Differentiation as a Determinant of the Reaction of Rat Embryos to X-Irradiation.* (20026)

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Two previous papers(1,2) have dealt with the effects of irradiating rat embryos on the 9th and 10th days of gestation. The principal effects in both instances were slowing of the embryonic growth rate, the appearance of developmental malformations and an increase in intrauterine mortality. After a given effective dosage on either day the rate of mortality was generally similar, but growth retardation and the number of malformations were always greater following 9th-day treatment. Thus, on the whole, the rat embryo appeared to be more sensitive to irradiation on the 9th than on the 10th day of gestation. The greater reactivity of the 9th-day embryo was thought to be related to the lesser degree of embryonic differentiation at this age.

The present experiment is an extension of these studies to include the effects of x-irradiation on the 8th day of gestation. Embryos of this age show no differentiation; they consist merely of a homogeneous mass of randomly arranged blastomeres at the embryonic pole of the egg cylinder. This is distinctly more primitive than the condition seen on the 9th day when the embryo is a laminated plate composed of the 3 primary germ layers, ectoderm, mesoderm, and endoderm; and it is in even greater contrast to the 10-day embryo in which actual organogenesis is beginning in several systems.

The *method* was the same as that employed in the previous experiments and only the essential features need be mentioned here. On the 8th day of pregnancy female rats were anesthetized with Nembutal, an incision made in the ventral abdominal wall, and one uterine horn brought to the surface of the incision. Lead plates were so arranged as to shield the mother and all but 2 to 5 of the implantation sites. The unshielded sites were exposed to 12½, 25, 50, 100 or 200r of x-rays† after which the uterine horn was replaced within the abdominal cavity. Before the incision was closed, the number and position of all irradiated and non-irradiated embryos were noted on a diagram to facilitate later identification. Pregnancy was allowed to continue for 3, 5, or 7 days post-irradiation before the embryos were removed, fixed, weighed, and prepared for histologic study. The distribution of irradiated embryos as to dosage, post-irradiation interval, and number of non-irradiated siblings is presented in Table I.

Observations. The rate of intrauterine death among embryos irradiated on the 8th day was not increased above that of their non-irradiated siblings after doses of 12½ to 100r. Exposure to 200r, however, killed 14 of 15 embryos within 3 days and caused severe growth retardation in the survivor. During the same period only one of 24 non-irradiated siblings from the same pregnancies died. A dose of 200r, therefore, was regarded as rapidly lethal and no effort was made to obtain survivors after longer post-irradiation intervals. This dosage has been shown to be lethal also to embryos exposed on the 9th and 10th days (1,2). A difference, however, was noted in the reaction of 9- and 10-day embryos to doses less than 200 r. They showed an appreciable elevation of the mortality rate above control values after exposure to only 100r, and the 9-day embryos exhibited a moderate increase after as little as 50r. Thus, although embryos at the 8th, 9th or 10th days of gestation were equally susceptible to the lethal

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† The beam was that produced by the factors: 85 kvp, 10 ma, and 1.0 mm aluminum added filter. Further characteristics were: HVL of 2.0 mm of aluminum, and the 1.0-cm depth dose exactly equivalent to the air dose. The target-object distance varied between 10 and 20 cm and the duration of exposure never exceeded 2 minutes.

TABLE I. Distribution of Irradiated Embryos as to Dosage, Post-Irradiation Interval, and Number of Non-Irradiated Siblings.

Post-irradiation interval, days	No. of irradiated and control embryos in each dosage group									
	12½r		25r		50r		100r		200r	
	I*	C	I	C	I	C	I	C	I	C
3									15	24
5	9	10	13	15	16	18	10	10		
7	4	3	14	19	12	14	17	13		

* I = Irradiated; C = Control.

TABLE II. Retardation of Growth in Surviving, Irradiated Embryos Expressed as Mean Wt of Control—Mean Wt of Irrad. / Mean Wt of Control = % Retardation.

Age when exposed, days	Post-irrad. interval, days	% retardation following			
		12.5r	25r	50r	100r
8	5	1	12	18	30
8	7	5	6	5	14
9*	4		6	10	17
9*	6		1	1	17
10*	3			0	6
10*	4			0	9
10*	5			0	14

* Data taken from previous papers(1,2).

TABLE III. Representative Malformations Produced by Irradiation with 100r.

Day of irradiation	Organ or region affected	Incidence, %
8	None	0
9	Eyes	90
	Brain	45
	Aortic arches	27
	Heart	27
	Spinal cord	18
	Situs inversus	18
	Urinary tract	5
10	Eyes	75
	Urinary tract	25
	Brain	11

action of 200r, those treated on the 8th day were resistant to the lethal effects of smaller doses, whereas those treated on the 9th or 10th days showed a gradation of response.

