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LATE EFFECTS OF CHRONIC ^{90}Sr INGESTION IN MINIATURE SWINE*

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INTRODUCTION

In attempting to define the potential biohazards of ^{90}Sr in man, many studies have been performed over the past 30 years utilizing numerous animal models to evaluate the biologic damage. In fact, much of the data presented at this conference will be directed toward this very point. The majority of these studies have, however, utilized single or multiple injections of radiostrontium in rodents or dogs. This mode of administration, as opposed to ingestion, results in uneven skeletal distribution and areas of high strontium concentrations with production of severe focal bone damage.

This laboratory initiated a study in 1958, using miniature swine exposed to ^{90}Sr by daily ingestion, in order to more realistically simulate the most probable route of ^{90}Sr entry and its resultant distribution and effects in man. Miniature swine have several desirable characteristics for such studies since they are omnivores and attain a mature weight and bone mass similar to man, in addition to having a life span in excess of 12 years. The use of this species also circumvents one of the major problems with rodent studies, i.e., the loss of ~ 70% of the ^{90}Sr radiation energy from bone because of the small bone mass, (Parmley, et. al., 1962). Palmer, et. al. (1970), found this energy loss in miniature swine to be about 13%, which compares favorably to the estimated 12% loss in man, (Parmley et. al.).

MATERIALS AND METHODS

The experimental design for this study has been previously reported in detail by McClellan, et. al., (1962). Briefly, Original generation female Pitman-Moore miniature swine were started on a daily ^{90}Sr feeding regimen at 9 months of age and subsequently bred to control males. The resultant F1 generation animals were weaned at 6 weeks of age, at which time they were started on daily ^{90}Sr feeding at 1/4 their dam's level and gradually increased so that at 6 months of age they received the same amount of ^{90}Sr daily as their dam. This feeding schedule permitted the dietary calcium - strontium ratio to remain constant during the rapid growth of adolescence. The F1 generation pigs were bred to control males to produce the F2 generation, which were subsequently subjected to the same ^{90}Sr feeding regimen as the F1 generation.

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Over 700 miniature swine, representing 3 generations, have been exposed to daily ^{90}Sr ingestion at 1, 5, 25, 125, 625, and 3100 μCi over the past 14 years. In addition, there have been ~ 200 control animals. Only females have been maintained for the chronic toxicity studies. About 250 animals of both sexes have been killed at various ages to determine tissue distribution of the deposited ^{90}Sr , and to evaluate the early effects of the radionuclide ingestion.

A second major group, comprising 47 miniature swine, was exposed to ^{90}Sr during five different periods of development by litter switching procedures (Table 1). This study, at the 125 μCi ^{90}Sr level, was initiated primarily to correlate the period of ^{90}Sr exposure to the subsequent development of myelopathies.

Table 1. Experimental Design: Uterine-Milk Exposure Study

Group	Dam	^{90}Sr Exposure	No.
I	125 $\mu\text{Ci}/\text{day}$	in utero only	10
II	control	nursing only	10
III	125 $\mu\text{Ci}/\text{day}$	in utero + nursing	8
IV	control	nursing + feed	10
V	125 $\mu\text{Ci}/\text{day}$	in utero + nursing + feed	9

RESULTS

Radiation Doses: The methods of dosimetry and assays for ^{90}Sr accumulation in miniature swine, ingesting 1 thru 625 μCi daily from birth to ~ 8 years of age, have been reported by Palmer, et. al. Original females, started on daily feeding at 9 months of age, attained a body burden about 7.5 times their daily ^{90}Sr intake, whereas the F1 and F2 generations accumulated ~ ten times the daily intake. Less than 1% of the retained ^{90}Sr was located in soft tissue, including the ovaries, resulting in a radiation dose to the extraosseous tissue ~ 0.001 that received by the skeleton. Radiation dose rates to developing fetuses were determined using thermoluminescent dosimeters (LiF) at various periods in gestation. The ^{90}Sr contained within maternal tissues did not contribute significantly to the fetal radiation dose, (Palmer, et. al.). Table 2 contains a summary of average skeletal radiation doses received by miniature swine in the different groups at various periods during their life.

Table 2. ^{90}Sr Dose Rates to the Skeleton of Miniature Swine

$\mu\text{Ci}/\text{day}$ fed	RADS/DAY TO BONE		
	Original Adults	OFFSPRING	
		in utero	Adults
1	0.064	0.004	0.072
5	0.32	0.02	0.36
25	1.6	0.1	1.8
125	8	0.5	11
625	40	2.5	

Life Span Effects: Currently, 72 miniature swine from the original experimental design are alive after 10-13 years chronic exposure to ^{90}Sr . These include 30 controls, twenty-four 1 μCi offspring, eleven 5 μCi offspring, and one 25 μCi original sow and five of their offspring. Cumulative mortality data (Fig. 1) have been analyzed using the method of Cutler, (1958), as modified by Rosenblatt, (1971). Although the data are incomplete at this time, certain trends are apparent. Mortality in parent generation swine started on ^{90}Sr feeding at 9 months of age have not been significantly different than controls at the 1, 5, and 25 $\mu\text{Ci/day}$ levels thru 12 years of age. However, original animals of the 125 and 625 μCi groups have experienced a decreased survival after 6 and 1 years, respectively.

