FROM THE DIVISION OF RADIOBIOLOGY (DIRECTOR: DOCENT A. NELSON),
RESEARCH INSTITUTE OF NATIONAL DEFENCE, SUNDBYBERG, SWEDEN.

PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF ^{90}Sr IN MICE

Development of carcinomas in the mucous membranes of the head

by

AGNAR NILSSON

Much work has been done on ^{90}Sr-induced neoplasms but it seems that the attention has been mainly concentrated on the induction of osteosarcomas in the skeleton for a study of the histogenesis and development of such lesions, and their possible dose dependency (ANDERSON, ZANDER & KUZMA 1956, FINKEL 1959, FINKEL & BISKIS 1959, FINKEL, BERGSTRAND & BISKIS 1961, KOWALEWSKI & RODIN 1964, NILSSON 1962, LITVINOV 1963, OWEN, SISSONS & VAUGHAN 1957, and others). With respect to skeletal malignancies, the problem of dose relationships has not yet been unambiguously solved, and even less is known concerning neoplasms deriving from other tissues, such at hematopoietic organs and mucous membranes in close contact with bone.

A comprehensive study has been made in mice on dose relationships and the pathologic effects and carcinogenic influence of varied doses of ^{90}Sr, in particular on the skeleton, the hematopoietic tissues and the mucous membranes of the head. The results of these investigations will be reported in separate papers, the present

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Table 1

Experimental conditions and doses employed

Dose of ^{90}Sr in μCi/g body-weight	Total number of mice	Number of mice killed in groups of 5 every month	Duration of experiment in days	Number of animals dead before sacrifice
1.6	120*	65	300	50
0.8	121	75	360	46
0.4	122	95	480	27
0.2	120**	100	540	17
Control	95	94***	570	1

* Out of these animals five were lost during the experiment
** Out of these animals three were lost during the experiment
*** Only four animals were sacrificed in the last test group

dealing mainly with carcinomas in the mucous membranes of the head. Many of the mice with these carcinomas had osteosarcomas as well, or leukemia, or both. Reports concerning carcinomas are sparse (FINKEL 1959, NILSSON 1962, VAUGHAN 1962) and it has been the aim of this investigation to obtain more precise information on latency time, frequency and sites, histogenesis, development and dose relationships.

Material and Methods. Four groups of CBA mice, 75 days old, were treated intraperitoneally with $^{90}Sr(NO_3)_2$. In addition, a group of 95 animals without ^{90}Sr-treatment were used as controls for a study of the natural incidence of tumours. At intervals of 7, 14, 21, and 30 days after injection of ^{90}Sr, and then at monthly intervals, five mice from each group were selected at random and sacrificed, until all mice in each series had been utilized. The experimental conditions and the ^{90}Sr doses employed are recorded in Table 1.

It was not possible to keep the number of killed mice in each dose group the same, because with increasing doses the survival times became much shorter. However, both the sacrificed mice and the mice dead before sacrifice have been investigated, though handled separately.

The mice were decapitated. The head was divided in the median plane during dissection, fixed in Stieve's fluid and decalcified in 20 % formic acid for histologic examination. Conventional methods were used, the sections being stained according to the van Gieson method, with Ehrlich's haematoxylin-eosin, and Lillie's azur-eosinate. The animals were fed during the experiment on a standard diet ad libitum and kept under similar environmental conditions in the

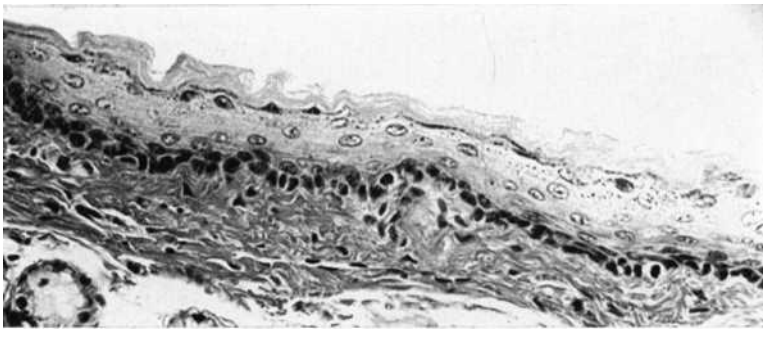

Fig. 1. Hard palate. Normal mucous membrane from a 225-day-old male mouse. van Gieson × 280.

