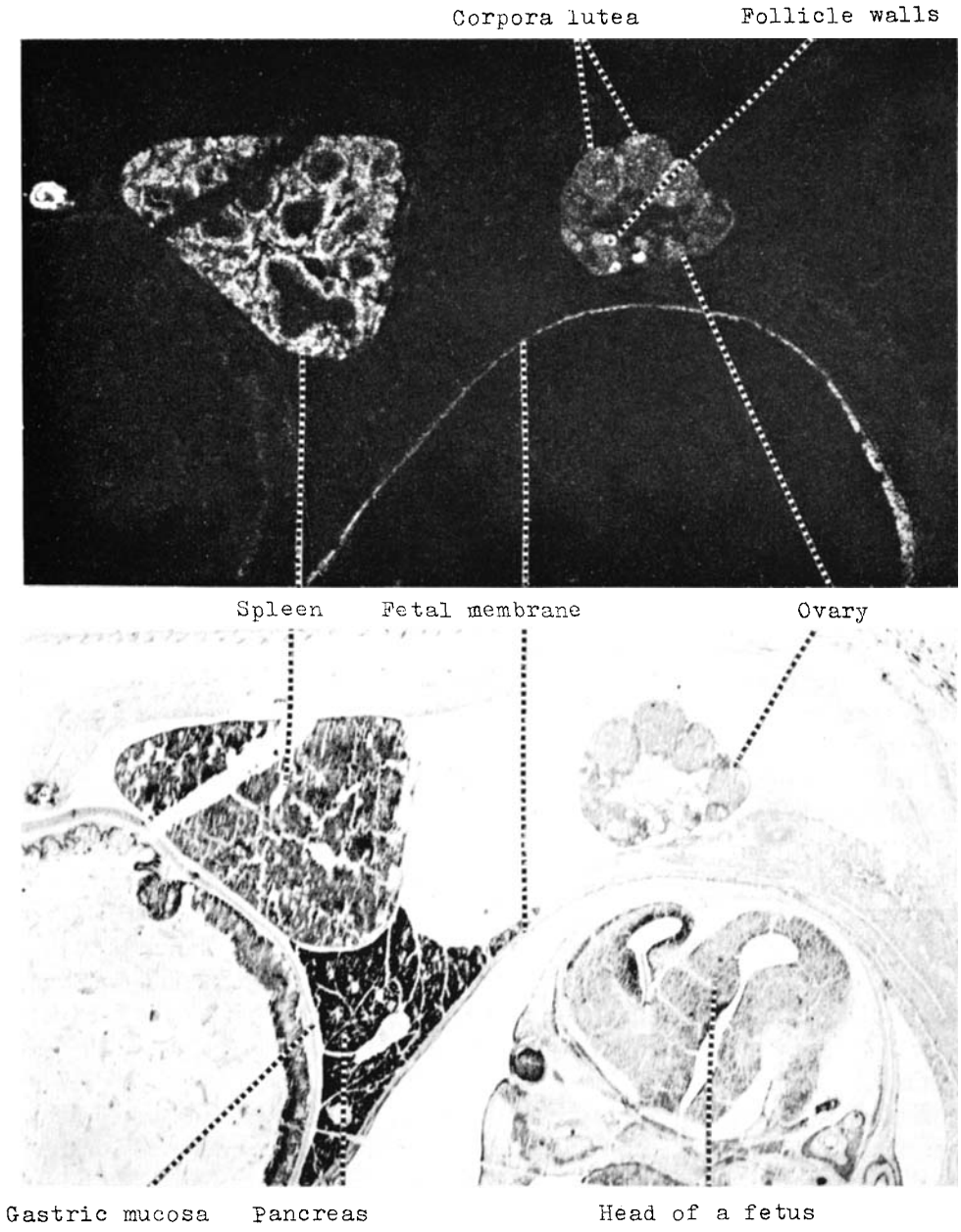

Fig. 4. Detail of autoradiogram (upper image) of a pregnant mouse (15th day of gestation) 4 hours after intravenous injection of ^{241}Am, and the corresponding stained section (lower image) depicting the distribution of ^{241}Am in the ovary, spleen and adjacent placenta; high uptake in some follicle walls of the ovary.