F1 and F2 generations from the 1 and 5 $\mu\text{Ci/day}$ groups had no evidence of life-shortening thru 12 years of age when compared with control animals. However, offspring from the 25 μCi group had an increased mortality after 8 years of age. The cause of death in individual animals in this latter group were not always readily apparent. Usually, the primary factors necessitating euthanasia in all of our aging miniature swine regardless of dose level have been related primarily to uterine pathology and/or faulty dentition.

Although the uterine-milk exposure study is still in a relatively early stage, mortality rates of the two groups that received ^{90}Sr after weaning are of particular interest (Fig. 2). Those animals exposed to ^{90}Sr exclusive of the gestation period, (Group IV), had a 70% mortality by 36 months of age, whereas those exposed to ^{90}Sr throughout gestation, nursing and subsequent feeding, (Group V), experienced only a 22% death loss over the same time period. Deaths in both group generally were due to bone marrow hypoplasia, with leukopenia, thrombocytopenia, and anemia. However, there was one case of acute myelogenous leukemia in each of these groups. The only other deaths in this study have both occurred in the group exposed in utero only, (Group I). One was a non-radiation related acute necrotizing pancreatitis, and the other a severe myeloid metaplasia. This latter case is difficult to explain, on the basis of an estimated radiation dose of ~ 50 rad received in utero only, with death occurring 41 months later.

Farrowing Performance: An extensive statistical analysis of the farrowing performance of miniature swine ingesting ^{90}Sr has been previously published, (Clarke, et. al., 1970). Except for smaller weaning weights of offspring from the original 625 $\mu\text{Ci/day}$ dams, there were no differences in litter size, percentage of stillborn, birth weights or weaning weights between the control animals and those ingesting ^{90}Sr . Original dams at the 3100 μCi level failed to survive the gestation period, and 625 $\mu\text{Ci/day}$ offspring died before sexual maturity, so that there were no F1 nor F2 generations, respectively in these two groups.

Farrowing parameters of the F1 generation dams ingesting 0 through 125 $\mu\text{Ci/day}$ were indistinguishable with the exception of larger litters from the 25 $\mu\text{Ci/day}$ dams. The reason for this difference was not apparent, and probably does not have biologic significance.

Osseous Lesions: Although a high incidence of bone tumors was predicted as an end result of ^{90}Sr feeding, this has not proved to be the case, since only seven animals have developed detectable bone tumors. Five of the animals were 125 $\mu\text{Ci/day}$ offspring that had received ^{90}Sr throughout their life before dying of hematopoietic tumors at 744-1210 days of age. The two other animals with bone tumors were 625 μCi offspring that received 156 $\mu\text{Ci/day}$ for approximately six weeks after weaning, and then lived for 1093 and 1561 days before death. Accumulated skeletal radiation doses ranged from 9000-15000 rads in both the 125 and 625 μCi offspring. Nine of the 11 tumors were located in the head, and one each in a rib and an ulna. Histologically, 73% of the neoplasms were classified as osteosarcomas and 27% as giant cell tumors or osteoclastomas. In most cases, these tumors appeared to develop from the periosteal surface. A detailed description of these neoplasms has been reported by Howard, et. al., (1969).

Non-neoplastic ^{90}Sr induced bone lesions histologically consisted of irregular calcification, increased numbers of resorption cavities, reactive bone, and fibrosis progressing to severe osteonecrosis at 625 $\mu\text{Ci/day}$. Detailed analyses of the histologic and radiographic bone changes by dose levels have not been completed at this time due, to the diminished effects of ingested ^{90}Sr on bone at the lower dose levels. These analyses are further complicated by osseous changes also present in the aging control pigs.

Hematopoietic Effects: Definitive effects of chronic ^{90}Sr ingestion in miniature swine have been related primarily to bone marrow irradiation and subsequent depression of the cellular blood components. These effects were unequivocally time-dose related at feeding levels greater than 25 $\mu\text{Ci/day}$, and consisted of thrombocytopenia, neutropenia, lymphopenia and a terminal, rather rapidly progressive anemia. Peripheral blood values at 1 $\mu\text{Ci/day}$ were not significantly different than the controls, whereas at 5 and 25 $\mu\text{Ci/day}$ a significant ($P < 0.05$), chronic neutropenia was observed. After about 8 years at the lower dose levels, i.e., 1, 5, and 25 μCi , separation of radiation effects from those of aging became tenuous in regards to peripheral blood values. The two most common causes necessitating euthanasia, uterine tumors and dental problems, had a pronounced effect on the hemogram. A detailed description of the hematopoietic changes will be presented at the conference.

