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CYTOPLASMIC ULTRAVIOLET EXTINCTION OF STRONTIUM-90-INDUCED FIBROBLASTIC OSTEOSARCOMAS CORRELATED TO HISTOLOGIC APPEARANCE AND ULTRASTRUCTURE

by

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The development of ^{90}Sr-induced fibroblastic osteosarcomas through different morphologic stages of oncogenesis and the histologic appearance of overt tumours have been well established in mice (NILSSON 1962, NILSSON & ULLBERG 1962). The present study is concerned with the general histology of ^{90}Sr-induced tumours, and the question whether they follow the general tumour pattern, with correlation between cytoplasmic RNA, active cell proliferation and the histologic and clinical signs of malignancy (AMBS & THORELL 1959, CASPERSSON & SANTESSON 1942, MOBERGER 1954, MOBERGER et coll. 1952, THORELL 1947). This has been investigated histologically, by ultraviolet cytophotometry and by electron microscopy of ^{90}Sr-induced tumours.

Material and Methods

Male CBA mice, 75 days old, were injected intraperitoneally with ^{90}Sr, corresponding to 0.7 to 0.8 μCi/g bodyweight. The mice weighed between 22 and

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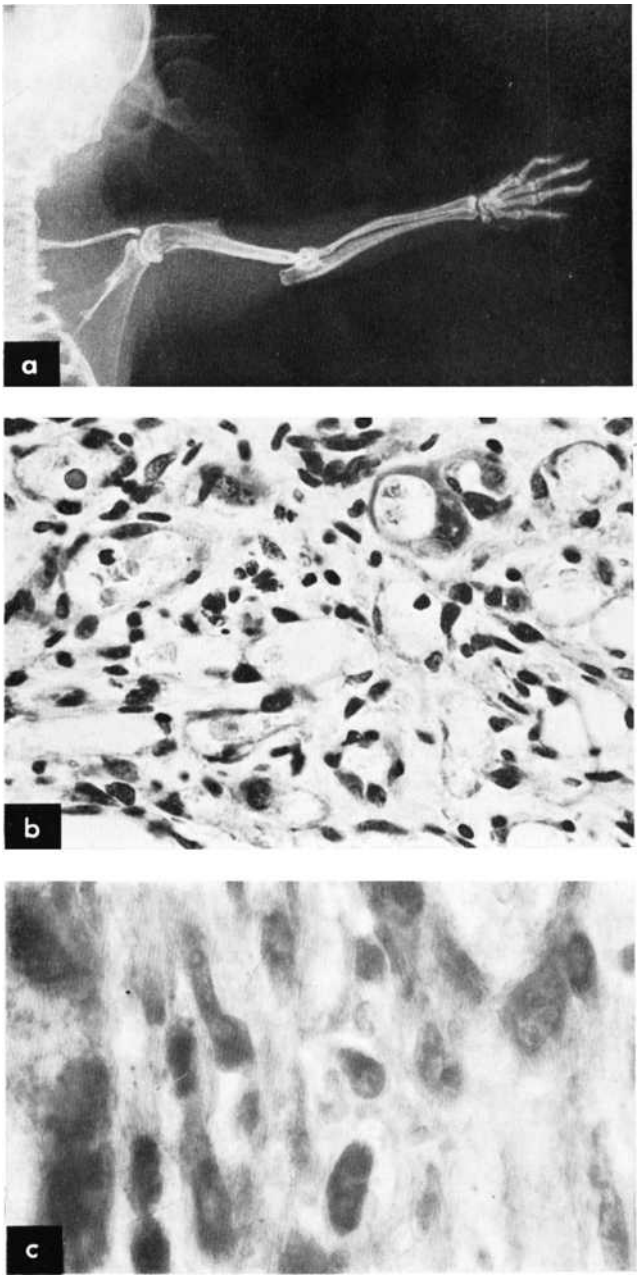

Fig. 1. Group 3. Tumour from left humerus, 276 days after ^{90}Sr injection. Proliferation of reticular cells and pleomorphism. a) Roentgenogram. b) van Gieson. ×500. c) Ultraviolet. ×1000.