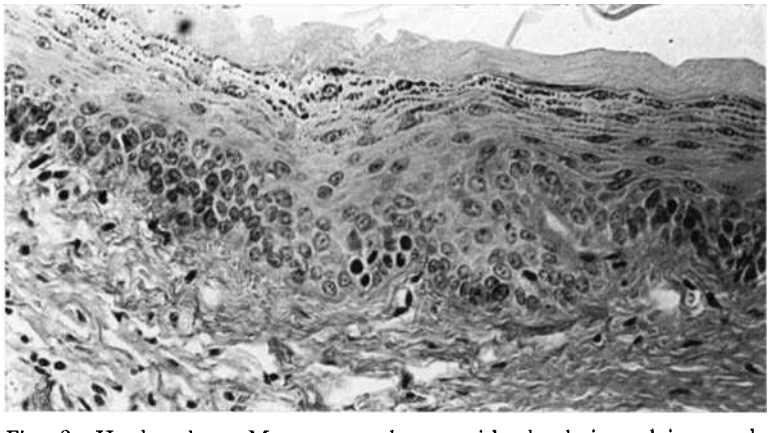

Fig. 2. Hard palate. Mucous membrane with dysplasia and increased mitotic activity from a mouse killed 150 days after treatment with 0.8 μCi ^{90}Sr/g bodyweight. van Gieson ×280.

same room. In all the series, the mice were housed in groups of ten animals per cage throughout the whole experiment.

The nomenclature used is in accordance with the general principles in human pathology.

Results

Tumour development. The earliest morphologically detectable signs of disturbance of the mucous membranes of the ^{90}Sr-treated animals consisted of enhanced mitotic activity, mostly in the stratum germinativum of the epidermis, as com-

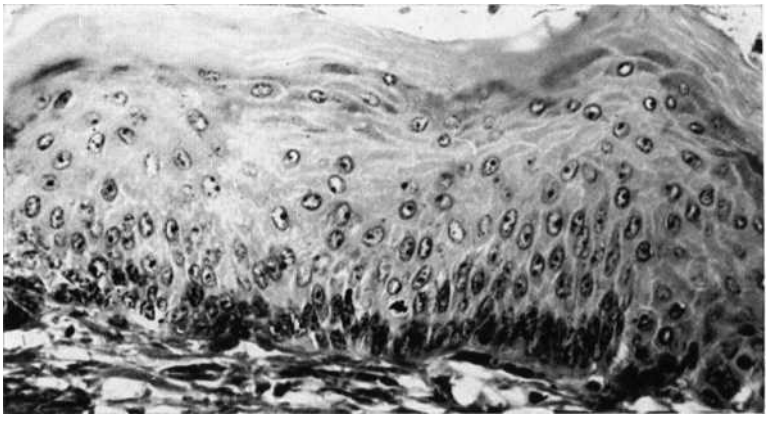

Fig. 3. Hard palate. Mucous membrane with increasing degree of hyperplasia, and dysplasia with disarranged, slightly atypical basal cells from a mouse, 300 days after injection of 0.8 μCi ^{90}Sr/g bodyweight. van Gieson $\times$280.

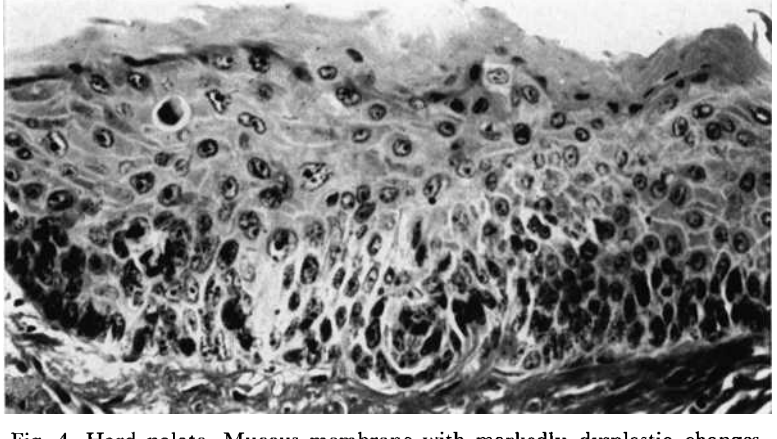

Fig. 4. Hard palate. Mucous membrane with markedly dysplastic changes and, predominantly, slightly atypical basal cells extending into the stratum spinosum from a mouse, 360 days after injection of 0.8 μCi ^{90}Sr/g bodyweight. van Gieson $\times$280.

