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# THE PATHOLOGY OF AMERICIUM 241

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Americium 241 is a transuranic element which has found use in industry. On account of this and its metabolic behaviour and high energetic $\alpha$-irradiation ($\sim$5.45 MeV) it may present a considerable biologic hazard. It is taken up mainly in the skeleton and the liver and also for long duration in the adrenal cortex, the ovary, the dental pulp as well as in the testes (HAMMARSTRÖM & NILSSON 1970). Americium is like plutonium a 'surface seeker' in the skeleton but its initial uptake is, however, about 1.4 times less than that of $^{239}$Pu while the rate of loss from the skeleton is almost the same for the two nuclides (TAYLOR et coll. 1969). Among various bones the concentration of $^{241}$Am (ROSEN et coll. 1972) varies within a factor of 4, that in the vertebrae being the highest and that in the small bones the lowest.

LANGHAM & CARTER (1951), BENSTED et coll. (1965) and TAYLOR & BENSTED (1969) have reported that $^{241}$Am-in doses of 63 to 2.5 $\mu$Ci/kg has a large tumour spectrum including tumours of the bone, adrenal glands and haematopoietic tissues in rats. Using doses between 25 and 2.5 $\mu$Ci/kg to rats RUDNITSKAYA & MOSKALEV (1970) have also found tumours of the liver and degenerations in the liver, kidney, myocard and testes. They also conclude that the irradiation of some endocrine organs particularly the pituitary and the thyroid may create a state of endocrine imbalance.

A comparison between $^{239}$Pu and $^{241}$Am in rats have revealed that the former is a much more effective carcinogen than americium when the two nuclides are administered in approximately the same doses (BENSTED et coll.).

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**Table 1**

*Experimental design*

| Dose of $^{241}$Am $\mu$Ci/kg | Number of mice | Handling |
|---|---|---|
| 16 | 25 | One or two mice killed at certain intervals up to 9 months |
| 16 | 40 | Survivors |
| 8 | 100 | Survivors |
| 0.4 | 50 | Survivors |
| 0.2 | 48 | Survivors |
| 0.04 | 51 | Survivors |
| Control | 50 | Survivors |

Most $\alpha$-emitters, however, seem to be more effective cancerogens than nuclides emitting $\beta$-irradiation. In the present report longevity, causes of death, dysplastic and dystrophic changes, carcinogenicity and the development of tumours induced by various doses of $^{241}$Am are presented. In some respects the late effects induced by the $\alpha$-emitting $^{241}$Am have been compared with those induced by the $\beta$-irradiation from $^{90}$Sr described previously (NILSSON 1962, 1970).

## Material and Methods

Totally 314 CBA male mice aged $75 \pm 5$ days were injected intraperitoneally with $^{241}$Am-citrate and 50 were used as untreated controls (Table 1). Before autopsy the animals were examined roentgenologically and the films were used as a guide for locating tumours. From all mice i.e. both long time survivors and those killed at certain intervals by cervical dislocation, both femurs, tibias and humeri, the spine, the sternum and the head were fixed in Stieve's fluid and decalcified in 20 % formic acid for microscopy.

The liver, kidney, testes, adrenals and spleen were weighed. In addition to these organs also the lungs, the thyroid and the heart were fixed in Stieve's fluid. Other tissues were fixed only when macroscopic lesions were found. Conventional histologic methods were used, the sections routinely being stained according to the van Gieson method and with haematoxylin-eosin. Occasionally Masson's trichrome method, Lillie's allochrome, the van Kossa method for calcium, azure-eosinate, Einarson's gallocyanine, toloudine blue and congo red for amyloid, PAS by the method of Hotchkiss, Foot and Foot's silver method and Weigert's elastin were also applied.

## Results

### *Non-malignant lesions*

*Haematopoietic tissues.* In almost all cases a moderate to severe effect on the haematopoietic and lymphatic systems was found in the two highest dose groups,

ranging from a complete bone marrow aplasia to a slight hypoplasia. In the lower dose groups, however, these changes were insignificant. Usually all types of cells were depleted, the erythroid series, however, being most sensitive. The majority of the marrows were loaded with fat and had heavily congested sinusoids. In many aplastic marrows the sinusoids were besides completely destroyed leading to formation of large blood lakes. In a few aplastic marrows an increase of reticular cells occurred with a slight intramedullar formation of argyrophilic and fuchsinophilic fibres. By histologic methods it was also found that the effect of $^{241}$Am was more severe in the vertebral marrow than in that of the long bones, whereas in the sternum the effect was intermediate. In the long bones the injury was most marked in the distal end of the femur and in the proximal end of the humerus and tibia. In the diaphyseal parts the lesion was less prominent and when regeneration occurred it was usually most evident here. In the regenerative phase myeloid elements and megakaryocytes predominated, the erythroid elements being in many cases practically absent. Thrombosis of the marrow occurred in a few per cent in all $^{241}$Am groups, i.e. even the lowest ones.

The spleen and other lymphatic tissues were persistently depleted of lymphatic cells (Table 2) and at the time of death the spleen was almost atrophic in most cases in the two highest dose groups. A slight or moderate extra-medullary haematopoiesis of all kinds, however, could still exist. A considerable predomination of myeloid elements and megakaryocytes generally existed and in many mice there was also a heavy intramedullary and paraosteal proliferation of myeloid elements.

*Skeleton.* In the 16 $\mu$Ci groups mice were killed with certain intervals (14 days, 1, 2, 3 up to 9 months after administration of the nuclide). Some increase of the number of osteoblasts was found after 1 month in the distal metaphysis of the femur and after 2 months there was still some osteoblastic and osteoclastic activity. Between 3 to 5 months the number of these cells decreased successively giving place to a sparse appearance of fusiform cellular elements which increased in number furthermore during month 8 to 9. At this time also a slight formation of fuchsinophilic fibres could be found. Devitalized bone was abundant and at many sites it was being attacked by osteoclasts.

In mice dying between 160 and about 250 days after injection of 16 and 8 $\mu$Ci $^{241}$Am/kg the bone tissue was generally heavily damaged. Both in vertebrae and long bones the nucleated cells of the epiphyseal cartilage were few and in the metaphysis the trabeculae had more or less completely disappeared (Fig. 1 a). The edge of the epiphyseal cartilage was quite even in correspondence with a complete disappearance of osteoclasts. The thickness of the compact bone of the diaphysis was strongly reduced. The endosteal cells, both osteoclasts and osteoblasts, had disappeared completely. The osteocytes were shrunken and appeared pycnotic and their lacunae were conspicuously increased in size or empty (Fig. 1 b). This could be observed everywhere but was most evident in the vertebral bodies. Here the walls sometimes were

**Table 2**

*Mean weights*

| Dose of $^{241}Am$ $\mu$Ci/kg | Number of mice | Weight of | | | | | Survival days |
|---|---|---|---|---|---|---|---|
| | | Mice x±SE g | Liver x±SE g | Spleen x±SE mg | Testes x±SE mg | Adrenal x±SE mg | |
| 16 | 39 | 14.1±0.28 | — | 30.3±3.8 | 23.1±1.4* | — | 174.9±9.5 |
| 8 | 100 | 17.8±0.39 | 0.7289±0.0963 | 33.7±3.2 | 57.5±1.6** | 4.6±0.10 | 301.8±6.5 |
| 0.4 | 50 | 21.3±0.20 | 2.4829±0.0731 | 85.9±7.6 | 45.0±2.0 | 7.0±0.85 | 684.9±18.2 |
| 0.2 | 48 | 22.1±0.90 | 2.5796±0.2556 | 76.1±5.4 | 47.2±3.6 | 5.6±0.22 | 697.8±25.8 |
| 0.04 | 51 | 22.7±0.94 | 2.6710±0.273 | 76.8±1.2 | 49.4±3.1 | 5.7±0.22 | 731.7±17.6 |
| Control | 50 | 20.8±0.76 | 1.9467±0.0187 | 93.1±7.5 | 49.2±3.9 | 5.8±0.44 | 779.7±17.4 |