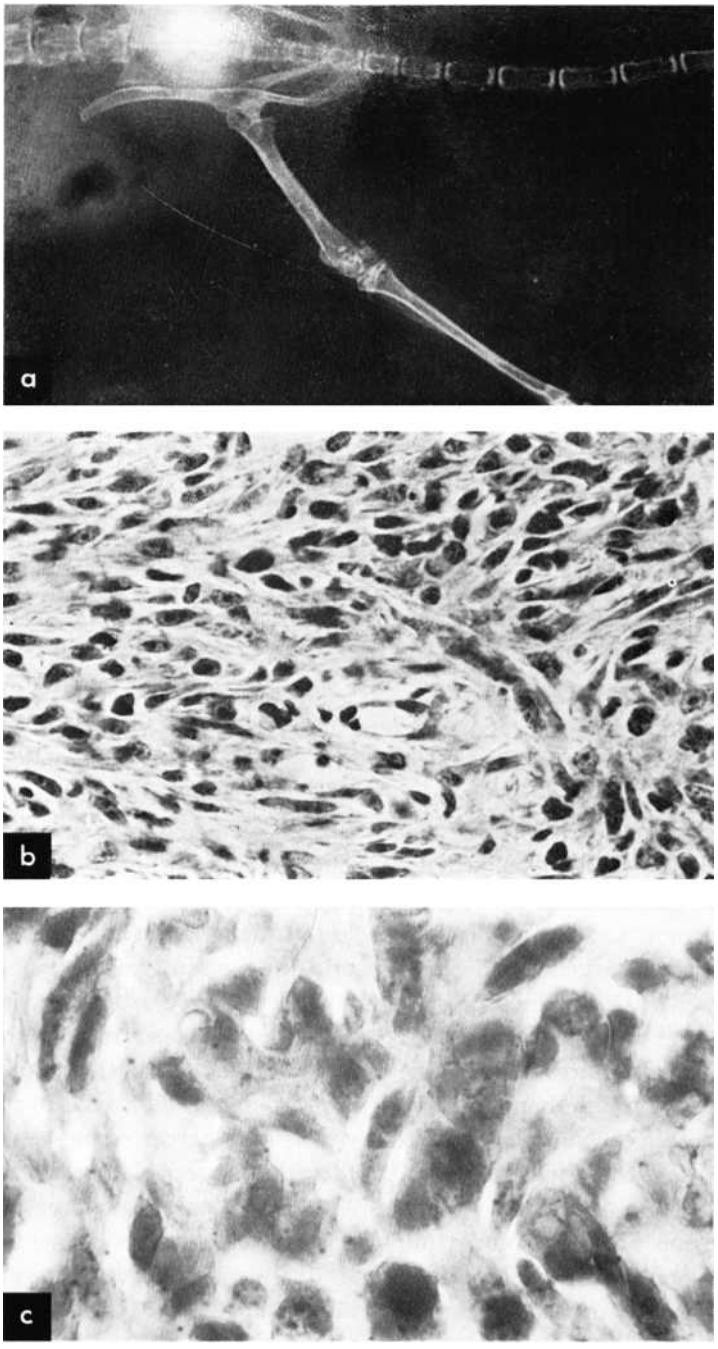

Fig. 2. Group 4. Intramedullary tumour in right femur, 269 days after administration of ^{90}Sr. a) Roentgenogram. b) van Gieson. ×500. c) Ultraviolet. × 1000.

