Effects of Inhaled $^{144}CeO_2$ on Influenza Virus Infection in Mice[1] (37564)

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In certain nuclear accident situations, the potential exists for the inhalation exposure of individuals resulting in chronic, nonuniform irradiation of the lung by beta-emitting radionuclides. In assessing possible post-inhalation exposure medical complications in individuals thus accidentally exposed, it would be useful if the influence of chronic irradiation of this type on the susceptibility to a commonly occurring respiratory infection such as influenza was known. Numerous studies of the effects of relatively uniform whole-body ionizing irradiation on the course of influenza virus infection in mice have yielded conflicting conclusions. Ionizing radiation in the form of X- or gamma-irradiation of mice has been reported to (a) increase survival time of infected mice (1, 2), (b) increase susceptibility to infection (3–7), (c) decrease mortality (1), or (d) have only slight or no effect on mortality and virus content of the lungs (3, 5, 8, 9). The lack of uniformity in these observations probably has resulted from differences in the strains of mice studied (5), differences in the degree of mouse adaptation of the influenza virus used (1–3, 10, 11), and differences in radiation dosages and the intervals between irradiation and experimental infection (12). Since there are no studies known that relate influenza virus infection to chronic lung irradiation from internally deposited beta-emitting radionuclides, the disparity of experimental results indicated above make correlation or prediction difficult. To address this potentially practical problem, mice were inoculated with influenza virus at various intervals after the inhalation of relatively insoluble ^{144}Ce (in equilibrium with its daughter, ^{144}Pr) oxide and the course of infection was observed.

Materials and Methods. Eight to 10-wk-old female mice of the C57BL/6J strain (Jackson Laboratory, Bar Harbor, ME) received a single (20 min) inhalation exposure to a $^{144}CeO_2$ aerosol. A suspension of ^{144}Ce hydroxide stabilized with diethylenetriaminepentaacetic acid (13) was aerosolized, passed through a heating column at 1100°, mixed with dry, cool diluting air and directed into an essentially nose-only inhalation exposure chamber in which a plenum was used to direct the air flow to unanesthetized mice (14). The aerosol was characterized by analysis of cascade impactor (15) and electrostatic precipitator samples collected during the exposures. The $^{144}CeO_2$ particle size distribution was approximately log-normal with an activity median aerodynamic diameter of 1.4 μm and a σ_g of 1.8. Whole-body and relative lung retentions of ^{144}Ce with time postinhalation exposure were determined by periodic whole-body counting, sacrifice, dissection and tissue analysis in a well-type liquid scintillation counter. An initial lung burden (ILB) was estimated for each animal based on the average relationships determined. A three-component curve was fit to the lung retention data [$LB(t) = 27.2e^{-1.14t} + 50.9e^{-0.027t} + 18.6e^{-0.0047t}$] and used with each animal's estimated ILB to calculate individual cumulative beta-radiation dose to the lungs. For dosage calculations, an average lung weight of 0.25 g was used. Other constants used were 1.29 MeV, the average beta energy for ^{144}Ce–^{144}Pr in equilibrium; and 0.35, the estimated

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fractional beta energy absorption in mouse lung (16).

A bacteriologically sterile 10% suspension of mouse adapted influenza virus (Type A_0/PR8/34; Research Reference Reagents Branch, NIH, Bethesda, MD) infected mouse lungs in phosphate buffered saline (PBS) containing 10% heat inactivated horse serum and stored at −60° until needed was used for all experimental infection studies. Groups of mice were inoculated by the intranasal (IN) route with approximately 0.05 ml of a suspension containing approximately 10^2 mouse IN 50% infective doses of virus at 8, 12 and 20 wk postinhalation exposure to $^{144}CeO_2$. They were housed in groups of 5 in 5 × 7 × 12 in. plastic cages with wire tops and given food and water *ad libitum*.