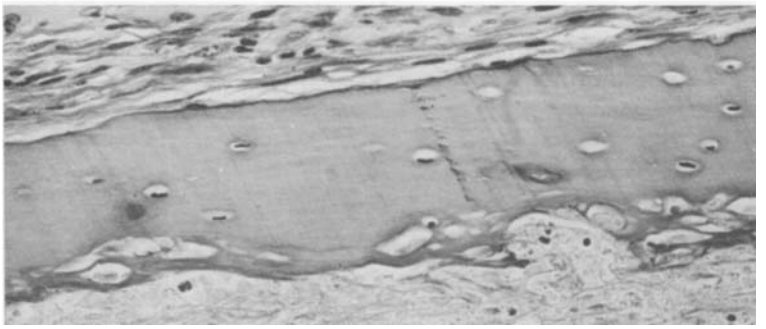
**Appendix**

*Absolute and relative weight of testes, liver, spleen and adrenals for five normal serially killed mice*

| | Days | Absolute weight (mg) | Relative to body weight (per cent) |
|---|---|---|---|
| * Testes, killed at | 180 | 131.4±4.0 | 0.37 |
| ** Testes, killed at | 300 | 141.9±3.2 | 0.35 |
| Liver, killed at | 180 | 1.2647±0.0665 | 5.3 |
| | 300 | 1.4663±0.0339 | 4.8 |
| | 570 | 1.7709±0.0778 | 5.3 |
| Spleen, killed at | 180 | 65.2±1.7 | 0.18 |
| | 300 | 75.1±3.2 | 0.19 |
| | 570 | 86.2±8.9 | 0.26 |
| Adrenal, killed at | 180 | 2.9±0.13 | 0.008 |
| | 300 | 3.7±0.10 | 0.009 |
| | 570 | 3.3±0.06 | 0.010 |

extremely thin and the structure was kept together only by the periosteal tissue. Complete fractures of the vertebral body without reaction or only a slight attempt to formation of a callus could be detected. This destruction of the bone was obviously a process of osteolysis and necrosis since osteoclastic activity was with some exceptions insignificant. Up to about 240 to 280 days in the 8 $\mu$Ci/kg group the microscopy of the bone tissue was totally dominated by this destructive phase generally without any capacity for repair reactions. At this time osteoclasts started to appear, destroying circumscribed necrotic tissues or more diffusely distributed devitalized bone. In many bones the epiphyseal cartilage was almost completely absent. The surrounding of the nutritional foramen was also often attacked and the foramen significantly widened. Around 280 days after the injection of 8 $\mu$Ci $^{241}Am$/kg the first evident signs of repair could be detected mostly in lacunar areas with osteoclastic activity (Fig. 2 a). These cells were fusiform or sometimes osteoblastlike elements with the ability to osteoid formation (Fig. 2 b). Such areas of reparation were usually

a



b

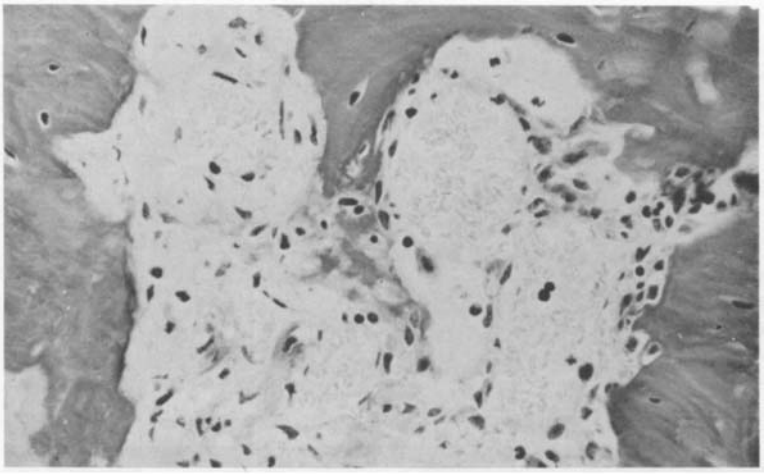
Fig. 1. a) Femur, epiphyseal region, mouse 364 days after injection of 8 $\mu$Ci $^{241}$Am-citrate kg body weight. The compact bone and epiphyseal cartilage almost completely destroyed. Heavy periosteal reaction. van Gieson $\times$100.

b) Femur, diaphyseal part, mouse 364 days after injection of 8 $\mu$Ci $^{241}$Am-citrate kg. Enlarged osteocyte lacunae. Numerous lacunae are empty or contain pycnotic osteocytes indicating bone necrosis. The endosteal bone is strongly mottled and no osteoblasts appear. The periosteal tissue (upper part) is broadened by formation of fibrous tissue. The bone marrow is completely necrotic. van Gieson $\times$250.

most frequent in the vertebrae. They were, however, not particularly conspicuous and did never predominate over the destructive lesions.

The first microscopic osteosarcoma was detected in the 16 $\mu$Ci group after 231 days in the proximal epiphysis of the tibia and after 280 days in the wing of the first sacral vertebrae of the 8 $\mu$Ci group. As a rule these processes started inside Howship's lacunae or in narrow tunnels in the bone. Most tumours appeared as small buds in the angel between compact bone and the epiphyseal cartilage in the vertebrae and in the epiphyseal part of the long bones.

In the 0.4, 0.2 and 0.04 $\mu$Ci groups the abnormalities found in the skeleton at high



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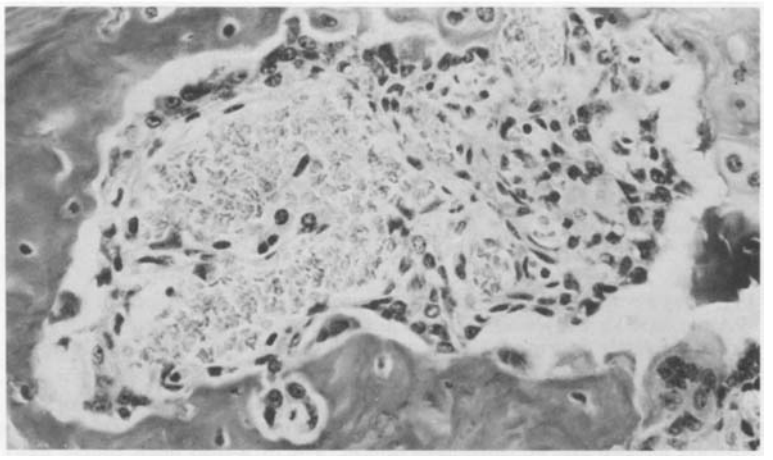

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Fig. 2. a) Lumbar vertebra, mouse 306 days after injection of 8 $\mu$Ci/kg $^{241}$Am. Large lacunae with a few osteoclasts remaining. Beginning of reparation by proliferation of fusiform cells in the marrow. van Gieson ×250.

b) Thoracic vertebra, mouse 313 days after injection of 8 $\mu$Ci/kg $^{241}$Am. Lacunae with a few osteoclasts and a heavy proliferation of osteoblast-like cells and apposition of immature bone. van Gieson ×250.

age such as devitalization and osteoporosis were usually more developed than the age related lesions in the control group. In the control animals the cortical bone particularly in the femur generally grew thinner with advancing age or could be built up by a number of thin bone spiculae separating large lacunae. In some cases there was also a strong formation of endosteal bone.