pared to the normal mucous membranes, and there was furthermore usually a tendency towards concentration of the mitotic activity to circumscript areas. Slight disarrangement, and often a changed polarity and slightly increased size of the basal cells could be observed. These usually focally situated changes became

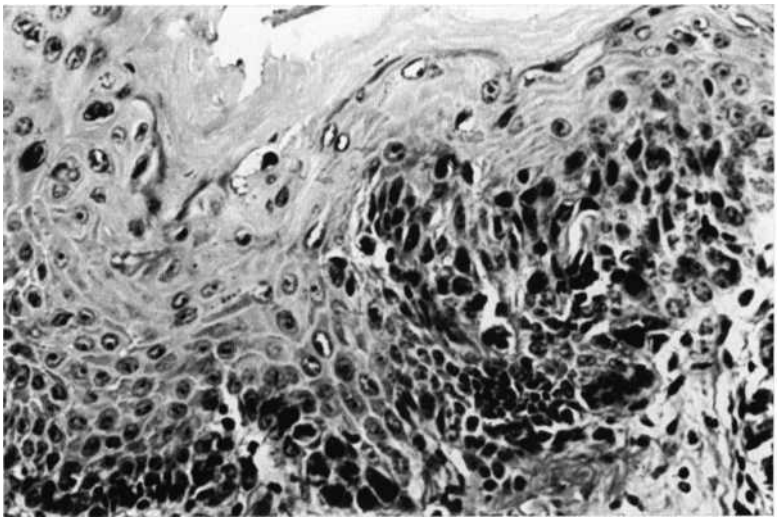

Fig. 5. Hard palate. Mucous membrane with marked dysplasia and scattered, moderately atypical nuclei situated in different layers of the epidermis from a mouse, 240 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. van Gieson $\times 280$.

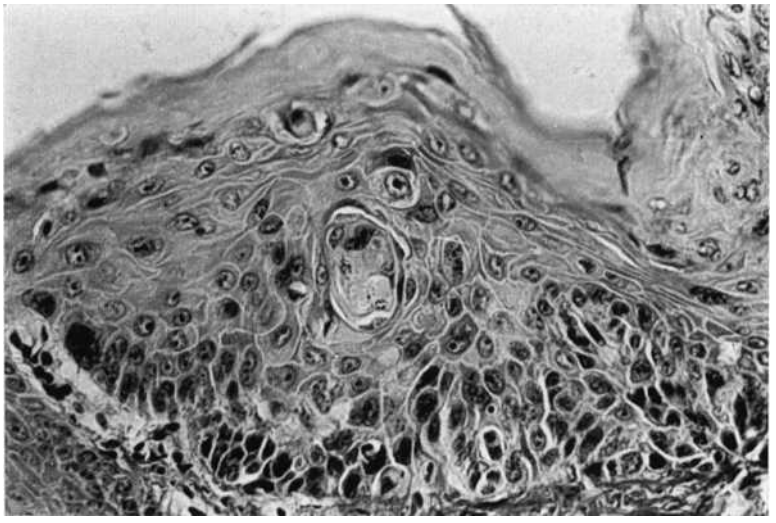

Fig. 6. Hard palate. Mucous membrane with carcinoma in situ from a mouse, 120 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. van Gieson $\times 280$.

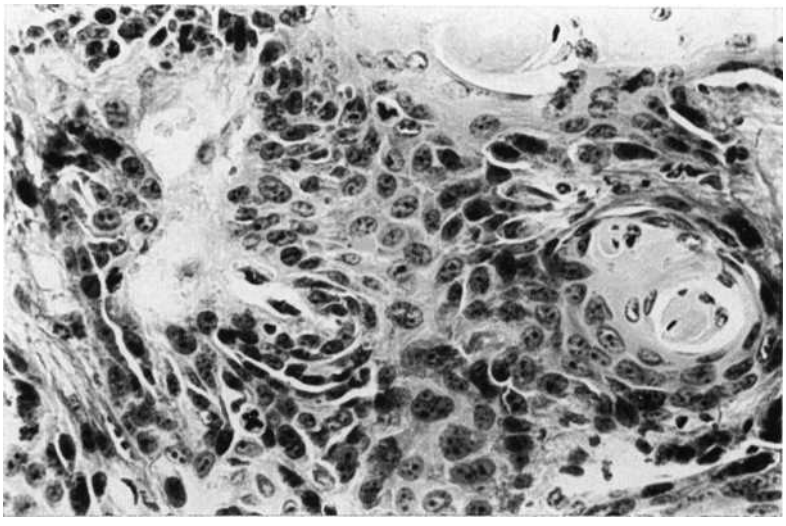

Fig. 7. Oral mucous membrane with a highly differentiated squamous carcinoma from a mouse, 270 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. van Gieson $\times$280.

successively more accentuated. The basal cell layer was broken up and cell buds or scattered cell elements from this layer began to extend into the stratum spinosum. The basal cells were usually hyperplastic with hyperchromatic and slightly atypical nuclei. Changes of the now mentioned types have in this investigation been termed *dysplasia with slight atypism* (Figs 2 to 4).