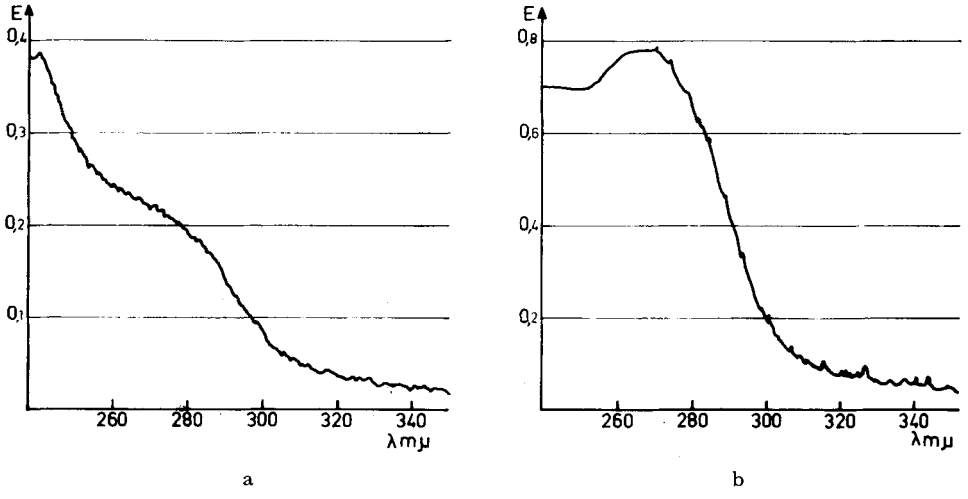

Fig. 3. Ultraviolet extinction spectrum of cytoplasm. a) Reticular cell with normal structure. b) Tumour cell group 4. A specific nucleic acid absorption peak is visible.

23 grams initially and were fed and kept under constant conditions before being killed at intervals between 190 and 377 days after the ^{90}Sr injection.

For the histologic study, bone tissue were fixed in 5 % neutral formalin for 6 hours, rinsed in tap water for 24 hours and decalcified under vacuum in 20 % formic acid for 3 hours, dehydrated, embedded in paraffin and sectioned at 5 μ. All sections were stained by van Gieson's method.

All the tumours used for this study were predominantly fibroblastic osteosarcomas with scanty osteoblastic elements and little osteoid formation. The ultraviolet analysis was performed on cells with evident fibroblastic character.

The material could be classified by the microscopic appearance of the reticular cells in the bone marrow into nine stages of tumour development. Group 0 represented hypoplastic bone marrow with morphologically normal reticular cells and group 1 aplastic bone marrow with swelling and increased number of reticular cells. In group 2 there was a distinct proliferation of reticular cells but the tissue had the histologic appearance of fibrosis without neoplastic character. Group 3 (Fig. 1), group 4 (Fig. 2) and group 5 represented tumours situated completely within the bone marrow. The different groups were distinguished by the size of the tumour, cellularity and pleomorphism. Groups 6 to 8 consisted of tumours infiltrating and breaking through the compact bone and infiltrating the sur-

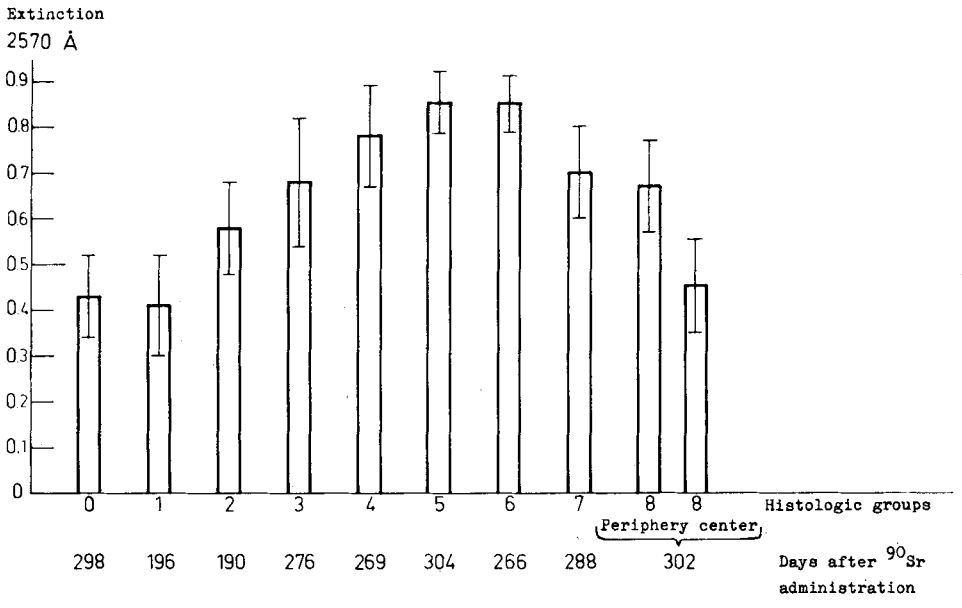

Fig. 4. Cytoplasmic extinction in cells from successively more advanced tumours (see text). Each column represents mean value for 20 cells and standard deviation.