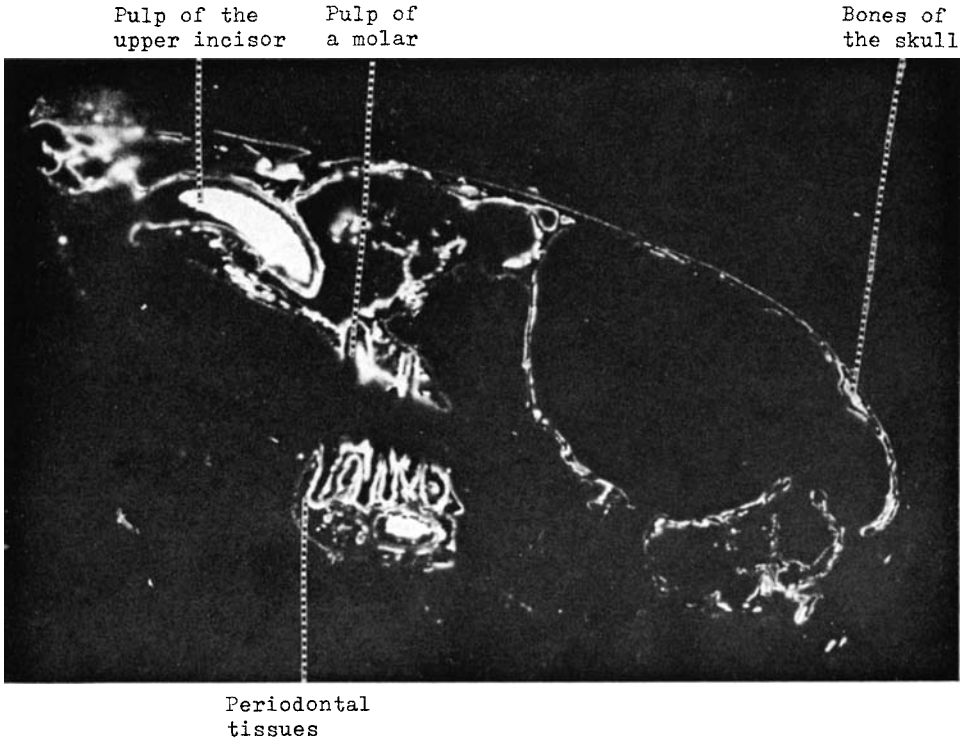

Fig. 5. Autoradiogram. Distribution in the head of a mouse 24 hours after intravenous injection of ^{241}Am. High concentration in the bone, periodontal tissues and dental pulp.

activity in these glands seemed to persist when no radioactivity was demonstrable in the circulating blood.

Gonads. The uptake in the testes was low but appeared to increase moderately with time. The radioactivity was mainly localized in the interstitial tissue. The ovaries of the pregnant mice had a high concentration in some follicles and a moderate concentration in the interstitium, while the corpora lutea displayed a lower concentration (Fig. 4).

The central nervous system presented no evidence of radioactivity.

Lymphatic tissues. The red pulp of the spleen had a fairly high concentration, which seemed to remain unchanged during the whole period of investigation. The marginal sinuses had a higher concentration than the red pulp (Fig. 4). No radioactivity was present in the white pulp of the spleen or in the lymph glands.

Muscles. The muscles had no detectable concentration.

Skin and subcutaneous tissue. The vibrissa follicles had an uptake of americium that gradually disappeared during the first day after the injection (Fig. 2). No

Fig. 6. Autoradiogram. Distribution of ^{241}Am in a mouse 15 days after intravenous injection. Attention may be drawn to the outer zone of the adrenal cortex and the marginal sinuses of the spleen.

uptake was noted in other hair follicles or in other parts of the skin or subcutaneous tissue.

Brown fat. A moderate concentration was seen in the brown fat. The disappearance from this tissue was slower than from the blood.

Placenta. No radioactivity was present in the placenta at the intervals studied.