Two hours after virus inoculation and at 2-day intervals thereafter for 8–14 days, 5 irradiated and 5 nonirradiated control mice were sacrificed by exsanguination via the medial canthus of the eye using capillary pipets. Sera, collected from the blood samples, were tested for hemagglutination inhibiting (HI) antibody. The lungs of each mouse were aseptically removed, grossly examined and lesions were scored numerically from 0 to 4 by estimating the percentage of lung consolidation. Each lung was then weighed, ground in a mortar with sterile alumina abrasive and a 10% suspension was prepared in PBS containing 10% heat inactivated horse serum.

Twofold serial dilutions of each homogenate were prepared and tested for virus by hemagglutination (HA) of guinea pig red blood cells (RBC) at room temperature. Equal portions of the homogenates from the mice in each sacrifice group were pooled, tenfold serial dilutions were prepared and 0.1 ml was inoculated into each of five rhesus monkey kidney (MK) cell culture tubes (Flow Laboratories, Inglewood, CA) per dilution. One week later, the inoculated MK cell culture tubes were tested for virus by HA with guinea pig RBC at room temperature, and the tissue culture 50% infective dose ($TCID_{50}$) was calculated (17).

All sera were tested for HI antibody after treatment with receptor destroying enzyme for 18 hr at 37° and then heat inactivated at 56° for 30 min. Twofold serial dilutions of sera, beginning at 1:4, were tested for antibody by mixing each dilution with 4 HA units of the influenza virus. After 1 hr incubation at room temperature, the virus–sera mixture was tested for HA by the addition of a 0.5% suspension of guinea pig RBC.

Three weeks after inoculation of influenza virus, 5 nonirradiated control mice and 5 mice exposed to $^{144}CeO_2$ were exsanguinated via the medial canthus for histological examination. At the same time, 5 normal noninfected mice and 5 mice exposed to $^{144}CeO_2$ but not inoculated with virus were also sacrificed. Immediately after death, the lungs were perfused with buffered 10% formol, removed and fixed in the same solution. After fixation the lungs were embedded in paraffin, sectioned *in toto* and hematoxylin–eosin stained sections and autoradiographs were prepared.

Results. Absorbed beta-radiation dose to the lungs to the day of virus inoculation. The average beta-radiation doses accumulated by the lungs of mice that inhaled $^{144}CeO_2$ prior to the inoculation with influenza virus are summarized in Table I. The lower average beta-radiation dose absorbed by the lungs of mice inoculated with virus 20 wk postinhalation of $^{144}CeO_2$ was due to their relatively lower ILB. Mice with the highest estimated ILBs of ^{144}Ce had died prior to the 20-wk inoculation time. The mice inoculated with virus 8 wk after they inhaled $^{144}CeO_2$ particles had estimated ILBs (± 1 SD) of 7.0 ± 2.1 μCi, those at 12 wk, 6.2 ± 1.7 μCi and those at 20 wk, 4.2 ± 1.8 μCi.

Mortality in mice. Mortality among the mice inoculated with virus 8 and 12 wk postinhalation of $^{144}CeO_2$ was significantly greater ($p < 0.01$) than in the virus inoculated nonirradiated control mice (Table I). Mortality after inoculation of influenza virus was not significantly different ($p > 0.05$) among the mice 20 wk after inhalation of $^{144}CeO_2$.

Lung weights and lesions. The lung weights and lung lesion scores of virus infected irradiated mice and virus infected control mice are shown graphically in Fig. 1. The statistical significance of the lung weights is given in Table I. In the mice that had inhaled $^{144}CeO_2$ there were no significant differences ($p > 0.05$) in lung weights between irradiated

TABLE I. Beta-Radiation Dose to Lungs of Mice from $^{144}CeO_2$ Inhaled Prior to Virus Inoculation, and Significant Differences in Mortalities and Lung Weights After Influenza Virus Inoculation.