rounding soft tissue. The main differences between groups 6, 7 and 8 were successively increasing cellularity and size.

The femurs collected for electron micrography belonged to the histologic groups 0, 2, 5 and 8. The material was fixed in Os_4, embedded in epon and sectioned with an LKB-ultratome with a glass knife, stained with lead citrate and examined in a Siemens Elmiscop IA.

For the ultraviolet microphotometry, paraffin sections, 3 μ thick, were deparaffinized in chloroform, placed on quarz slides, immersed in glycerol for 2 days and mounted in glycerol. Cytoplasmic areas of fibroblastic cells were analysed in a UV spectrophotometer (CHANCE et coll. 1959) by recording continuous spectra from 240 to 350 mμ. The instrument was used in the present investigation with a Zeiss UV achromat condensor stopped down to NA 0.4 and ultrafluar objective NA 0.85. In the cytoplasm of tumour cells a specific absorption peak could generally be registered close to 260 mμ. In apparently normal fibroblasts this peak was lower, or could not be detected at all (Fig. 3).

An estimate of the RNA contribution to the absorption at 260 mμ was obtained by recording the absorption before and after digestion and extraction of the RNA. Digestion was done with 1 % RNase at 37° C for 60 minutes and

Table

Percentage reduction of cytoplasmic extinction values of group 5 tumour cells after digestion with RNase and extraction with perchloric acid. E_{2570}

Before digestion	After digestion	Percentage reduction
0.75	0.56	29
0.76	0.42	45
0.76	0.40	47
0.76	0.47	39
0.64	0.33	48
0.23	0.12	48
0.70	0.41	44
0.48	0.23	52
0.46	0.22	48
0.43	0.31	28
0.38	0.22	42
0.60	0.30	50
0.43	0.32	26
0.57	0.42	26
0.58	0.37	36
0.84	0.46	45
0.40	0.24	40
0.49	0.36	26
0.51	0.35	31
0.57	0.33	42
0.85	0.43	51
0.53	0.30	43
0.41	0.25	39
0.50	0.27	46
0.48	0.28	41
	Mean ± SD	40 ± 8

extraction with 1 % perchloric acid at 4° C for 20 minutes followed by rinsing in water for 2 hours (PERRY et coll. 1961). Identical points in the cytoplasmic areas were measured according to THORELL (1947) before and after digestion and extraction in phosphatic buffer saline pH 7.2.

Results and Discussion

The tumours will be considered here solely in relation to their histologic appearance and not in relation to their induction time since these are not necessarily correlated with each other.

