in the plasma glucose 140 times as high as that of blood acetate and higher ^{14}C activity in the lactose than in the butterfat. Much of the acetyl-CoA produced by beta-oxidation of the butyrate-2-^{14}C could well be incorporated into glucose within the hepatic cell via the TCA cycle since glucose is withdrawn rapidly for lactose synthesis(12). After injection of norvaline-3-^{14}C, maximum specific activity of the plasma glucose was 98 times as high as that of the blood acetate.¶ Norvaline, as indicated in Table I, also has a relatively high ketogenicity. Both norvaline and butyrate exhibit glucogenic behavior in addition to showing a relatively high ketogenicity as compared with the other metabolites investigated.

Summary. The relative transfer of carbon-14 from propionate-2-^{14}C, carbonate-^{14}C, glucose-1-^{14}C, glycine-1-^{14}C, butyrate-2-^{14}C, and norvaline-3-^{14}C into the 2, 3, 4 carbon atoms of urinary B-hydroxybutyric and acetoacetic acid, and acetone has been determined. Carbon-14 atoms of norvaline-3-^{14}C and butyrate-2-^{14}C were more ketogenic than those present in the other compounds investigated.

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Effects of Low Doses of X-Rays on Embryonic Development in the Mouse. (23158)

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Irradiation of mice in various stages of pregnancy has shown that the effect observed in the young subsequently born is closely correlated with the embryonic stage at which they were irradiated. Irradiation during the first 5 days, *i.e.*, prior to the embryo's implantation in the uterus, leads to all-or-none effects: there is a high incidence of death shortly after irradiation but those embryos that survive are normal. On the other hand, irradiation during the next 8 days, the period of major organogenesis, has little effect on

* Operated by Union Carbide Nuclear Co. for U. S. Atomic Energy Com.

survival to birth of the embryos, but almost all the animals born are abnormal, the exact type of malformation depending closely on the exact stage reached at the time of irradiation(1-6). All of our detailed reports to date have concerned experiments in the range from 100-400 r, and particularly doses of 200 and 300 r. From comparisons within that range it was quite evident that there must be different types of dose-effect curves for the production of different abnormalities at the respective sensitive stages. For the purpose of getting more extensive information on that point, lower dose ranges were chosen for the following reasons: (a) the total number of abnormalities in a given animal is smaller and the chance of abnormalities interfering with one another in expression therefore reduced; (b) while no threshold in production of certain abnormalities could be detected in the higher dose range, lower doses were needed to show whether or not the dose-incidence relation in such cases was truly continuous; and (c) from the point of view of hazards of radiation to human beings, it seemed desirable to have direct experimental evidence in a dose range occasionally encountered in fluoroscopy (7,8).

Because evidence on the third point is of practical importance, a preliminary report of the data is presented here, although analysis of the large amount of material collected(9) is not yet complete.

Material and methods. The developmental stages at which radiation was applied in the present experiment were 2 that, on the basis of our earlier results(2), were expected to yield not only malformations but also several quantitative changes of a type suitable for certain kinds of analysis. These quantitative effects include homeotic changes in the axial formula, and changes in the number of costo-sternal junctions and number of sternebrae. Day 8½ postconception has been shown to be a stage highly sensitive to the induction of these changes by radiation(2). Day 7½ is somewhat less sensitive to the same quantitative changes; it is, on the other hand, highly sensitive to the induction of various malformations of the axial skeleton.

The axial formula is variable within each of a number of inbred strains of mice studied (10). The mean value is characteristic of each strain, indicating that genetic constitution determines location of the strain on a scale of developmental potencies, while environmental factors cause individuals to be distributed about this mean. Although the final expression of the character is discontinuous (*e.g.*, either 25 or 26 presacral vertebrae) due to developmental thresholds, the continuous distribution in underlying processes can be mathematically derived(11). The strain chosen for the experiment here reported was the *BALB/c*, whose position with regard to the thresholds is such that even small induced shifts should be readily detectable; *i.e.*, the strain is naturally variable in the position of the thoraco-lumbar border, lumbo-sacral border, number of costo-sternal junctions, and number of sternebrae.

Females of the *BALB/c* strain were irradiated with 250 kvp X rays either 7½ or 8½ days after observation of a vaginal plug (following mating with *BALB/c* males). Doses used were 25, 50, and 100 r. Control females were taken through all the motions of the irradiation procedure. Litters were allowed to come to term. Newborns were killed, observed for various external and visceral characters, then processed for skeletal observation. Details of the technic have already been described(1,2). Skeletal observation was for vertebral column and thorax only. To date, 253 skeletons have been studied.

Results. Although both malformations and quantitative characters were recorded for each animal studied, this preliminary communication will present the former only for animals irradiated day 7½ postconception (Table I), the latter only for animals irradiated day 8½ (Fig. 1).