In local foci there was a continuously increasing number of more atypical basal cells extending into all the layers of the epidermis. Giant nuclei also began to appear. In some cases, premature keratinization was evidenced by cells containing eosinophilic homogenous droplets of intracellular keratin. The destruction of the normal histologic structures was extensive. This stage has in the present communication been termed *dysplasia with moderate atypism* (Fig. 5).

In the next stage the cells were of definitely carcinomatous type, and all layers of the epidermis were infiltrated by these. A distinction has, however, been made between *carcinoma in situ* (Fig. 6) and *invasive cancer* (Fig. 7). The first type is represented by local carcinomatous noduli of limited extent, not infiltrating surrounding tissue or the basal membrane.

The epithelium of the tongue of the same animal has been used as an unirradiated histologic control. Since the average ranges of the β-particles from ^{90}Sr and its ^{90}Y daughter in soft tissues are, respectively, 0.3 and 2.1 mm, this mucosa

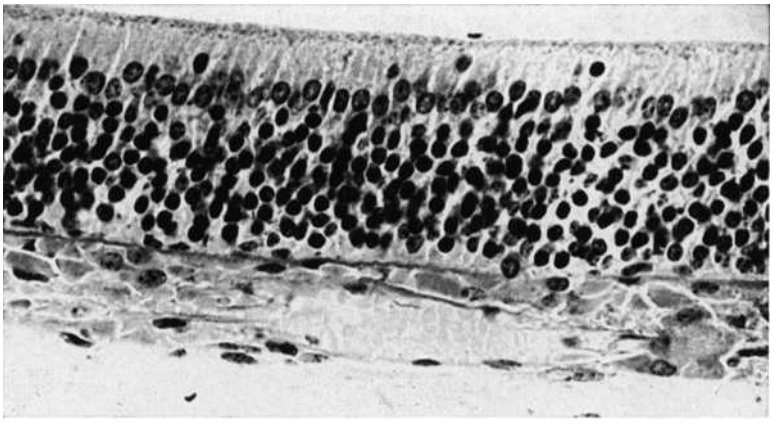

Fig. 8. Regio olfactoria. Normal mucous membrane from a 285-day-old mouse. van Gieson ×450.

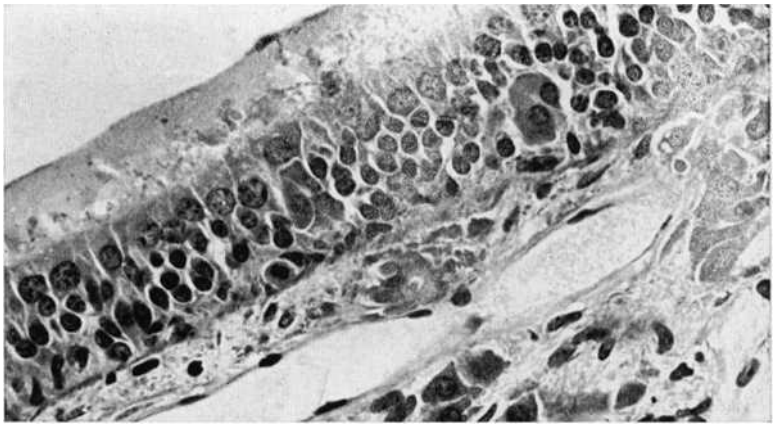

Fig. 9. Regio olfactoria. Mucous membrane from a mouse, 270 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. Depletion of nervous olfactory cells; marked sustentacular cells; mitotic figure along the basal membrane. van Gieson ×450.

may be regarded as unirradiated in comparison with parts of the oral mucosa irradiated from underlying bone structures.