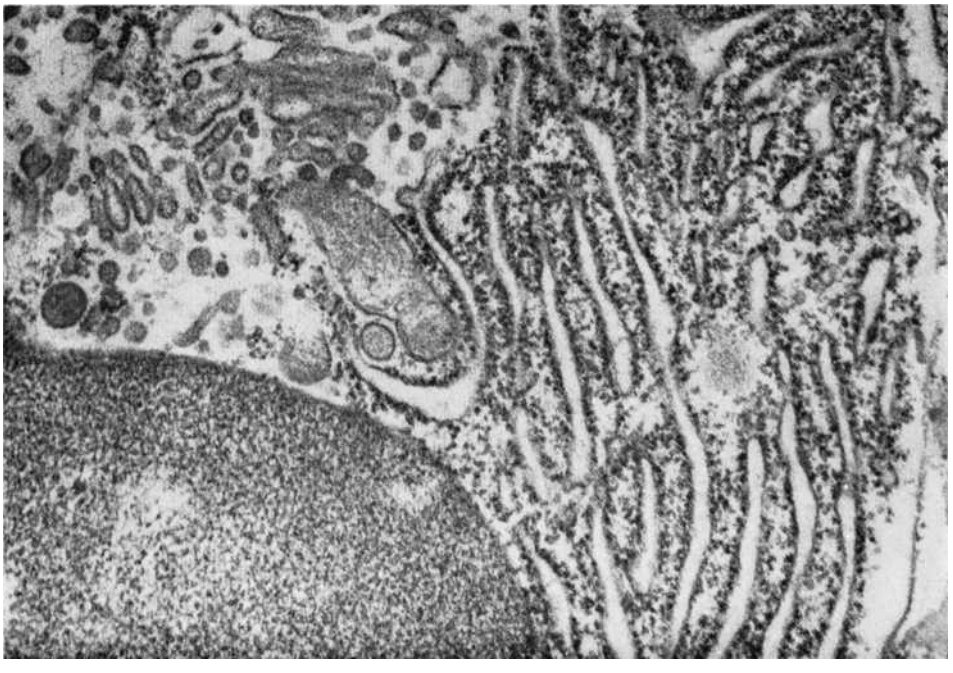

Fig. 5. Group 8. Tumour cell with abundant rough endoplasmic reticulum and aggregation of ribosomes. × 54 000.

Digestion with RNase indicated that about 40 % of the cytoplasmic ultraviolet extinction values were attributable to RNA (see Table). The values could clearly be correlated to the histologic groups (Fig. 4). The morphologically more advanced stages of intramedullary tumour development were associated with increasing extinction values. The extramedullary tumour cells, however, had lower extinction values than the peak values obtained for intramedullary tumours. In overt tumours, the cells towards the periphery also contained apparently greater amounts of cytoplasmic RNA than those in the center (cf. CASPERSSON & SANTESSON 1942).

The cytoplasmic ultraviolet extinction reflects both ribosomal and soluble RNA. The ribosomal fraction can be further analysed with the ultrastructural technique.

The electron micrographs revealed no significant differences between normal reticular cells and group 2 tumour cells while the established tumour cells had some significant alterations.

The ribosomes were generally much more abundant in cells from established

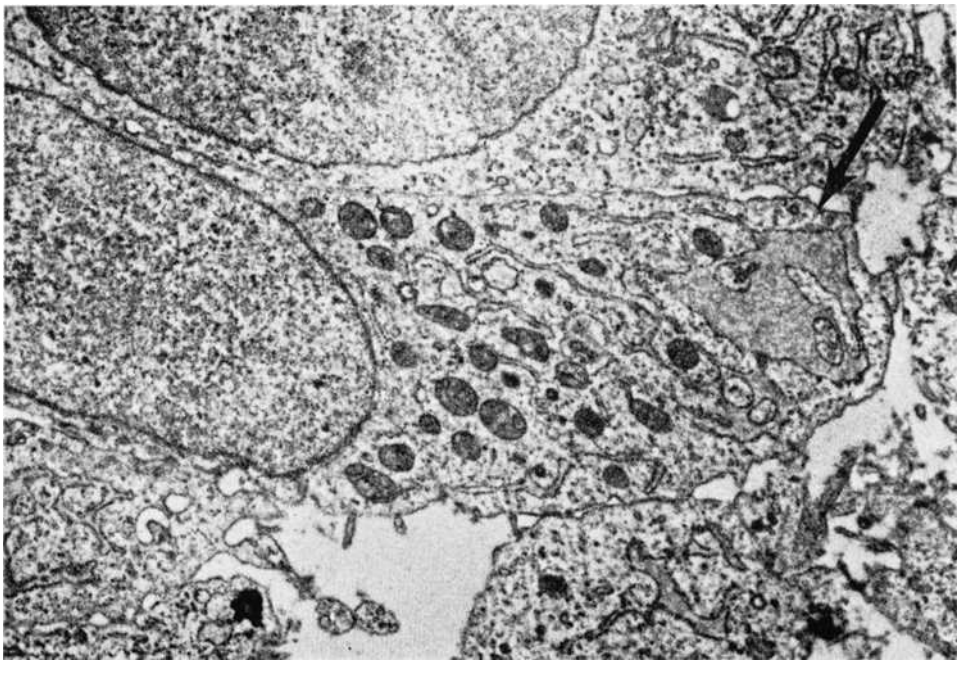

Fig. 6. Group 5. Tumour cell with numerous mitochondria and sac-like distension of endoplasmic reticulum (arrow). ×12 000.