Peripheral blood values of the uterine-milk exposure study are consistent with those previously observed in animals ingesting 125 μCi $^{90}\text{Sr/day}$. Cellular elements from those groups not fed ^{90}Sr , i.e., exposure in utero only, milk only, or in utero and nursing (Groups I, II, III), have not been different than controls. Examination of aspirated sternal bone marrow from the five groups at 33 months of age revealed a left shift in maturation curves of both the erythroid and myeloid series in those animals that continued to ingest ^{90}Sr (Groups IV, V). However, the percentage of immature cells at the blast and "pro" stages was greater in the animals not exposed in utero, (Group IV), than in those born to dams ingesting ^{90}Sr and then continuously fed ^{90}Sr , (Group V).

A diverse spectrum of proliferative disorders involving the stem cell, lymphoid, and myeloid compartments has been encountered in the animals ingesting ^{90}Sr daily (Table 3). The high incidence of myeloid metaplasia in the 625 μCi animals was associated with severe bone marrow stress resulting in profound thrombocytopenia, leukopenia and anemia after a relatively short exposure. At 125 $\mu\text{Ci/day}$ the incidence of metaplasia was low, whereas frank neoplasia was evident. Clinically these neoplasms were characterized by being very acute, with only a few weeks elapsing between the presence of blast forms in the peripheral blood and death of the animal.

With lower accumulated radiation doses (1, 5, and 25 $\mu\text{Ci/day}$) the incidence of metaplasia and neoplasia was reduced and there was a greater tendency toward the development of lymphoid neoplasms.

In our control population, during the past 14 years, two cases of myelogenous leukemia and one reticulum cell sarcoma, occurring at 98-105 months of age have been observed. There have been no cases of myeloid metaplasia in the control swine.

Other Neoplasms: The incidence of all tumors observed during this chronic toxicity study are outlined in Table 3. The number of animals shown does not constitute the animals "at risk", but is indicated merely to give some information regarding totals per group. These data are

Table 3 Neoplasia and Myeloid Metaplasia in Miniature Swine Ingesting ^{90}Sr Daily

^{90}Sr ($\mu\text{Ci/day}$)	Generation	No. of Animals	Annual Skeletal Dose ($\text{RAD} \times 10^3$)	Bone	Stem Cell	Myeloid	Lympho-reticular	Uterine	Ovarian	Hepatic	Other	Total Neoplasms	Metaplasia
0	-	183	-	0	0	2	1	17	1*	2	3	26	0
1	P ¹	24	0.02	0	0	0	1	6*	0	1	0	8	1
	O ²	64	0.03	0	0	0	4	11	0	1	3	19	1
5	P	10	0.12	0	0	0	0	2	0	0	0	2	0
	O	36	0.13	0	0	0	1	8	0	1	0	10	0
25	P	19	0.6	0	0	2	0	7**	1	2	3	15	0
	O	41	0.7	0	0	2	2	5*	3	4	4	20	6
125	P	16	3	0	0	3	1	2	0	0	0	6	3
	O	27	4	9	3	14	7	0	0	0	0	33	2
625	P ³	14	15	0	0	0	0	0	0	0	0	0	3
	O ³	13	20	2	0	1	1	0	0	0	0	5	15
3100	P	5	72	0	0	0	0	0	0	0	0	0	2

¹ = Parent

² = Offspring

³ = Removed from ^{90}Sr feeding at ~ 3 mo. of age

* = 1 metastatic adenocarcinoma

** = 1 leiomyosarcoma

being analyzed as a continuum of animals at risk for each neoplasm. Tumors in the "other" column of this table include: Control - 1 intestinal carcinoid, 1 pituitary adenoma, and 1 adrenal lymphoma; 1 $\mu\text{Ci/day}$ - 1 skin fibroma, 1 adrenal adenoma, and 1 thyroid carcinoma; 25 $\mu\text{Ci/day}$ - 2 intestinal adenocarcinomas, 1 metastatic pheochromocytoma, 1 astrocytoma, 1 mammary adenocarcinoma, 1 pancreatic adenoma, and 1 adrenal adenocarcinoma; and at 625 $\mu\text{Ci/day}$ - 1 meningioma.