Tumours formed in the cutaneous membranes of the nose follow the same pattern of development as the oral carcinomas. In some cases, tumour formation has however also been observed in the olfactory membranes. The most important early observation was a reduction, and a later disappearance, of the nervous olfactory cells, swelling of the sustentacular cells and the appearance of mitotic

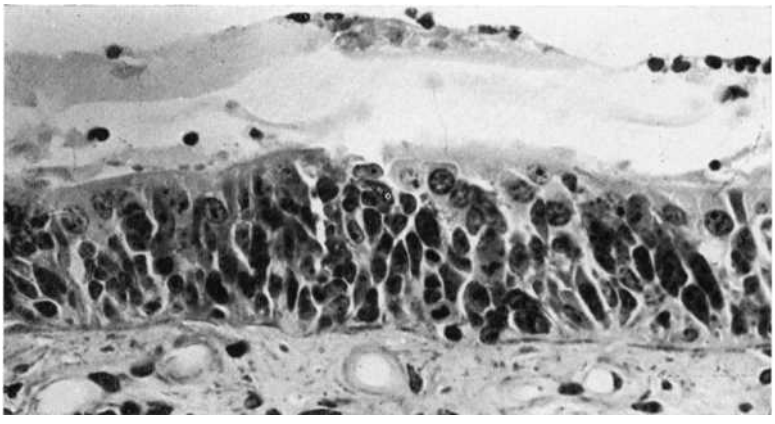

Fig. 10. Regio olfactoria. Mucous membrane from a mouse, 300 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. Most of the olfactory nerve cells have disappeared; abundance of atypical cells and mitotic figures. van Gieson $\times$450.

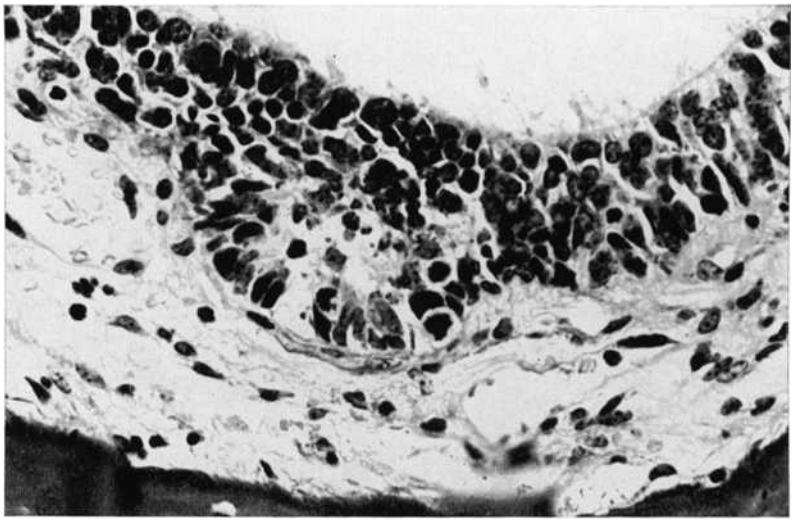

Fig. 11. Regio olfactoria. Mucous membrane from a mouse, 240 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. Bud of atypical cells showing a focal expansion of the basal membrane into underlying connective tissue. van Gieson $\times$450.

figures along the basal membrane. The cells replacing the nervous elements were usually elongated, hypertrophic and with strongly hyperchromatic nuclei which seemed to invade the mucosa from the basal membrane. It seemed to be basal cells that comprised the greater bulk of these cells (Figs 8 to 12).

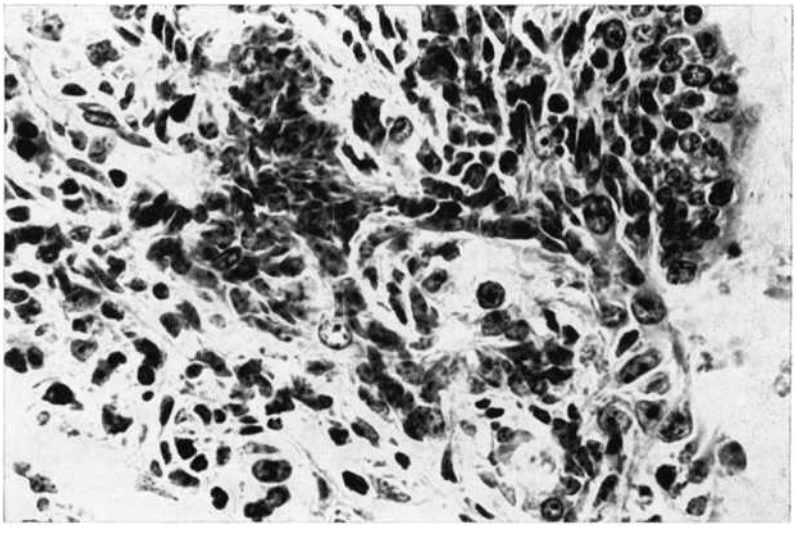

Fig. 12. Nasal cavity. Invasive carcinoma in a mouse, 300 days after injection of 1.6 μCi ^{90}Sr/g bodyweight. van Gieson $\times$450.