*Liver.* A slight periportal fibrosis was a common finding in a majority of the livers given 8 $\mu$Ci. Parenchymal changes varying from a slight pleomorphism of the liver cell nuclei to an extensive probably hypoxemic fatty degeneration and occurrence of multiple focal necrosis were also observed in numerous cases. In some livers the number of binucleate cells and the degree of nuclear pleomorphism appeared abnormally high in the 16 to 8 $\mu$Ci groups. In a significant number of livers the epithelial cells of the biliary canalicule appeared to be swollen and in a few livers heavy multifocal proliferations of these structures occurred (Fig. 3). In a few cases numerous microabscesses also existed.

In the 0.4, 0.2 and 0.04 $\mu$Ci groups and in the control groups dystrophic changes of various types such as fatty degenerations, necrosis and microabscesses appeared approximately with the same frequency.

*Adrenal glands.* The weight of the adrenals was in many mice in the 8 $\mu$Ci group higher than normally. In Table 2 it is also evident that the mean weight for the whole group of animals was significantly increased as compared to that of normal mice of the same age. In some cases the reason for the increased weight seemed to be a hyperplasia of the cortex sometimes evidenced by small nodular cortical hyperplasia. In some cases extracapsular hyperplasia nodules were encountered.

In mice of advanced age the weight of the adrenal glands continued to rise. The mean weight of the adrenals for mice 685 to 732 days old in groups 0.4, 0.2 and 0.04 and the control mice (780 days) were thus statistically significantly increased over that of mice of 300 and 570 days of age (Table 2 and appendix). No such difference, however, existed between the low dose groups and their controls which means that the adrenals are increasing in weight with age. One reason for the increased weight of the adrenal in mice of old age seemed to be related to varying degree of hyperplasia of the adrenal medulla and at the same time an atrophy of the cortex. This hyperplasia which sometimes was difficult to separate from a tumour (phaeochromocytoma) was generally more frequent and marked in the 0.4 $\mu$Ci group than in the controls (Fig. 4). In most cases with medullary hyperplasia a more or less conspicuous destruction of Zona reticularis and a narrowing and atrophy of Zona fasciculata with a dilatation of its sinusoids appeared. In many cases the neurons of the medulla appeared greatly increased in size and had a large prominent nucleus. Sometimes the hyperplastic medulla was in direct contact with the adrenal capsula.

*Kidney.* In the 16 $\mu$Ci group only serially sectioned mice were examined microscopically. Lesions, usually focally situated, were found 6 months after the injection of $^{241}$Am. In some glomeruli at such sites the epithelium was stratified or desquamated and in a few cases the glomeruli tufts were slightly fibrotic. Also some tubuli walls were hyalinized and strongly thickened and the epithelium almost occluded the lumen. A slight interstitial focal fibrosis was also observed as well as a few hyaline casts.

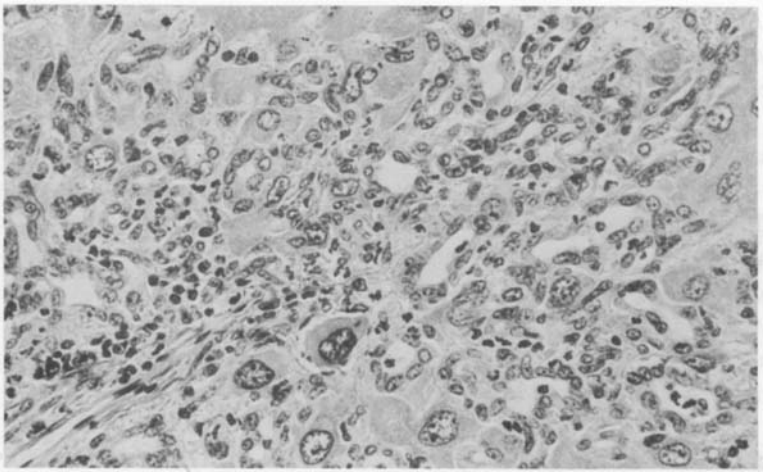


Fig. 3. Liver, mouse 191 days after injection of 8 $\mu$Ci $^{241}$ Am/kg. Proliferation of biliary canaliculi. Atypic and pleomorphic hepatocyte nuclei. van Gieson $\times 250$.

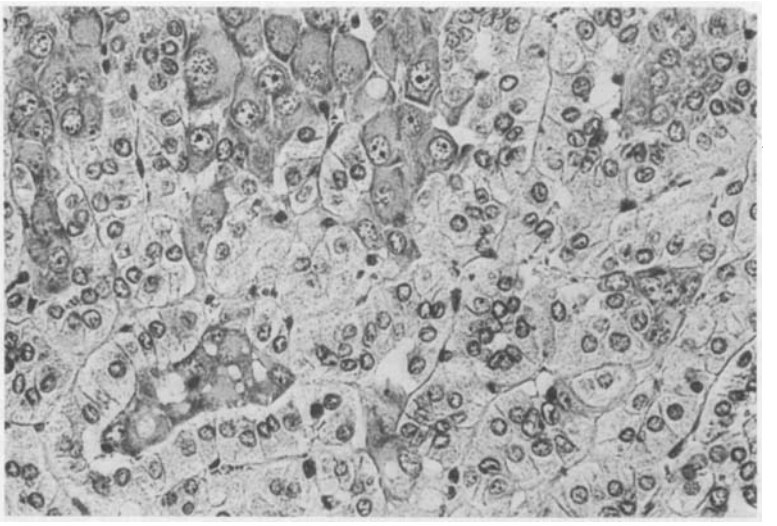


Fig. 4. Hyperplasia of the adrenal medulla, mouse 553 days after 0.4 $\mu$Ci $^{241}$Am/kg. Weight of the glands 9.6 mg. H–E $\times 250$.

With increasing age these changes grew progressively worse within focal areas. Thus, after 9 months the lesions were conspicuous with numerous hyaline casts in the tubuli or inside the Bowman capsule. Locally the glomeruli were slightly to moderately fibrotic and in many cases contracted. In these areas the interstitial con-

**Table 3**

*Kidney lesions. Percentage in relation to dose*

| Type of lesion | Dose of $^{241}$Am $\mu$Ci/kg | | | Control |
|---|---|---|---|---|
| | 0.4 | 0.2 | 0.04 | |
| I | 30.9 | 24.3 | 10.3 | 37.0 |
| II | 35.7 | 13.5 | 46.3 | 37.0 |
| III | 30.9 | 51.4 | 28.2 | 22.2 |
| IV | 2.4 | 2.7 | 2.6 | 3.7 |
| V | — | 8.1 | 10.3 | — |

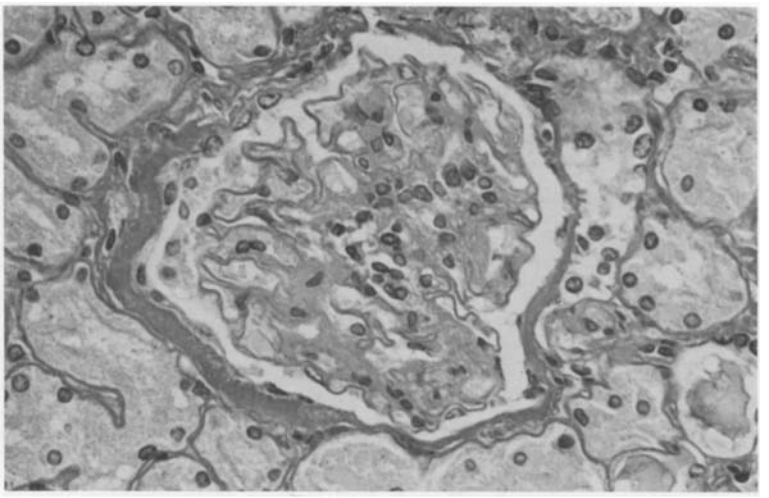
nective tissue was increased. Lesions of this type were not found in control material of comparable age.