Weeks post-$^{144}CeO_2$ inhalation	Absorbed dose rads ± 1 SD	Mortality/mice inoculated[a]		Mortality greater in irradiated mice than in controls	Lung wt greater in irradiated mice than in controls
		Irradiated	Control		
8	17,000 ± 6700	17/58	2/60	<0.01[b]	>0.05[c]
12	19,000 ± 5200	20/71	3/55	<0.01	<0.01
20	16,000 ± 6900	11/56	19/64	>0.05	<0.01

[a] Deaths in mice not sacrificed through Day 14 after virus inoculation.
[b] p values computed by chi-square test.
[c] p values computed by Student's t test.

mice and controls with virus inoculation at 8 wk postinhalation. With inoculation at 12 and 20 wk postinhalation, the lung weights of the irradiated mice were significantly higher ($p < 0.01$) than those of the controls. The lung lesions in the irradiated mice inoculated at 8 wk postinhalation were similar to or less than those in the control mice, whereas inoculations at 12 and 20 wk after inhalation resulted in a greater number of lesions in irradiated mice than in controls.

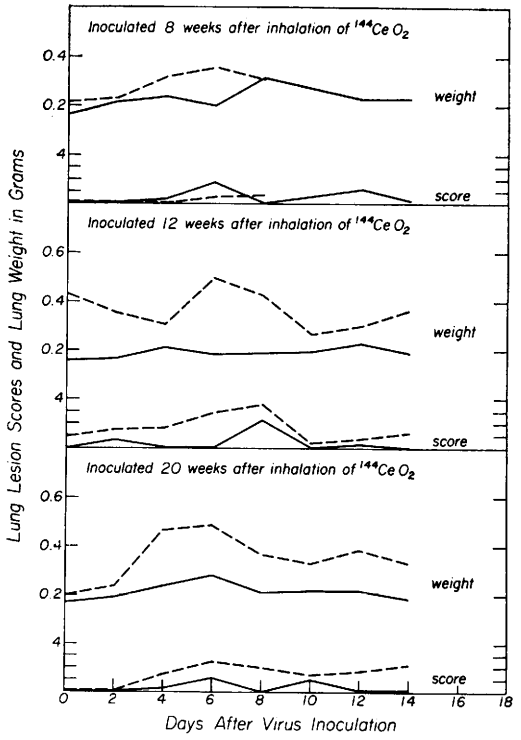

FIG. 1. Lung weights and lung lesion scores of influenza virus infected mice at intervals after they inhaled $^{144}CeO_2$ (- - -) compared to infected control mice (—).

Virus content of lungs. Peak HA titers in all mouse lung homogenates were reached between Days 4 and 8 postvirus inoculation. The geometric means of the peak HA titers ranged from 1:10 to 1:300 in all groups tested. The mice that inhaled $^{144}CeO_2$ and were subsequently inoculated with influenza virus consistently had slightly higher HA titers in their lung homogenates than the non-irradiated infected controls. These differences, however, were not significant at the $p = 0.05$ level.

Infectious virus concentrations of 10^4 to 10^6 $TCID_{50}$ were present in lung homogenates from all mouse groups by the second day after virus inoculation, remained within this range until Days 8 to 10 and then declined rapidly to undetectable levels (< 1 $TCID_{50}$) by Day 12. There was no significant difference between infectious virus concentration in the mice that had inhaled $^{144}CeO_2$ and control mice at the $p = 0.05$ level.

HI antibody responses. Demonstrable HI antibody was evident in all groups of mice 6 to 8 days after influenza virus inoculation. Antibody responses among the infected irradiated and infected control mice were one to twofold serial dilutions lower in the irradiated mice than the controls at 8 wk after inhalation of $^{144}CeO_2$: similar in both groups at 12 wk postinhalation of $^{144}CeO_2$ and suppressed at Days 6 and 8 only in the irradiated mice at 20 wk postinhalation of $^{144}CeO_2$.