The changes successively leading to the development of carcinoma follow nearly the same pattern in the different dose groups. They commence earlier, however, and develop more rapidly with increasing ^{90}Sr doses. This is exemplified in Fig. 13, representing the results obtained from each group of animals sacrificed at 30-day intervals. Only in the highest dose group have carcinomas of the olfactory mucous membrane been detected.

Tumour frequency. The number of tumours occurring in the different dose groups are recorded in Table 2, and in Fig. 14 the accumulated total number of tumours are represented in relation to the time elapsed after the ^{90}Sr administration.

A total of 80 carcinomas and carcinomas in situ were found in the whole experiment when the sacrificed mice and the animals dead before sacrifice were counted together. Of these, 56 or 70 % were found in the 1.6 μCi group, 21 or 26 % in the 0.8 μCi group, and only 3 or 4 % in the 0.4 μCi series. It should be pointed out that the number of tumours obtained is a minimum since no serial sectionings were performed.

Latency time. A comparison of the various time intervals between injection of ^{90}Sr and the occurrence of dysplastic changes, carcinomas in situ and carcinomas has been made for the different dose groups in Table 3.

Table 2

Tumour frequency in the different dose groups

Dose μCi ^{90}Sr/g body-weight	Number of mice investigated	Number of sacrificed mice	Number of tumour-bearing mice	Total number of tumours	Number of tumours per mouse	Percent mice with tumours
1.6	115	65	38	56	0.49	33.0
0.8	121	75	17	21	0.17	14.0
0.4	122	95	3	3	0.02	2.5
0.2	117	100	0	0	0.00	0.0
Control	95	94	0	0	0.00	0.0

Table 3

Comparison of latency time in the development of dysplastic changes and carcinomas in mucous membranes in the different ^{90}Sr dose groups

Dose μCi ^{90}Sr/g body-weight	Dysplasia		Carcinoma in situ + carcinoma			
	Sacrificed animals		Sacrificed animals		Mice dead before sacrifice	
	Number	Days Mean $\pm$ SE	Number	Days Mean $\pm$ SE	Number	Days Mean $\pm$ SE
1.6	17	152$\pm$19.0	18	242$\pm$11.2	20	232$\pm$3.9
0.8	17	249$\pm$15.6	11	330$\pm$ 8.1	6	349$\pm$8.4
0.4	23	335$\pm$16.6	2	465	1	407
0.2	7	497$\pm$24.4	0	—	0	—

Table 4

Percentual tumour distribution at different sites

Group	Hard palate	Incisivous part		Nose
		Upper jaw	Lower jaw	
Whole experiment	58.8	11.3	13.8	16.3
1.6 μCi group	60.7	10.7	12.5	16.1
0.8 μCi group	61.9	9.5	9.5	19.0
0.4 μCi group		1.3	2.6	