tumours, and in some of the tumour cells the endoplasmic reticulum was dense and almost exclusively of the rough type (Fig. 5). In other cells it was scanty, and the ribosomes were mostly free. Small aggregations of ribosomes were found in reticular cells of group 0 and 2 but they were larger and more numerous in cells of established tumours, especially in those with a scanty endoplasmic reticulum. In some of the group 8 tumour cells there were few free and bound ribosomes; in these cells, other cytoplasmic organelles were also poorly developed, and they seemed to correspond to the centrally situated cells with low UV extinction values. The endoplasmic reticulum of tumour cells characteristically had sac-like distensions filled with electron dense material (Fig. 6). Such distensions have been described in strontium-induced fibroblastic osteosarcomas and other tumours (NILSSON 1962) but also in non-tumourous inflammatory tissue (PEACH et coll. 1961), and it has been demonstrated by GOLDBERG & GREEN (1964) that they are the site for collagen formation.

The size and shape of the mitochondria were irregular both in tumourous and non-tumourous reticular cells but the internal mitocondrial architecture was un-

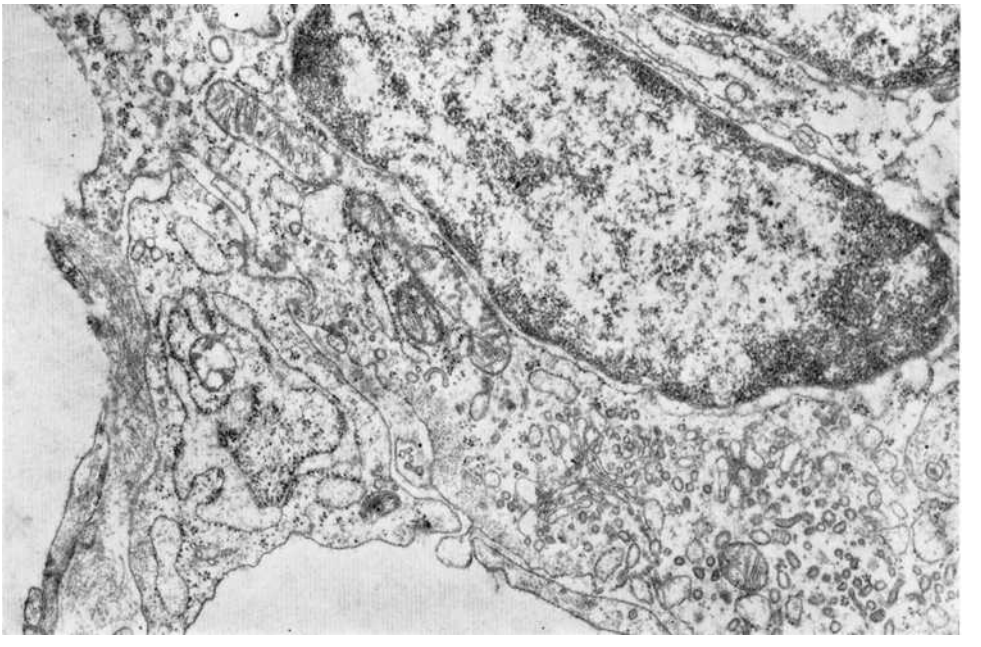

Fig. 7. Group 0. Reticular cells with scanty electron dense material in the nucleus, scanty endoplasmic reticulum and few aggregations of free ribosomes. × 24 000.

changed. In some of the cells from the central parts of the group 8 tumours, however, the number of mitochondria was markedly decreased.