Histopathology. Control mice. Lung sections

from the 15 mice that were not exposed to either $^{144}CeO_2$ or influenza virus had some vascular congestion, rare interstitial petechiae and rare to frequent interstitial collections of inflammatory cells.

$^{144}CeO_2$ exposed mice. In the 5 mice exposed to $^{144}CeO_2$ and sacrificed at 11 wk postinhalation exposure, the histological responses consisted of small focal areas of interstitial chronic inflammation, occasional hypertrophy of alveolar epithelial cells, hyperchromic metaplastic alterations of alveolar duct and bronchiolar epithelium and fibrillar thickening of septa walls. The lungs of the 5 mice that had inhaled $^{144}CeO_2$ 15 wk earlier showed mild changes: a few scattered histiocytes in the alveoli, some bronchiolar epithelial cells with peg-like configuration, some focal interstitial edema and mild inflammation with accumulation of a few granulocytes. Examinations of the lungs of the 5 mice that inhaled $^{144}CeO_2$ 23 wk earlier revealed scarcely any effects in 3 of them. The 2 remaining exhibited histologic features of diffuse radiation reaction with edema, fibrillar thickening of septal walls and fibrin deposits. Cellular reactions consisted of alveolar accumulation of histiocytes, hypertrophy and hyperchromatism of alveolar epithelium and loss of some bronchiolar epithelium while the remainder showed atypical hyperplasia. Autoradiographs of the lungs of all 15 mice revealed scattered to numerous deposits of ^{144}Ce along alveolar walls, most often emanating from histiocytes.

Virus infected mice. In the lungs of the 15 mice not exposed to $^{144}CeO_2$ but sacrificed 3 wk postinfluenza virus inoculation, there were multiple and rather dense patches of chronic inflammation, edema, atelectasis, congestion and local proliferation of alveolar epithelium in a few places. The infiltrate consisted of lymphocytes, plasmacytes, fibrocytes, granulocytes and histiocytes.

$^{144}CeO_2$ exposed virus infected mice. The 5 mice inoculated with influenza virus 8 wk after inhalation of $^{144}CeO_2$ and sacrificed 3 wk later showed scattered clusters of alveoli containing large, swollen and vacuolated histiocytes with a few hypertrophic alveolar lining cells. Occasional groups of alveoli contained fibrin deposits and a few leukocytes. The lungs of one mouse had local vascular intimal proliferative thickening with some bronchiolar epithelial alterations marked by hyperchromatism and an atypical peg-like appearance. Histological changes in the lungs of the 5 mice inoculated with influenza virus 12 wk after they inhaled $^{144}CeO_2$ and sacrificed 3 wk later consisted of rare tiny foci of chronic inflammation, small accumulations of histiocytes in alveoli, occasional hypertrophy of alveolar lining cells and some alterations of bronchiolar epithelium with a few peg-like cells. There were also some fibrin deposits and early fibrosis. In the lungs of the 5 mice inoculated with influenza virus 20 wk after they inhaled $^{144}CeO_2$ and sacrificed 3 wk later, there were distinctive histologic alterations of pulmonary tissue differing considerably from the lesions seen in the groups exposed to $^{144}CeO_2$ or virus influenza alone. These alterations were most evident in the lungs of 3 mice that contained histologic changes of mild radiation reaction and broad areas of scarring. These areas enclosed distorted remnants of alveoli lined by an adenomatous epithelial proliferation. This feature had progressed, in several places, to a clear squamous cell differentiation (Fig. 2). This was particularly noticeable at the peripheral margins of the involved area where the nests of squamous cells progressively filled the alveolar spaces, giving a pseudoneoplastic appearance to the lesions. However, the distinctive, infiltrative and invasive features of a true malignant neoplasm were absent. In 1 of these 3 animals, the proliferation of metaplastic cells all but obliterated one-half of the pulmonary lobe. The autoradiographs of lungs of these 5 animals revealed numerous deposits of $^{144}CeO_2$.