In the 8 $\mu$Ci group practically all mice had lesions of the kidney varying from a slight to a relatively strong interstitial peri- and intraglomerular fibrosis. In some cases the glomerular tufts were sclerotic. The tubuli were often dilatated and could have an atrophic epithelium.

In the three lowest dose groups 0.4, 0.2, 0.04 and in the control material practically all kidneys had pathologic changes of the same type and frequency. The glomeruli were as a rule more heavily affected than the interstitial tissue. The lesions were approximately divided into different groups (Table 3). In the first group (I) the glomeruli capsule was thickened and hyalinized and the epithelium transformed to a stratified usually single-layered epithelium or absent. The glomerular tufts were usually slightly hyalinized and slightly fibrotic. In the second group (II) the abnormalities were more aggravated and besides those mentioned in group I there was also a considerable fibrosis of the Bowman capsule and periglomerularily (Fig. 5 b). In the third group (III) there was in addition a heavy interstitial fibrosis which in some cases could involve the whole organ. In a few cases (group IV) a chronic pyelonephritis was found. The fifth group (V) consisted of kidneys with only minor changes, such as a more or less evident dilatation of the tubuli or the glomeruli.

*Testis.* As compared to the normal group there was an accelerated relative and absolute testicular weight loss in the $^{241}$Am treated mice (Table 2). In most testes from mice in the higher dose groups there was aspermia or a marked hypospermia and tubular degeneration. In older mice a slight interstitial fibrosis and a concomitant reduction of Leydig's cells occurred.

A congestion was macroscopically detectable of the superficial testicular vessels in numerous testes. Microscopically there was a necrosis of the vessels which, however, stained intensely blue with H–E or black with the van Kossa method on account of calcium deposits (Fig. 6 a). In the 8 $\mu$Ci group 81 per cent of the mice had these lesions. The mean survival for these mice was 302 days. Similar abnormalities



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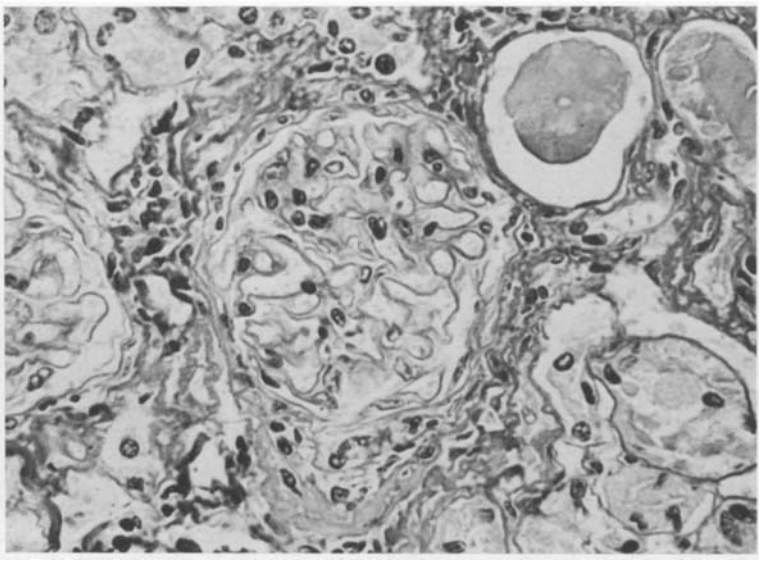
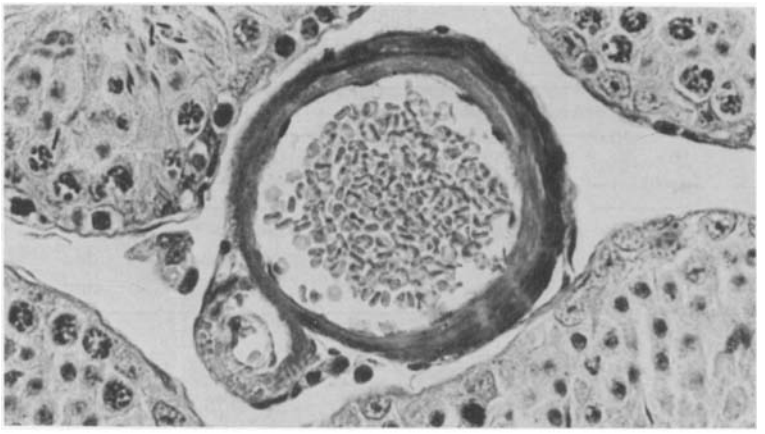


b

Fig. 5. Glomeruli. a) Untreated control mouse 650 days after start of experiment. Hyalinized Bowman capsule and glomerular tuft. Degeneration of tubular epithelium. Lillie's allochrome ×400.

b) Untreated control mouse 675 days after start of experiment. Hyalinization of the Bowman capsule. Slight fibrosis of the capsule and its surrounding, hyaline casts, dilated tubuli with degenerated epithelium. Cellular proliferation within the glomerular capsule. Lillie's allochrome ×400.



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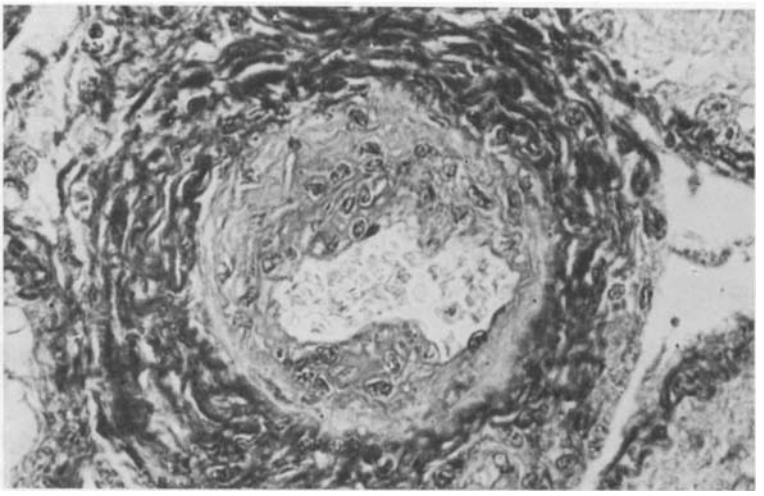


b

Fig. 6. Testis mouse. a) Vascular dystrophically calcified necrosis 289 days after injection of 8 $\mu$Ci/kg $^{241}$Am.

b) 659 days after injection of 0.2 $\mu$Ci $^{241}$Am/kg. Proliferation of the intimal layer and partly occlusion of the vascular lumen. Considerable perivascular fibrosis. Lillie's allochrome ×400.

were found also in the three lowest dose groups (0.4, 0.2, 0.04) and in the control material and were 73 per cent (survival time 658 days), 53 per cent (survival time 731 days), 56 per cent (survival time 708 days) and 93 per cent (survival time 769 days), respectively. In the lowest dose group the first lesions appeared after 452 (0.4), 473 (0.2) and 473 days (0.04), respectively, and in the control group after 580 days. In the 8 $\mu$Ci group the first case was found after 165 days.