The nuclei of tumour cells were often lobulated and contained electron dense material with over-all spread in the nucleus (Figs 5 and 6), while the normal reticular cells had scanty dense material concentrated along the nuclear membrane (Fig. 7).

The abundance of endoplasmic reticulum seems to vary considerably in different kinds of tumour cells. In many carcinomas investigated, the ergastoplasm has been found to be scanty, and the majority of the ribosomes are free. In other types of tumours — Rous sarcomas, osteosarcomas, myelomas and pituitary adenomas — the amount of ergastoplasm has been reported to be increased in comparison with the corresponding normal cells (EPSTEIN 1957, PEACH et coll. 1961). It is obvious that the cells with abundant ergastoplasm of the rough type have a very high UV extinction, and it is possible that they are responsible for the formation of collagen, which is present in large amounts in these tumours. Further investigations are in progress to elaborate on this point.

SUMMARY

^{90}Sr-induced osteosarcomas in different stages of development were investigated by means of ultraviolet microphotometry and electron microscopy. Successively increasing UV extinction values could be correlated to morphologically more advanced stages of tumour development.

ZUSAMMENFASSUNG

Osteosarkome in verschiedenen Entwicklungsstadien, die durch Strontium 90 verursacht waren, wurden mittels der ultravioletten Mikrophotometrie und Elektronenmikroskopie untersucht. Successiv zunehmende UV-Auslöschungswerte konnten mit morphologisch fortgeschrittenen Stadien der Tumorentwicklung korreliert werden.

RÉSUMÉ

Des ostéosarcomes induits par le ^{90}Sr, à différents stades de développement, ont été examinés par microphotométrie en ultra-violet et par microscopie électronique. On a pu établir une corrélation entre les valeurs croissantes d'extinction du cytoplasme en ultra-violet et les stades d'avancement morphologique du développement des tumeurs.

REFERENCES

AMBS E. und THORELL B.: Die Cytogenese bei der Virus leukemia des Huhnes. Acta haemat. 21 (1959), 284.

CASPERSSON T. and SANTESSON L.: Studies on protein metabolism in the cells of epithelial tumours. Acta radiol. (1942) Suppl. No. 46.

CHANCE B., PERRY R., THORELL B. and ÅKERMAN L.: Highly sensitive recording microspectrophotometer. Rev. Sci. Instrum. 36 (1959), 735.

EPSTEIN M. A.: The fine structural organisation of Rous tumour cells. J. biophys. biochem. Cytol. 3 (1957), 851.

GOLDBERG B. and GREEN H.: An analysis of collagen secretion by established mouse fibroblast lines. J. Cell Biol. 22 (1964), 227.

MOBERGER G.: Malignant transformation of squamous epithelium. Acta radiol. (1954) Suppl. No. 112.

— RINGERTZ N. and HÅKANSSON E.: Study in nucleic acid metabolism in gliomas. Acta Un. int. Cancr. 8 (1952), 591.

NILSSON A.: ^{90}Sr-induced osteosarcomas. Acta vet. scand. 3 (1962), 122.

— Histogenesis of ^{90}Sr-induced osteosarcomas. Acta vet. scand. 3 (1962), 185.

— and ULLBERG S.: Uptake and retention of strontium 90 in mouse tissues studied by whole animal autoradiography and impulse counting. I. Acta radiol. 58 (1962), 81.

— — Uptake and retention of strontium 90 in strontium-90-induced osteosarcomas. II. Acta radiol. 58 (1962), 168.

PEACH R., WILLIAMS G. and CHAPMAN J. A.: A light electron optical study of regenerating tendon. Amer. J. Path. 38 (1961), 495.

PERRY R. P., ERRERA M. and HELL A.: Kinetics of nucleoide incorporation into nuclear and cytoplasmic RNA. J. biophys. biochem. Cytol. 11 (1961), 1.

THORELL B.: Studies on the formation of cellular substances during blood cell production. Henry Klimpton, London 1947.