Similar studies with mice having ILBs of approximately 0.7 μCi of ^{144}Ce in fused clay particles and infected with influenza virus at 4, 8 and 12 wk postinhalation exposure were also done. There were essentially no significant differences in the parameters observed in the present study among the mice that had inhaled ^{144}Ce in fused clay, stable Ce in fused clay and normal control mice following inoculation of influenza virus.

Discussion. The experiments described

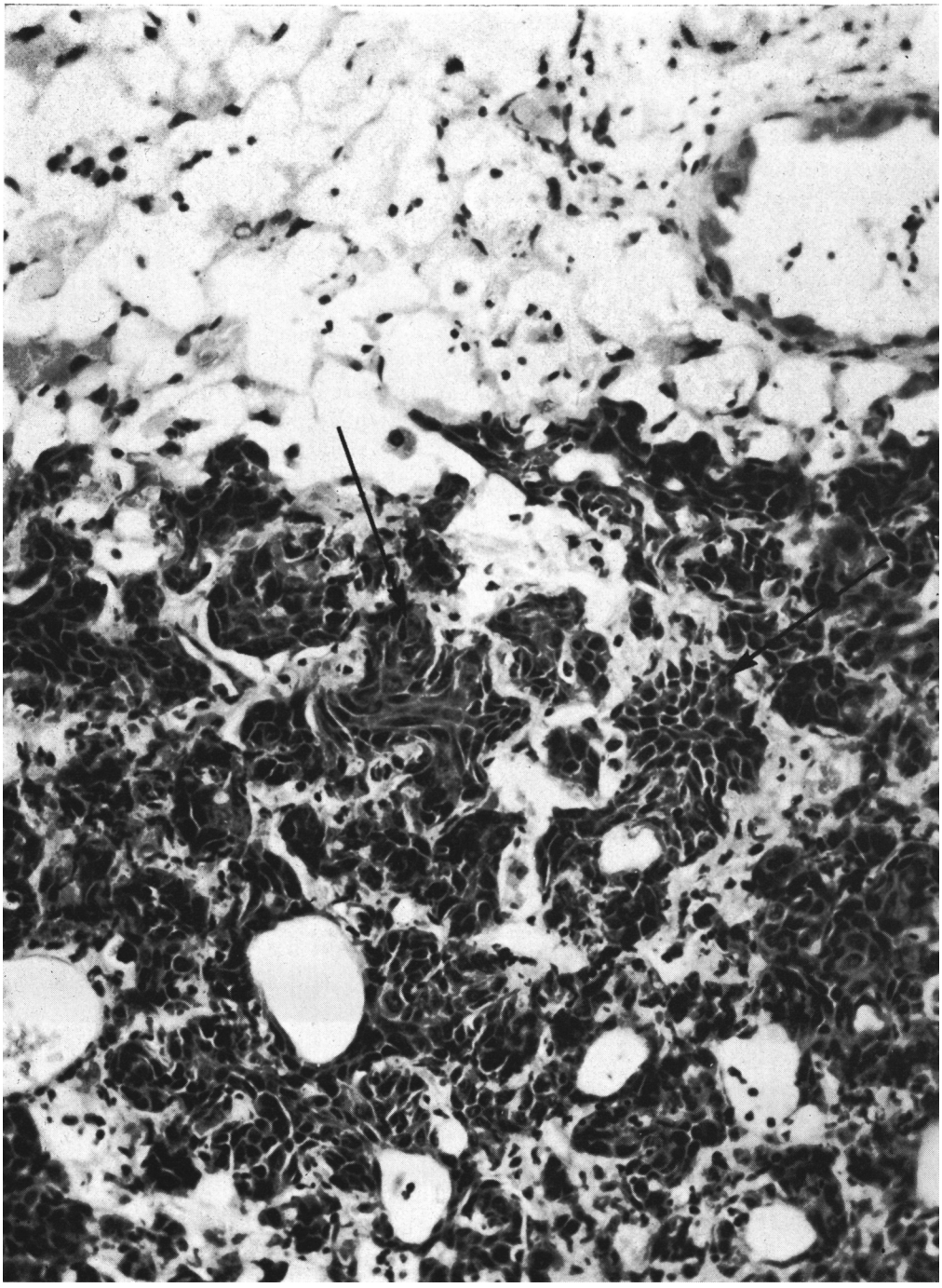

FIG. 2. Severe metaplasia of the lung with clear squamous cell differentiation resembling epidermoid carcinoma in mouse infected with influenza virus 20 wk postinhalation of $^{144}CeO_2$ and sacrificed 3 wk later. H + E stain. Original ×175.

demonstrate that chronic beta-irradiation from inhaled $^{144}CeO_2$ with cumulative doses to lung of 16,000 to 19,000 rads over a period of 8 to 20 wk did not appreciably alter the infectious influenza virus concentrations in homogenates prepared from the lungs of infected irradiated mice compared with control mice. There also was no significant difference in the influenza virus HA titers in the lung homogenates from the irradiated mice compared with the control mice although there was a trend for the irradiated mice to have higher HA titers. The observations that beta-radiation from ^{144}Ce had no significant effect on influenza virus production in mouse lungs *in vivo* are similar to the findings that beta-radiation from ^{90}Sr–^{90}Y did not affect the production of virus in *in vitro* studies (18).

Significant differences in the responses of irradiated and control mice to influenza virus infection were evident when the lung weights, lung lesion scores and mortality were compared. In general, the greater the accumulated radiation dose and the longer the chronic irradiation of the lung, the greater was the increase in lung weight and lung lesion development compared to the control mice. It has been reported that lung lesion development following influenza virus infection was greater in X-ray irradiated mice than that in control mice (3).

Slight suppression of antibody formation due to beta-radiation from inhaled ^{144}Ce in an insoluble form was observed in some groups of mice. Similar findings have been reported in mice inoculated with influenza virus following whole-body X-ray or gamma radiation (1, 4, 7).