**Table 4**

*Number and induction time (days) of tumours in relation to dose of $^{241}Am$*

| Tumours of | Dose of $^{241}$Am $\mu$Ci/kg | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16 n: 39 | | 8 n: 100 | | 0.4 n: 50 | | 0.2 n: 48 | | 0.04 n: 51 | | Control n: 50 | |
| | No. | Induction | No. | Induction | No. | Induction | No. | Induction | No. | Induction | No. | Induction |
| Bone | 4 | 254.0 | 45 | 352.5 ±9.1 | 2 | 708 | 1 | 783 | 1 | 785 | 0 | |
| Liver | | | | | 36 | 665 ±19 | 32 | 678 ±22 | 34 | 708 ±23 | 28 | 731 ±24 |
| Lung | | | 1 | 280 | 5 | 746 ±51.6 | 12 | 730 ±45.5 | 15 | 828 ±31.8 | 12 | 789 ±31.5 |
| Lympho-reticular system | | | 10 | 278 ±25.8 | 3 | 746 | | | 3 | 742 | 2 | 608 |
| Vessel | | | | | 1 | 779 | | | 1 | 847 | 1 | 964 |
| Adrenal glands | | | | | 1 | 452 | | | | | | |
| Kidney | | | | | | | 1 | 834 | | | | |
| Orbit | | | | | | | | | 1 | 847 | | |
| Coccyx (Chondroma) | | | | | | | 1 | 888 | | | | |

Besides these lesions of medium sized arteries, proliferation of the endothelium was found in numerous smaller arteries, generally combined with a heavy proliferation of the adventital layer (Fig. 6 b).

*Myocardium.* The lesions of the myocardium mainly found in the 8 $\mu$Ci group were characterized by multifocal occurrence of degeneration and necrosis with occasional signs of alterative inflammations of the myocardial tissues. Numerous necrosis were dystrophically calcified. In the other groups one case of myocardial degeneration was found in each of the 0.4 and 0.2 $\mu$Ci groups. In the control group no such lesions appeared.

*Other tissues.* The vertebral discs, particularly those between the 3rd and 4th sacral vertebrae and those between the 4th sacral vertebra and the first and second coccygeal vertebrae, were strongly increased in size and had a tendency to protrude bilaterally. No such lesions appeared in the two highest dose groups but they were common in the lowest dose groups (0.4, 0.2, 0.04) and in the control material, being 71.4, 64.6, 69.6 and 44.0 per cent, respectively. However, mice having these lesions were of a high mean age, being $763 \pm 25.8$, $789 \pm 20.4$, $773 \pm 24.8$ and $731 \pm 15.6$ days for the control material and the 0.04, 0.2 and 0.4 $\mu$Ci groups, respectively.

**Table 5**

*Anatomic distribution of $^{241}Am$ induced hard tissue tumours*

| Site | Number of tumours in relation to dose of $^{241}Am$/kg | | | | |
|---|---|---|---|---|---|
| | 16 n: 39 | 8 n: 100 | 0.4 n: 50 | 0.2 n: 48 | 0.04 n: 51 |
| *Long bones* | | | | | |
| Femur | 2 | 3 | | | |
| Tibia | 1 | 1 | | | |
| Humerus | | 1 | | | |
| Radius | | 2 | | | |
| Total | 3 | 7 | | | |
| *Spine* | | | | | |
| Cervical | | 1 | | | |
| Thoracic | | 14 | | | |
| Lumbar | 1 | 16 | | 1 | 1 |
| Sacral | | 3 | 1+ | | |
| Coccygeal | | | | 1** | |
| Total | 1 | 34 | | | |
| *Others* | | | | | |
| Pelvic bones* | | 1 | | | |
| Sternum | | 1 | | | |
| Head | | 2 | 1+ | | |
| Total | 4 | 45 | 2 | 2 | 1 |

* These bones were not sectioned routinely
\+ Osteomas
** Chondroma
n: number of mice

In a few mice of the 8 $\mu$Ci and in one of the 0.4 $\mu$Ci group a prominent thickening and induration of fuchsinophilic fibres was observed in the pachymeninges; evidently as a result of irradiation.

### *Malignant lesions*

The number and induction time of various types of neoplasia appear in Table 4.

*Tumours of the hard tissues.* In the 16 $\mu$Ci group a total of four microscopic osteoblastic osteosarcomas were detected in three mice, whereas a total of 45 tumours appeared in 27 mice in the 8 $\mu$Ci group. Ten tumours were macroscopically and 35 microscopically detected. All osteosarcomas were of osteoblastic type with a moderate bone formation. In some tumours an abundancy of osteoclasts was found. In the 0.4 $\mu$Ci group two microscopic tumours with morphologic characteristics of osteomas appeared. The two tumours found in the two lowest dose groups were microscopically osteosarcomas.

*Anatomic localization.* The site of the bone tumours is indicated in Table 5. The

**Table 6**

*Hepatomas*

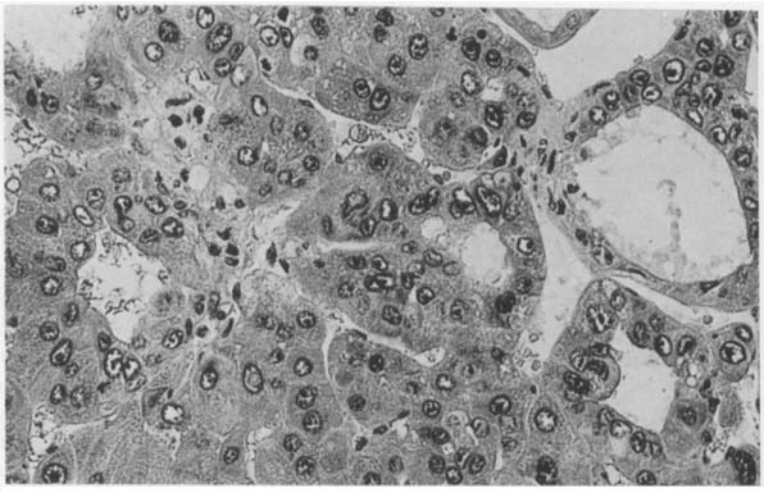
| Dose of $^{241}Am$ $\mu$Ci/kg | Number of mice | Number of hepatomas | Frequency (per cent) | 'Induction time' Days ± SE | Weight of liver x ± SE | Relative weight, liver x ± SE | Number and frequency of hepatomas with metastases (per cent) | |
|---|---|---|---|---|---|---|---|---|
| 0.4 | 50 | 36 | 72 | 665 ± 19 | 3.051 ± 0.238 | 13.7 ± 0.92 | 2 | 5.6 |
| 0.2 | 48 | 32 | 67 | 678 ± 22 | 3.192 ± 0.092 | 14.6 ± 1.01 | 4 | 12.5 |
| 0.04 | 51 | 34 | 67 | 708 ± 23 | 3.346 ± 0.072 | 14.4 ± 0.77 | 3 | 8.8 |
| Control | 50 | 28 | 56 | 731 ± 24 | 2.269 ± 0.167 | 10.3 ± 0.64 | 6 | 21.4 |

two osteomas in the 0.4 $\mu$Ci group were located in the skull and in a lumbar vertebrae, respectively. The chondroma was found in the first coccygeal vertebra.