1. Malformations. Two broad conclusions may be drawn from the results shown in Table I. First, it is apparent that the incidences of all but one of the abnormalities (simple split of thoracic centra) increase with dose, and that dose curves for individual abnormalities are of a variety of shapes (compare, *e.g.*, "jumbling" of cervical centra with

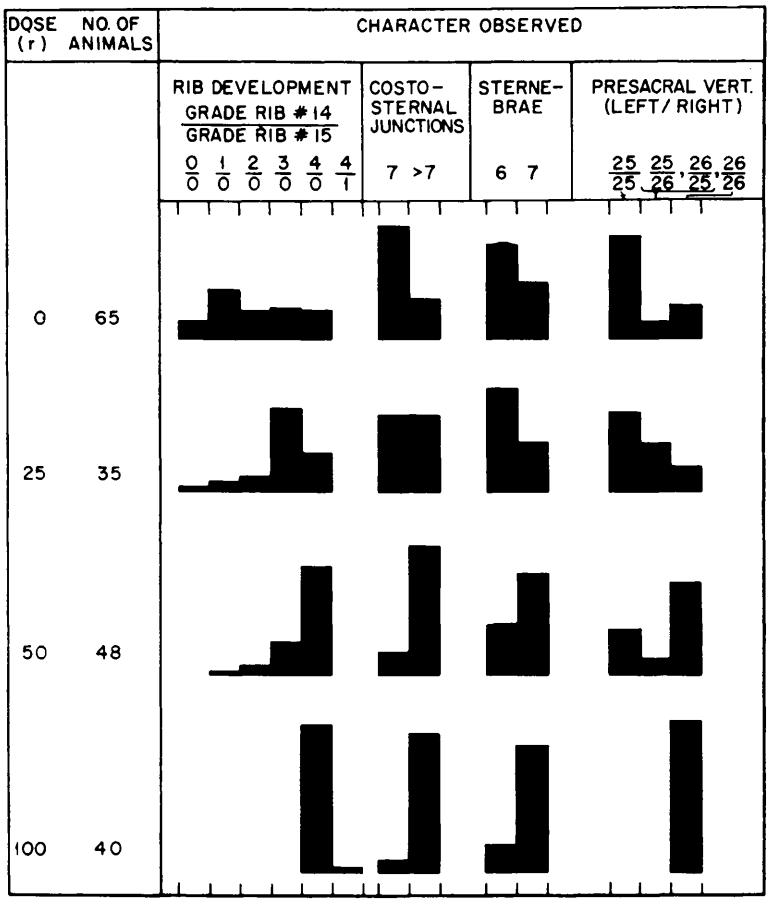

FIG. 1. Effects of irradiation on day 8½ postconception on various quantitative skeletal characters observed at birth in *BALB/c* mice. Histograms indicate percentages in the various categories.

fusion of ribs). It should, however, be pointed out that there are complications in making dosage comparisons. For one thing, a complete dosage comparison would have to take degree of abnormality into consideration. This has not been attempted here, Table I revealing only the incidence. Furthermore, it is quite likely that several of the abnormalities are correlated(2). Some of the abnormalities classified separately may not really be separate at all but merely represent different degrees of expression of the same basic effect.

A second conclusion that may be drawn from the results shown in Table I is that the lowest dose used in the present experiment, 25 r, gives effects that are detectable even with the relatively small sample sizes of this experiment. In the 25 r-group, the incidence of all 5 of the abnormalities normally found in *BALB/c* was increased and, in addition, 6 abnormalities were observed that were not present in controls. Although none of the *individual* comparisons that can be made is statistically significant by itself, comparison of the groups as a whole yields highly significant results. This is true regardless of which one of two opposite and extreme assumptions are made. Thus, if one considers all malformations completely uncorrelated with one another, *i.e.*, each embryo as the site of 17 possible independent malformations, the control group has 27 malformations in a total of 657 possible ones, while the 25 r-irradiated group has 57 in 507 possible ones ($\chi^2 = 20.69$, $P<0.001$). If one goes to the other

TABLE I. Percentage Incidence of Abnormalities of Axial Skeleton following Irradiation of Strain *BALB/c* Mouse Embryos 7½ Days Postconception.

Abnormalities†	Dose 0 r	25 r	50 r	100 r
	No. animals obs.* 39	30	29	6
Cervical				
atlas, arcus anterior	0	13.3	13.8	100
" , lateral masses	0	13.3	24.1	100
axis, dyssymphysis	23.1	43.3	48.3	100
centra,‡ reduced	5.4	17.2	19.2	75
" ,‡ absent	0	13.8	26.9	100
" , "jumbled"	0	3.4	23.1	66.7
arches, "	0	0	3.4	66.7
" , miscellaneous	0	0	3.4	16.7
Thoracic				
centra,‡ simple split	25.6	33.3	38.0	33.3
" ,‡ uneven split or split with reduction	5.1	20.0	41.4	100
" , "jumbled"	0	0	0	100
arches, "	0	0	0	83.3
" , other	0	0	0	16.7
Lumbar				
centra,‡ split	10.3	26.7	38.0	80
Thorax				
ribs,§ fused	0	0	0	100
costal cartilages,§ fused	0	3.3	10.3	100
sternum, "jumbled"	0	3.3	17.2	100

* A few percentages in the Table are based on somewhat lower numbers, since a few skeletons were damaged in certain regions or were otherwise unobservable.