DISCUSSION

Although relatively late effects of chronic ^{90}Sr ingestion in miniature swine have been manifest at several of the higher dose levels, there has been no evidence of increased fetal or neonatal mortality even at accumulated fetal radiation doses greater than 150 rads. This might have been predicted, since the fetus receives little radiation from maternal tissues, and the sensitive embryonic tissue is well differentiated prior to the time that bone-seeking ^{90}Sr begins accumulating. This does not, however, presume that bremsstrahlung radiation from maternal tissues during the early period of the embryo could not result in an embryonal rest of neoplastic propensity, which might become manifested under proper stimulation later in life. Vaughan, (1970), concluded, after a review of the literature, that the incidence of leukemia was increased in humans if the fetus had been exposed to the relatively low dose rates of diagnostic x-ray procedures.

In the uterine-milk exposure study, the 3-fold increase in mortality of those animals exposed exclusive of the gestation period, (Group IV), are of particular interest when compared to those exposed to ^{90}Sr during their entire life. Although a relatively small number of animals are involved, the difference appears too great to be incidental, particularly since the accumulated radiation dose rate between the two groups amounts to only about 50 rads. Therefore, it is interesting to postulate the evolvement of a relatively ^{90}Sr resistant cell population during embryogenesis, or simply a homeostatic increase in the hematopoietic stem cell compartment during this period which would require a greater accumulated radiation dose for depletion. The one case of extensive myeloid metaplasia 3 years after exposure in utero only (Group I), is equally perplexing and unexplained.

The low incidence of bone tumors in swine chronically ingesting ^{90}Sr is in direct contrast to the high incidence of bone neoplasia observed in rats and dogs injected with the isotope, (Finkel, et. al., 1968; Mays, et. al., 1969). Of particular interest is the observation that, in those cases where it was possible to determine the site of origin, swine bone tumors appear to arise from the periosteal surface rather than being of endosteal origin, as observed in animals injected with ^{90}Sr , (Mole, 1969). The more even skeletal distribution of the isotope following chronic ingestion results in a relatively greater dose rate to the periosteum than after injected ^{90}Sr . However, these studies with miniature swine tend to confirm the observation of Vaughan, (1970), that the latent period for leukemia induction is considerably shorter in the human than that for osteosarcoma development.

The sequence of events terminating in hematopoietic neoplasms in these pigs is unknown, but several possible mechanisms acting independently or in concert may be speculated. Early cell death and/or a maturation defect resulting in chronic leukopenia would influence the humoral homeostatic control mechanisms presumably by elevating "leukopoietin" levels, in addition to reducing the chalone effect. Bierman, (1967), found a prolonged granulocyte generation time in human acute leukemia patients, along with defective maturation and altered release of cells to the peripheral blood. A prolonged granulocyte generation time has also been demonstrated in six non-leukemic miniature swine ingesting 625 μCi ^{90}Sr /day, (Cooper, et. al., 1970). Bone marrow examinations from the uterine-milk exposure pigs also indicates an accumulation of immature forms in those animals exposed chronically to ^{90}Sr . This increase in blast and "pro" forms could be either a quantitative increase in these cell types, or a relative increase if more distal cells have failed to mature properly.

Impaired immunocompetence, with a viral and/or neoplastic clonal etiology, must also be considered in the pathogenesis of these hematopoietic neoplasms. Viruses with certain characteristics common to known leukemia viruses have been isolated from some of the leukemic pigs, but their role in the etiology of this disease is unknown. An impaired immune response has also been demonstrated in animals at the 125 μCi /day level, but has not been correlated with subsequent neoplastic development. These important aspects are currently under rigorous investigation.

A scheme depicting the possible pathogenesis of ^{90}Sr induced hematopoietic tumors is shown in Figure 3. Bone marrow irradiation may produce some subtle somatic mutation, resulting in an enzymatic change and a maturation defect. This clone of cells might then become the dominant cell line by several means: 1) progressive death of the normal cell line by constant irradiation, 2) proliferative increase in the defective clone because of particular susceptibility to the cellular and humoral regulators that have been altered due to the marrow damage, and/or, 3) a failing immune system which had previously prevented proliferation of the neoplastic cells. Myeloid metaplasia, in this scheme, is not a part of the progression to neoplasia, but more logically a physiologic response to the pathologic processes occurring in the bone marrow.

The "gross" effects of ^{90}Sr have been well documented in several species, but there is a need for additional studies of in-utero and neonatal exposure of the hematopoietic system correlated with the subsequent development of neoplasia. This is imperative, not only for clarification of cellular radiation effects, but also because nonradiation bone marrow insults may have a similar pathogenesis in the development of neoplasia. Because of the proved leukemogenicity following chronic ingestion, ^{90}Sr is particularly applicable as a tool for such studies.

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MINIATURE SWINE INGESTING ^{90}Sr
DAILY (COMBINED GENERATIONS)

MINIATURE SWINE EXPOSED TO ^{90}Sr AT VARIOUS PERIODS DURING THEIR LIFE

PROPOSED SCHEME OF ^{90}Sr INDUCED MYELOID METAPLASIA AND HEMATOPOIETIC NEOPLASIA