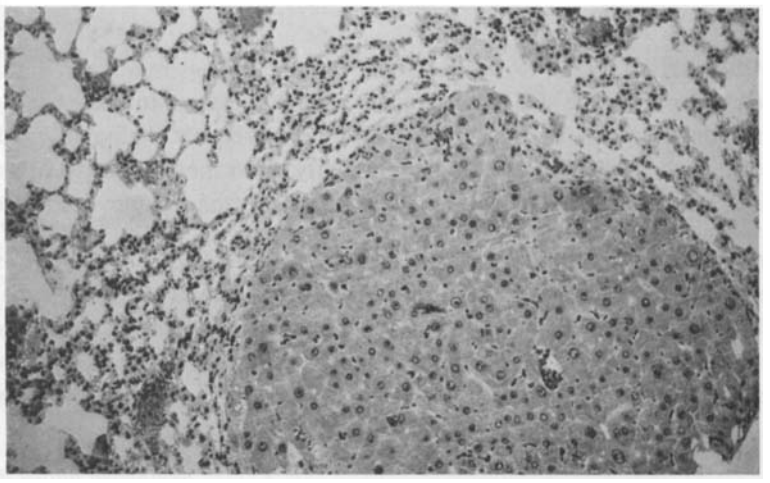
*Liver tumours.* The frequency, induction time and other data of the liver tumours are recorded in Table 6. In the two highest dose groups no liver tumours were observed. In the 0.4, 0.2 and 0.04 groups of mice as compared to controls there seems to be a general trend to a shortened tumour induction time. For the groups this difference is at the 95 per cent interval. The frequency of liver tumours is also slightly higher in the $^{241}Am$ treated mice, though not statistically separated from the control material. As regards the weight of the livers both the absolute ($p < 0.01$) and the relative weights ($p < 0.001$) are statistically increased over those of the controls. The tumours were of hepatocellular type and were classified as hepatomas according to the EULEP nomenclature. Metastasing hepatomas have been classified as malignant hepatomas (Fig. 7 a). Metastases were usually multiple and restricted to the lungs (Fig. 7 b) and thoracic wall and in some cases also to the kidneys.

*Lymphoreticular tumours.* Ten lymphosarcomas were found in the 8 $\mu$Ci group. They were of lymphatic and non-thymic type. A varying infiltration of lymphoid cells usually appeared in the tissues surrounding the lumbar and thoracic vertebrae. The mean latency time (Table 4) was 278 ± 26 days. In the 0.4 $\mu$Ci group three lymphosarcomas appeared after 660, 714 and 863 days, respectively. Two of these were restricted to the mesenteric lymph node and one to the spleen. In the 3 cases in the 0.04 $\mu$Ci group the thymus, spleen and lymph glands, but not the marrow, were involved in all cases. Around the thoracic vertebrae a 'lymphoma' also appeared in one case. The induction times were 691, 749 and 785 days, respectively. The two cases of lymphosarcomas in the control group appeared after 594 and 621 days, respectively and mainly affected the mesenteric lymph node.

*Lung tumours.* In Table 4 the number and induction time of the lung tumours are recorded. Generally the age of the animals and not the dose of $^{241}Am$ was decisive



a



b

Fig. 7. a) Liver malignant hepatoma, (hepatocellular carcinoma) mouse 665 days after injection of 0.2 $\mu$Ci/kg $^{241}$Am. H–E $\times$ 250.

b) Lung with metastases of malignant hepatoma. Same animal as in Fig. 6 a. H–E $\times$ 100.

of the frequency of the lung tumours. Thus no tumours were found in the 16 $\mu$Ci group and only one in the 8 $\mu$Ci group whereas a considerable number occurred in the older mice of the lowest dose groups and the control material. All tumours were alveolar cell carcinomas (Fig. 8), in one case combined with lung adenomatosis (Table 7). They occurred either as solitary or multiple tumours. The solitary tumours

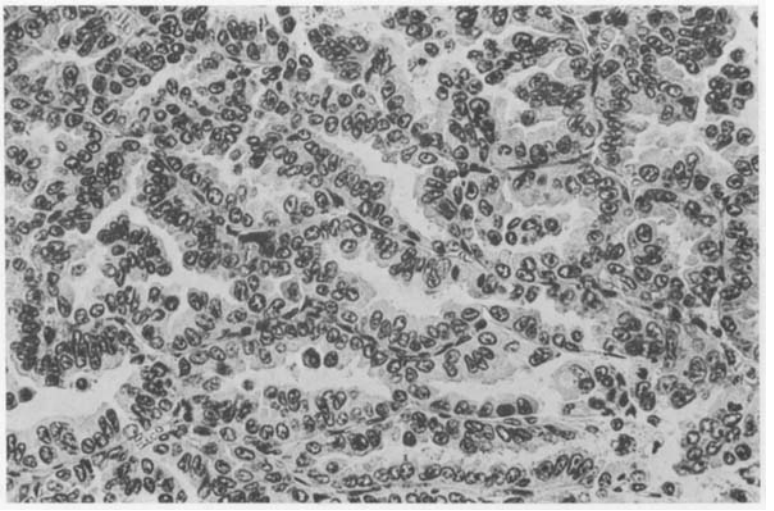


Fig. 8. Alveolar cell carcinoma, mouse 610 days after injection of 0.1 $\mu$Ci/kg $^{241}$Am. H–E ×250.

could reach a considerable size (hazel-nut or larger) and were in these cases the only cause of the death of the animal. The tumours were highly malignant with a considerable ability to spread inside the pleural cavity or to metastasize to other tissues. In one case metastases were found in both adrenals, in one kidney and in the liver.

*Other tumours.* Three tumours of the vessels were detected. Two of those which were found in the 0.4 and 0.04 $\mu$Ci group, respectively, were cavernous haemangiomas of the subcutaneous tissue and the other in the control material was an angiosarcoma involving the sternal and vertebral marrows and surrounding paravertebral tissues and the spleen. One cortical carcinoma of the kidney was found in the 0.2

**Table 7**

*Lung tumours*

| Dose of $^{241}$Am $\mu$Ci/kg | Number of alveolar cell carcinomas | Induction time Days ± SE | Percentage |
|---|---|---|---|
| 16 | | — | — |
| 8 | 1 | 280 | 1 |
| 0.4 | 5 | 746 ± 51.7 | 8 |
| 0.2 | 11 | 730 ± 45.4 | 25 |
| 0.04 | 15 | 828 ± 31.9 | 29 |
| Control | 12 | 789 ± 31.5 | 24 |