† Descriptions of most of these abnormalities may be found in an earlier publication(2).

‡ One or more.

§ Two or more.

extreme and makes the most conservative assumption, namely, that all malformations are completely correlated (which is obviously not the case), the comparison between the groups is made on the basis of animals with or without malformations, namely, 21 versus 18 in controls, 26 versus 4 in 25 r-irradiated animals ($\chi^2 = 6.97$, $P<0.01$). (On the same assumption, but omitting from the abnormal classification those animals that have no abnormalities other than simple split of thoracic centra—the one character apparently not affected by increasing the dose—the significance of the difference between the control and 25 r-groups is increased: $\chi^2 = 9.75$, $P \doteq 0.003$). The true case lies somewhere between the extremes set up here; *i.e.*, there is probably some correlation between abnormalities(2), but this correlation is far from complete. Since the difference between the control and 25 r-irradiated groups is significant even with the most conservative assumption, it is clear that 25 r gives effects that are detectable in samples of the size used here.

2. Quantitative changes. All of the 4 characters shown in Fig. 1 are clearly affected by irradiation on day-8½ postconception. In all cases, the effect increases with dose and in 3 of the 4 it is already apparent after only 25 r. The difference between the 25 r-group and the controls is highly significant, both with respect to rib development and to the number of costo-sternal junctions ($P<0.001$ and $P = 0.0018$, respectively). The effect of 25 r on number of presacral vertebrae is on the borderline of significance ($P = 0.037$) when the data are divided into 3 categories (25/25; asymmetrical, *i.e.*, 25/26 and 26/25; 26/26).

Discussion. Low dose irradiation of mouse embryos, at stages known from our earlier work to be sensitive to induction of certain abnormalities in vertebral column and thorax, has shown (a) that there are different types of dose-effect curves for different abnormalities, and (b) that a dose as low as 25 r gives effects that are detectable even in relatively small samples.

Analysis of dose-effect curves, when completed, will contribute to our knowledge of mechanisms of radiation effect on developing organisms. The sensitivity of the system, as demonstrated in this experiment, gives it a valuable place among the few biological systems that can be explored in the low dose range.

The results must also be examined from the point of view of human hazards. Individual skeletal abnormalities that may be called drastic are found only with low incidence after 25 r. However, the average number of minor skeletal defects per animal is markedly increased. Since only one organ system was examined here, it seems likely that the overall effect of 25 r on all organ systems would add up to a certain load on the individual. The present results, therefore, reinforce our earlier recommendation(5,6) that, whenever possible

pelvic irradiation of women of childbearing age should be restricted to the 2 weeks following menstruation when the chance of an unsuspected pregnancy is small. This recommendation is based on the fact that the stages found most vulnerable in the mouse correspond developmentally to weeks 2-7 in human embryonic development, *i.e.*, to stages so early that pregnancy may still be unsuspected. The earlier work was primarily in the 200-300 r range(1-4) and recommendations for the human case were made mainly by downward extrapolation, supported, however, by some data from 100, 50, and 25 r irradiations(9). The present data, forming an extension to the former meager information at 25 r, are of especial interest to the human hazard problem since they deal with a dose level that is, on occasions, actually encountered in medical practice.

Summary. Mouse embryos of the *BALB/c* strain were irradiated 7½ or 8½ days postfertilization with 25, 50, or 100 r of X rays (doses lower than any heretofore investigated in detail), allowed to come to term, and then observed for changes in vertebral column and thorax. It was found (a) that there are different types of dose-effect curves for different abnormalities, and (b) that a dose as low as 25 r gives effects that are detectable even in relatively small samples.

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Elemental and Amino Acid Composition of Purified Plague Toxin. (23159)

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During the past several years, we have been concerned with studies on the production, purification, characterization, and mechanism of action of the murine toxin of *Pasteurella pestis*(1,2). The isolation of the toxin in purified form(1) has led to an interest in its chemical composition. A detailed analysis of the purified material is presented in this report.

Materials and methods. Strain "Tjiwidej" (TJW) of *Pasteurella pestis* was employed for toxin production. The organisms were grown either in trypticase soy broth and the toxin extracted from the cells or in a casein hydrolysate-mineral-glucose medium(3) and the toxin salted out from solution after autolysis of the bacteria. For all analytical determinations, the toxin was purified either by the method described by Ajl, *et al.*,(1) or by the large scale ionophoresis procedure of Karler. Criteria of purity included homogeneity of the material in the ultracentrifuge and electrophoresis cell(1). By the gel precipitation technic there were a major component and a small minor component in the preparation used for most of this work; a sub-