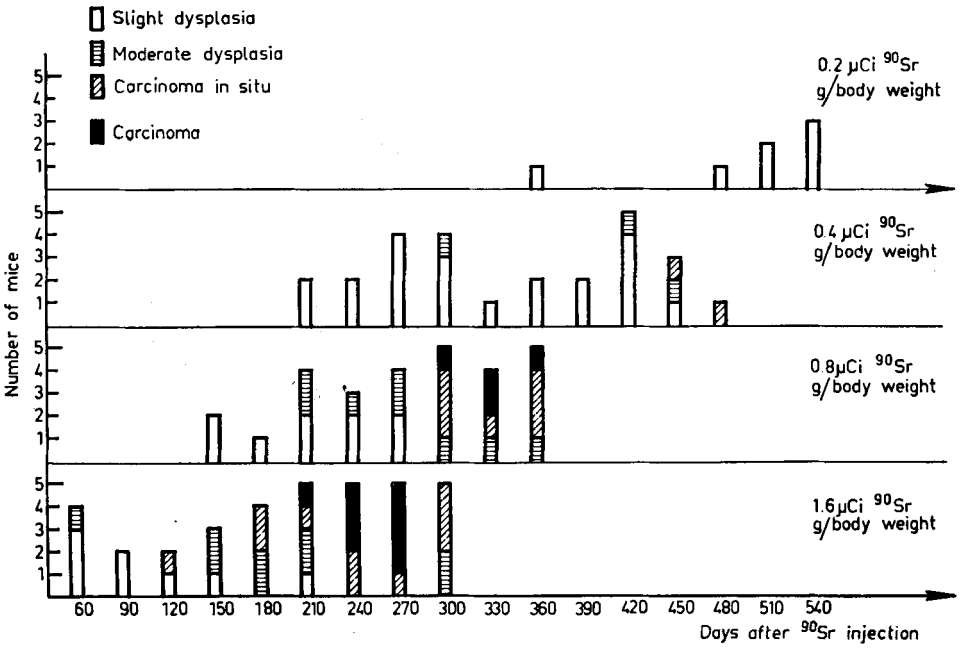

Fig. 13. Histograms showing start and development of changes leading to carcinomas. The mice were killed in groups of five at 30-day intervals.

Tumour sites. The sites of the carcinomas and carcinomas in situ are shown in Table 4. Out of the total of 80 tumours, 47 or 58.8 % were located in the mucous membranes lining the palatine bone. The most frequent site was in a limited area adjacent to the termination of the hard palate. No tumours were situated in the soft palate and only few in the rugae area of the hard palate. In an area lining the incisivous part of the mandible, 11 tumours or 13.8 % were detected, and in the opposite region of the upper jaw 9 or 11.3 % were found. Of 13 tumours, or 16.3 %, in the nasal cavities, three were situated in the olfactory region and the remainder in the cutaneous part of the nose.

Tumour type. All the carcinomas investigated, excepting those in the olfactory region, have been of the squamous type. The degree of differentiation varied, however. Of 31 tumours in the 1.6 µCi group, twenty-two were highly differentiated squamous carcinomas, five were moderate, and four were low-differentiated. Of the 9 tumours in the 0.8 µCi group, the corresponding figures were four, four, and one. The carcinomas defined as highly differentiated were strongly keratin-producing, the moderate type slightly, and the low-differentiated tumours did not show any evidence of keratin formation.

Discussion

The results of this investigation seem to bear in evidence that the squamous cell carcinomas in the cutaneous membranes of the mouth and nose start at local sites in the stratum germinativum. It is possibly basal cell elements, damaged by the irradiation from the underlying bone, which initiate the changes ultimately leading to overt neoplasia.

With respect to the mucous membrane from the olfactory region of the nose it also seems possible to assume from the few cases investigated that the lesion started from basally situated cells of epidermal origin. In the evaluation of the relationship between these changes and irradiation, the localization of the tumours is of the greatest importance. As may be seen from Table 4, most of these tumours emanated from the mucous membrane of the hard palate. They were usually formed in small foci in an area between the rugous part and the termination of the hard palate. They could also be found around the incisivous part of the upper and lower jaw. Histologically, this may be explained by the fact that in these parts the contact between the mucous membranes and the underlying bone structures is very intimate.

In contrast to these observations, no carcinomas were detected in the epithelium of the tongue. It is also obvious from Table 4 that the dose inside a range between 1.6 and 0.8 μCi of ^{90}Sr/g bodyweight has little influence on tumour localization. Exceptions are carcinomas in the olfactory membranes, which have been detected only in the 1.6 μCi group.

A comparison of the latency time for development of dysplastic changes as well as carcinomas in situ and carcinomas also seems to indicate dose dependency. Thus, dysplastic changes started on an average 90 days earlier in the 1.6 than in the 0.8 μCi group. This time relationship still exists if the latter series is compared with the 0.4 μCi group. Between the 0.2 and 0.4 μCi groups this time interval was however increased to 162 days (see Table 3). A possible explanation is that the development of dysplastic changes may be delayed for a considerable time by an intact recovery mechanism. Also for the carcinomas, the latency time was still approximately 90 days shorter for the 1.6 μCi group as compared to the 0.8 μCi group. Between the latter and the 0.4 μCi group, the time interval increased to 135 days. It may be pointed out that only carcinomas in situ developed in the 0.4 μCi group. If the mice in this group had been allowed to survive for a longer period it seems probable that more tumours would have had time to develop. On the other hand, animals given the 0.4 μCi dose seemed to have a greater ability to resist cellular transformations ultimately leading to tumour.