**Table 8**

*Causes of death*

| Diagnoses | $^{241}$Am $\mu$Ci/kg body weight | | | | | Control |
|---|---|---|---|---|---|---|
| | 16 | 8 | 0.4 | 0.2 | 0.04 | |
| Bone marrow aplasia | 5 (276±7) | 10 (306±9) | — | — | — | — |
| Bone marrow aplasia in combination with | | | | | | |
| Liver degeneration | — | 9 | — | — | — | — |
| Myocardial degeneration | — | 5 | — | — | — | — |
| Haemorrhagic diathesis and liver degeneration | — | 3 | — | — | — | — |
| Intestinal haemorrhagic +inanition | 26 (134±6) | 4 (287) | — | — | — | — |
| Inanition | 8 (207±20) | 20 (313±12) | 3 (807) | 1 | 1 | 11 (800±16) |
| Liver degeneration | — | 6 | 1 | 2 | 3 | 1 |
| Lympho-reticular tumours | — | 10 (278±26) | 3 (746) | — | 3 (742) | 2 (608) |
| Osteosarcomas | — | 7 (352±15) | — | — | — | — |
| Hepatomas | — | — | 24 (661±22) | 21 (698±25) | 26 (687±29) | 17 (718±32) |
| Alveolar cell carcinomas | — | 1 | 4 | 7 | 7 | 6 |
| Adrenal cortical carcinoma | — | — | 1 | — | — | — |
| Infections | — | 2* | 4*** | 3**** | 3***** | 2** |
| Other diagnoses | — | 2 | | 2 | 1 | 1 |
| Not stated (at autopsy) | — | 19 | 6 | 7 | 6 | 7 |
| Not stated (postmortal autolysis) | — | 2 | 4 | 5 | 1 | 3 |
| Total | 39 | 100 | 50 | 48 | 51 | 50 |

Days±SE within parenthesis

* One acute purulent bronchopneumonia. One fibrino purulent peritonitis.

** One chronic pyelonephritis. One acute purulent vesiculitis with peritonitis.

*** Two purulent panophthalmitis. One purulent peritonitis. One chronic pyelonephritis.

**** One acute purulent bronchopneumonia. One multiple abscesses in the head. One chronic pyelonephritis and multiple abscesses in the head.

***** One purulent pneumonia. One chronic pyelonephritis. One chronic glomerulonephritis.

$\mu$Ci group. In the 0.04 $\mu$Ci group a tumour was also found in the orbital tissue but could not be identified due to post-mortal autolysis.

In the 0.4 $\mu$Ci group one cortical carcinoma was found. There was also a considerable hyperplasia of the adrenal medulla. It was, however, not possible to determine whether these lesions were true tumours (phaeochromocytomas) or not (Fig. 4).

*Causes of death.* From Table 8 it is evident that in the two highest dose groups the most probable cause of death was, directly or indirectly, associated with a disturbance of the haematopoietic system. The disturbed haematopoiesis was also in many cases combined with other lesions as well (Table 8). Thus, the haemorrhages of the gut were probably related to an enteritis of the haemorrhagic type.

At autopsy the cause of death could not be stated in 19 mice belonging to the 8 $\mu$Ci group. All these mice had a bone marrow hypoplasia which, however, was insufficiently severe to explain the death.

Each of the 7 mice dying from osteosarcoma in the 8 $\mu$Ci group had all more than one tumour out of which at least one was of macroscopic extent.

In the degenerated livers multiple necroses and a severe wide-spread fatty degeneration, hyperemia and ectatic vessels existed.

In two cases (in the 8 $\mu$Ci group) spontaneous fractures of the vertebral bodies complicated with a compression of the spinal cord may be a probable cause of death (Table 8, 'other diagnoses'). Within the low level groups and between these and the control group no significant differences as regards the cause of death existed. In all the groups the majority of the mice died with hepatomas. The frequency was approximately the same, being 48, 44, 51 and 34 per cent in the 0.4, 0.2 and 0.04 $\mu$Ci groups and the controls, respectively. It should, however, be pointed out that many of the hepatomas occurred together with a severe chronic glomerulonephritis.

The adrenal tumours detected in the 0.4 $\mu$Ci group after 452 days had a weight of 31.7 mg. The tumour had ruptured and caused a severe bleeding to the peritoneal cavity. Under the heading 'other diagnoses' (Table 8) a carcinoma of the kidney and an angiosarcoma were found in the 0.2 $\mu$Ci groups, respectively. In the 0.04 $\mu$Ci group one mouse had mesenteric disease and one a tumour of the eye.

## Discussion

In the present investigation doses of 16 and 8 $\mu$Ci $^{241}$Am/kg induced a bone tumour incidence of 7.7 and 27 per cent, respectively. In the two lowest dose levels of 0.2 and 0.04 $\mu$Ci/kg tumours also appeared, but in a low frequency (2 %). In rats TAYLOR et coll. found an incidence of 47 per cent after a dose of 7.0 $\mu$Ci $^{241}$Am/kg and BENSTED et coll. 21 per cent after a dose of 2.5 $\mu$Ci $^{241}$Am/kg.

It is interesting to compare the carcinogenicity of $^{90}$Sr with that of $^{241}$Am. $^{90}$Sr doses between 1 000 and 800/$\mu$Ci kg seem to give an optimum bone tumour incidence of about 90 to 95 per cent whereas a dose of 200 $\mu$Ci/kg gives an approximate tumour incidence of about 35 per cent and 50 $\mu$Ci/kg about 6 per cent. Even if it could be anticipated that lower $^{90}$Sr doses could produce bone tumours, $^{241}$Am therefore seems to be a more effective carcinogen than $^{90}$Sr since it can induce tumours at significantly lower dose levels. On the other hand, the frequency of tumours induced by $^{241}$Am does not seem to be so high as that induced by optimum doses of $^{90}$Sr. Multiple tumours (2 to 3 or even more) in individual animals are frequent in $^{90}$Sr

treated mice at optimum doses. This, however, does not seem to be the case after $^{241}$Am-administration. The reason for this might be difficult to explain but may be associated with factors such as survival times, volume of bone tissue irradiated, localization and destructive effect in the body.

In the present investigation the bone destruction was in general more marked and reparative processes less evident after doses of 8 and 16 $\mu$Ci $^{241}$Am/kg than after 700 to 800 $\mu$Ci $^{90}$Sr/kg which may indicate that the Am-dose levels were superoptimum and leading to an 'over-kill' of cells. This is important since reparation and the ability to tissue proliferation seems to be a necessary prerequisite for tumour induction. $^{90}$Sr tumours like $^{241}$Am tumours also emanate from structures inside the bone. Those induced by $^{90}$Sr usually starts either from osteoblastlike cells along the endosteal linings as small bone producing buds or just below the epiphyseal cartilage in the diaphysis. They may, however, also arise like islands inside the bone marrow from multipotent reticular cells with the ability to form osteoid. The $^{241}$Am tumours on the other hand were preferably found in immediate contact with the epiphyseal cartilage or very often in lacunar areas of compact bone in close vicinity to the epiphyseal cartilage and not in the diaphyseal part. It is also notable that the majority of the $^{241}$Am tumours were found in the vertebrae, whereas after optimum $^{90}$Sr doses the long tubular bones are the most frequent site of osteosarcomas. At superoptimum $^{90}$Sr doses the spine is, however, the most frequent site of tumour induction, whereas at low doses there is a predomination in the diaphyses of the long bones (NILSSON 1970).

Most $^{90}$Sr induced tumours arising from endosteal linings are of osteoblastic type whereas those emanating as islands in the bone marrow are predominantly of fibroblastic type. After high and optimum doses osteoblastic osteosarcomas predominate, whereas lower doses give an increased incidence of fibroblastic osteosarcomas (NILSSON 1970). The $^{241}$Am tumours on the other hand were all of osteoblastic type.