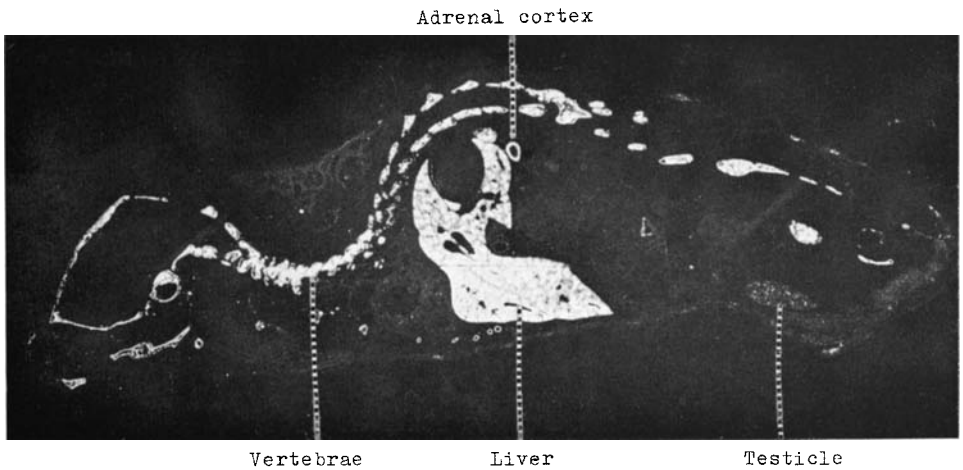

Fig. 7. Autoradiogram. Distribution of ^{241}Am in a mouse 60 days after intravenous injection. Attention may be drawn to the adrenal cortex, liver and bone.

However, a moderate concentration was noted in the fetal membranes (Figs 3 and 4).

Mammary glands had a moderate concentration at all the intervals studied.

Fetuses. Only little radioactivity was seen in the fetuses, and only a faint representation of the skeleton was obtained in the animals injected at the 18th day of gestation.

Discussion

The distribution of americium in the present investigation was predominantly characterized by an accumulation of the isotope in the bone and liver tissues. This is in close agreement with results obtained earlier with the impulse counting technique (SCOTT et coll. 1945) and seems generally to be a common feature for all the actinide elements (TAYLOR 1964). The preferential accumulation of americium at the endosteal surfaces of bone appears to be compatible with previous findings that its concentration is higher on resting and resorbing surfaces of bone than on those where bone formation is in progress (TAYLOR et coll. 1966, HERRING et coll. 1962). The mechanism of binding of americium to bone tissue is not known although some evidence that it is bound to bone glycoproteins (HERRING et coll. 1962, CHIPPERFIELD & TAYLOR 1968) exists. The finding in the present investigation that there was a latency in the uptake of the isotope in bone after an intravenous injection may indicate that americium is incorporated into a larger molecule that has an affinity for skeletal tissues.

Certain new sites of marked accumulation were also detected in the present investigation, i.e. the adrenal cortex, the ovary and the dental pulp. The accumulation in the adrenal cortex may be placed in relation to the americium-induced adrenal tumours in rats, observed by BENSTED et coll. (1965). However, ^{241}Am is not taken up in the adrenal cortex of young rats (HAMMARSTRÖM & NILSSON, to be published). This may indicate that the binding mechanism is in some way associated with steroid hormone production after sexual maturation. Of other actinide elements studied, both plutonium 239 (ULLBERG et coll. 1962) and uranium 233 (WALINDER et coll. 1965) have been reported to be accumulated in the adrenal cortex. The relative concentration in the adrenal cortex seems, however, to be considerably less for these two radioelements. Like americium, also these two actinides were accumulated and retained in the ovary and the interstitial cells of the testes. Local irradiation of the reproductive cells may have grave genetic consequences. Our preliminary results concerning the pathologic effect of ^{241}Am have revealed serious atrophy of the testes.

The accumulation in the dental pulp, noted in this investigation, does not seem to have been observed for other actinide elements. The concentration

appeared to be about the highest in the body and radiation injuries might well be expected. It would appear that a primary tumour of the dental pulp has never been reported and special attention will be paid to this tissue in a current long-time study of the radiopathology of americium 241.

SUMMARY

The distribution and retention in mice of ^{241}Am after a single intravenous injection were investigated by autoradiography. The technique is described and the findings are discussed in detail.

ZUSAMMENFASSUNG

Die Verteilung und Aufspeicherung des ^{241}Am wurde mittels Autoradiographie nach einer einzelnen intravenösen Injektion studiert. Die Technik der Methode und deren Resultate werden im einzelnen beschrieben.

RÉSUMÉ

La répartition et la fixation du ^{241}Am chez les souris après une injection intra-veineuse unique ont été étudiée par autoradiographie. Les auteurs décrivent la technique et analysent en détail les résultats.

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