Not only the site of the earliest changes and the latency time for tumour

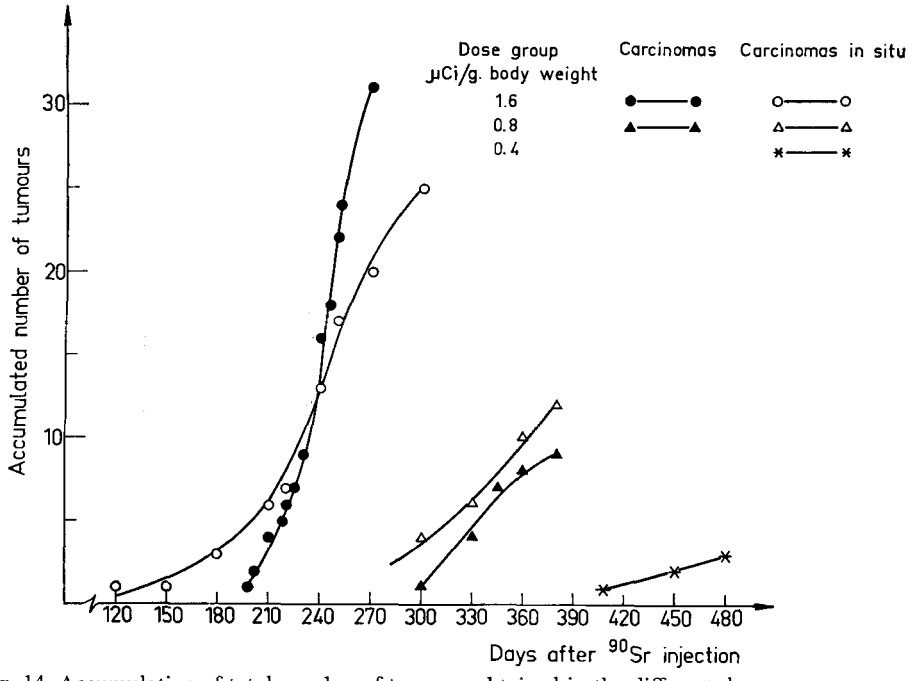

Fig. 14. Accumulation of total number of tumours obtained in the different dose groups.

development but also the carcinoma frequency and the total number of tumours obtained seem to indicate a dose relationship (Table 2, Fig. 14). Thus, there is approximately twice as high values for tumour-bearing mice, total number of tumours, and percent mice with tumours, in the 1.6 µCi as compared to the 0.8 µCi group. On the other hand, this correlation does not exist between the 0.8 and 0.4 µCi groups but this could possibly be explained by the fact that the mice had not survived long enough to give time for maximum tumour development in the lowest dose group.

SUMMARY

Radiostrontium-induced carcinomas of the mucous membranes of the head have been studied in mice. Four groups were injected with, respectively, 1.6, 0.8, 0.4 and 0.2 µCi ^{90}Sr per gram bodyweight. The development of carcinoma usually started in the stratum germinativum of the epidermis, usually at local sites, the most common being the mucous

membrane covering the palatine bone. A dose dependency seems to exist, since with increasing doses of ^{90}Sr the latency time was shortened and the tumour frequency augmented.

ZUSAMMENFASSUNG

Radiostrontium-induzierte Karzinome der Schleimhaut des Kopfes wurden in Mäusen studiert. Vier Gruppen wurden je mit 1,6, 0,8, 0,4 und 0,2 μ Ci ^{90}Sr per Gramm Körpergewicht intraperitoneal injiziert. Die Karzinome-Entwicklung fand erst in dem Stratum germinativum der Epidermis statt, meistens innerhalb begrenzter Regionen, häufigst in der Schleimhaut die das Gaumenbein bedeckt. Eine Dosisabhängigkeit scheint zu bestehen, da mit erhöhten Dosen von ^{90}Sr die Latenzzeit verkürzt wurde und die Tumor-Frequenz gesteigert wurde.

RÉSUMÉ

L'auteur a étudié les cancers des muqueuses de la tête induits par le strontium radioactif. Quatre groupes de souris ont reçu une injection intrapéritonéale de 1,6, 0,8, 0,4 et 0,2 μCi de ^{90}Sr par gramme de poids corporel respectivement. Les cancers ont commencé à se développer dans la couche germinative de l'épiderme, habituellement en certains sièges dont le plus fréquent a été la muqueuse qui recouvre l'os palatin. Ces cancers semblent dépendre de la dose puisque le temps de latence est raccourci et la fréquence des tumeurs augmente avec des doses croissantes de ^{90}Sr.

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