Using doses of $^{90}$Sr between 800 and 400 $\mu$Ci/kg angiosarcomas of the bone marrow do appear with an incidence of about 2 and 4.5 per cent, respectively. With $^{241}$Am no tumours of this type were found in the bone marrow in spite of the fact that the bone marrow was usually heavily injured in the proximal and distal parts of the long bones. This difference may be associated with the fact that the $\beta$-rays of $^{90}$Sr and $^{90}$Y has a much longer range, exposing larger volumes of the bone marrow to a 'cross fire'. It should also be pointed out that the lesion of the bone marrow after administration of $^{241}$Am differed from that of $^{90}$Sr. With $^{90}$Sr the lesions usually are located proximally and distally in the long bones during the first few months after the administration of the nuclide, whereas later on these parts regenerate and the marrow of the diaphysis atrophies. After injection of $^{241}$Am the lesions in the disphysis were never prominent.

With the high doses of $^{90}$Sr (1 600 $\mu$Ci/kg) carcinomas of the mucous membranes of the head in close vicinity of the bone, are induced in a high frequency (NILSSON

1968). This was not the case with $^{241}$Am with the doses employed. With $^{241}$Am as with $^{90}$Sr lymphosarcomas are induced. With high doses of $^{90}$Sr a fairly high number of these emanates from the thymus whereas with lower doses the majority seem to arise mainly in the bone marrow (NILSSON 1971). All the lymphosarcomas induced by the 8 $\mu$Ci $^{241}$Am were generalized lymphosarcomas of short induction time (278 $\pm$ 25.8 days) and probably originating from the bone marrow. The lymphosarcomas found in the lower dose groups and in the control group were not detected until after 600 to 700 days. They were usually restricted to a lymph node and were not generalized. Furthermore the frequency of the low dose lymphosarcomas did not differ from that of the control material.

As regards lesions in the soft tissues $^{241}$Am is as a consequence of its widespread localization much more destructive than $^{90}$Sr. In the testis the lesions induced by $^{241}$Am are largely irreversible and progressive. $^{90}$Sr on the other hand produces heavy abnormalities initially but they regenerate after about 2 months following administration of the nuclide. In the two highest dose groups $^{241}$Am had a highly destructive effect of the seminiferous epithelium whereas such an effect in the lower doses is questionable. Whether or not $^{241}$Am could influence upon the development of the vascular lesions found is difficult to prove. It might, however, be anticipated that the nuclide can accelerate the rate of development of these lesions. Thus not less than 81 per cent of the testes had vascular lesions in the 8 $\mu$Ci/kg group at a mean age of 301 days whereas the first case with such lesions in the control group—though reaching 93 per cent—were not found until 580 days after the start of the experiment. In the 8 $\mu$Ci group the first lesion was found already after 165 days. In the lower dose groups the tendency to an earlier appearance persists. The explanation for this might hypothetically be associated with a general effect of the physical health of the $^{241}$Am-treated animals. $^{241}$Am also induces lesions in the liver and adrenals, which, however, does not seem to be the case with $^{90}$Sr.

In the two highest $^{241}$Am groups liver tumours did not appear. The only reason for this seems to be the great reduction of the survival time. In the CBA strain the frequency of liver tumours is very high in males but the tumours do not start to occur until the mice have reached an age of about 450 days. In the low dose groups of the present material the frequency of these tumours is somewhat, although not significantly higher than in the control material (Table 6). On the other hand the shortened latency time and the increased liver weight of the $^{241}$Am-treated animals seem to confirm the general opinion that radiation may accelerate tumour formation in animals with a high normal incidence of tumours.

In a previous report (HAMMARSTRÖM & NILSSON) it has been stated that $^{241}$Am accumulates strongly and for a long time mostly in the zona glomerulosa of the adrenals. Others (BENSTED et coll., TAYLOR et coll.) have found cortical tumours in rats. The low incidence of adrenal tumours found in the present investigation is therefore most likely associated with the fact that the dose levels employed were not optimum for induction of cortical tumours. $^{241}$Am in the doses employed do

not seem to influence the incidence of lung tumours which also seems to be the case for the other soft tissue tumours found.

$^{90}Sr$ even in high doses does not seem to induce kidney lesions. In the present material the findings indicate a nephrotoxicity for the two highest $^{241}Am$ doses as evidenced by the early appearing fibrosis in mainly the cortical parenchyma. No such lesions were found among control mice of comparable age. The kidney lesions found in the low dose groups of mice and in the control material were on the other hand all of about the same type and frequency, including chronic glomerular nephritis with hyalinization of the glomerular capsule, productive inflammation, degeneration of tubular epithelium and a high frequency of hyaline casts. In the low dose range $^{241}Am$ therefore does not seem to have any significance for the development of the lesions found.

As regards the non-malignant lesions it is obvious that $^{241}Am$ had a very destructive effect of the bone marrow which developed aplasia or hypoplasia with destruction of the sinusoidal system and formation of a fatty marrow. In many cases also thrombosis of the vascular system occurred and a more or less evident fibrosis. Generally the spleen was also heavily injured contrary to what is found after $^{90}Sr$-administration. This explains the numerous cases of death from haematopoietic insufficiency and related findings such as hypoxemic degeneration of the liver, myocardial necrosis and evidence of haemorrhagic diathesis. The haemorrhages in the intestine might also be related to the haematopoietic disorder with or without complication with terminal bacterial infections. The high frequency of inanition like the increased frequency of infection such as pneumonia, purulent vesiculitis, peritonitis, purulent inflammations of the orbital tissues and in the paradental tissues etc. is probably also related to the impaired haematopoietic condition and an incapacitation of the immunological system.

## SUMMARY

Male CBA-mice were injected intraperitoneally with different doses of $^{241}Am$-citrate (16, 8, 0.4, 0.2, 0.04 $\mu$Ci/kg). The two highest doses were highly destructive of the haematopoietic tissues, testes and bone tissue. The highest frequency of induced tumours of the skeleton and haematopoietic tissue was found in the 8 $\mu$Ci group. In the liver, adrenal glands, kidney and heart degenerative lesions were found mainly in the higher dose groups. In the lower dose groups degenerative lesions seemed to appear earlier and at a higher frequency than in the control group.

## ZUSAMMENFASSUNG

Männlichen CBA-Mäuse wurden intraperioneal verschiedenen Dosen von $^{241}Am$-Zitrat (16, 8, 0,4, 0,2, 0,04 $\mu$Ci/kg) injiziert. Die beiden höchsten Dosen waren für das hämatopoietische Gewebe, die Testes und das Knochengewebe hochgradig destruktiv. Die höchste Frequenz an induzierten Tumoren des Skeletts und des hämatopoietischen Gewebes fand sich bei der 8 $\mu$Ci Gruppe. Vorzüglich bei den höheren Dosis Gruppen fanden sich degene-

rative Veränderungen in der Leber, der Niere und dem Herzen. In den niedrigeren Dosis Gruppen schienen degenerative Veränderungen frühzeitiger und in höherer Frequenz als in der Kontrollgruppe aufzutreten.

## RÉSUMÉ

Du citrate de $^{241}$Am a été injecté dans la cavité péritonéale de souris CBA mâles à diverses doses (16, 8, 0,4, 0,2, 0,04 $\mu$Ci/kg). Les deux doses les plus fortes ont eu un effet destructeur important sur les tissus hématopoïétiques, les testicules et le tissu osseux. C'est le groupe ayant reçu 8 $\mu$Ci qui a eu la plus grande fréquence de tumeurs induites du squelette et du tissu hématopoïétique. Des lésions dégénératives du foie, des glandes surrénales, des reins et du coeur ont été constatées surtout chez les groupes d'animaux ayant reçu les doses élevées. Dans les groupes ayant reçu de faibles doses, les lésions dégénératives semblent apparaître plus tôt et avec une plus grande fréquence que dans le groupe témoin.

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