Adenomatous epithelial proliferation in 3 of 5 mice inoculated with influenza virus 20 wk after inhalation of $^{144}CeO_2$ had progressed to a clear squamous cell differentiation at 3 wk after virus inoculation. These metaplastic changes superficially resembled an epidermoid carcinoma in the lung of 1 mouse. These findings suggest that the combined insult may be important in the development of late sequelae such as lung cancer. Studies in which squamous metaplasia and cancers have been produced in mice by sequential exposure to carcinogenic chemicals and influenza virus infection have been reported (19–23). The role of preexisting or secondary bacterial infections that may occur in the histopathological changes observed following infection with influenza virus is unknown. The findings reported here should be more thoroughly investigated including life-span observations on animals that have had virus infection and received chronic irradiation of the lungs from internally deposited radionuclides.

Summary. The effects of chronic beta-radiation from inhaled $^{144}CeO_2$ that resulted in beta-radiation doses of 16,000 to 19,000 rads to the lung during 8- to 20-wk intervals had little or no effect on the influenza virus concentration or hemagglutinin titers in the lungs of virus inoculated mice when compared with virus inoculated control mice. There was, however, a slight effect in suppressing hemagglutination inhibiting antibody formation. Lung weights, lung lesions and mortality of mice inoculated with influenza virus at 8, 12 and 20 wk after inhaling $^{144}CeO_2$ were reported. Increased mortality occurred at 8 wk, increases in all three parameters at 12 wk and increases in lung weights and lung lesions were observed at 20 wk. Mice that inhaled $^{144}CeO_2$ and were inoculated with virus had significant histopathological changes. These were most marked in the mice infected with virus at 20 wk postexposure in which squamous cell differentiation was a prominent finding progressing in one case to a superficial resemblance to epidermoid carcinoma.

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