The growth-retarding effects of irradiation, on the other hand, were more pronounced after treatment on the 8th day than at the older ages. Embryos receiving only 12½r on the 8th day showed a small deficiency in weight when compared with their non-irradiated controls 5 or 7 days later, and the disparity of weights became more striking after higher doses (Table II). Comparable data from previous experiments(1,2), although variable and not strictly equivalent in regard to the post-irradiation intervals, indicate that growth was inhibited to a lesser degree by irradiation on the 9th or 10th days.

No developmental malformations resulted from treatment on the 8th day. This is in sharp contrast to the results of 9th- or 10th-day exposure, after which the occurrence of congenital malformations was one of the most striking effects of irradiation (Table III). Although there was no evidence of aberrant developmental processes following treatment on the 8th day, general retardation in development was apparent in many embryos 5 to 7 days after exposure to 50 or 100r (Table II).

Discussion. These results demonstrate that the reaction of rat embryos irradiated on the 8th day of gestation was more limited than that of embryos exposed under similar conditions on the 9th and 10th days. After treatment at either of the older stages the general pattern of response was similar: the rate of mortality, the amount of growth retardation, and the incidence of malformations increased as the dosage increased—the only major difference being in the degree of response(1,2). After treatment on the 8th day, however, no malformations occurred and the mortality rate did not show a gradation as the dosage increased. This seems to constitute a different pattern of response.

Such a difference can best be accounted for in terms of differing susceptibilities to irradiation of the cells making up the embryos at the respective ages. The susceptibility of cells to irradiation is, in general, considered to be inversely related to the degree of cellular differentiation at the time of irradiation; *i.e.*,

the greater the susceptibility the less the differentiation, although exceptions to this generalization are well known. Indeed, the more limited response of the 8th- as compared with either the 9th- or 10th-day embryos seems to represent such an exception, whereas the greater sensitivity of the 9th- as compared with the 10th-day embryo is in accordance with the generalization. Then, in the present case, the diversity of response cannot be explained simply in terms of greater or lesser degrees of embryonic differentiation at the time of irradiation.

It is generally agreed that rapidly proliferating cells such as comprised these embryos may show any of 3 biological reactions to irradiation: 1) death may ensue without further cell division, 2) mitosis may be temporarily inhibited but later resumed at an approximately normal rate, or 3) subtle genic alterations (somatic mutations) may occur, but not be manifest until later in the faulty differentiation of descendant cells. The thresholds of sensitivity of these 3 reactions need not necessarily remain in a set ratio, nor need they change at the same rate, as differentiation progresses. The change in the embryonic response observed here can be explained by postulating that as differentiation began the component cells changed their propensity to show one or the other of the cellular reactions enumerated above. For example, the only observed effects of irradiation on the 8th day was smallness of size, until the dosage level was raised to 200r, at which level total mortality occurred. The simplest interpretation is that all cells which were "hit" or affected in any manner soon died. The death of a moderate number of these totally undifferentiated blastomeres would result in nothing more than a setback in the developmental schedule equivalent to the time required to replace the missing cells. This alone, or perhaps in combination with some mitotic delay in the surviving cells, could account for the retardation observed on the 5th and 7th post-irradiation days. Such destruction of blastomeres presumably could be tolerated up to a critical number, with no consequence other than generalized growth retardation; but destruction beyond this hypothetical point might be expected to cause embryonic death. The absence of an increase in mortality after all smaller doses and the total mortality after 200r are results compatible with such an explanation.

By the 9th day the embryo has already attained the first stage in differentiation, namely, the formation of the primary germ layers. Very likely some of the affected cells in the 9-day embryos, as in the 8-day embryos, were promptly killed by the irradiation; but there is strong evidence that other affected cells in the 9-day embryos reacted in a different way: they survived and continued to proliferate more or less normally until some critical stage in organ formation was reached. The malformations, many of which did not become apparent until several days after irradiation, undoubtedly included some that developed from surviving cells bearing altered developmental potentialities, that is, cells in which the genic make-up had been changed by the radiation. Malformations can result from the deletion of some or all of the cells already induced to form a particular organ, but this mechanism implies specific deficiency of tissues or parts in the abnormal organ. Several of the observed malformations(2) did not involve such deficiencies but, instead, were judged to be the result of aberrant developmental processes. Then, it is probable that certain of the cells that survived irradiation on the 9th day suffered genic alterations which became apparent only later in defective organ formation. It is also possible that some of these somatic mutations had lethal effects and thus secondarily contributed to the increased mortality among the irradiated animals.

On the 10th day differentiation had progressed to the point that overt organogenesis was present in several systems. The fact that embryos of this age reacted in a similar but less pronounced manner than did 9-day embryos, may be taken as an indication that the susceptibility of the cells to the various radiation-induced effects was also similar but that the threshold of reactivity to all such effects had risen. This proportionate lessening in all of the effects is thought to be related simply to the greater differentiation within the

10-day embryo.

The observations presented here warrant the conclusion that the pattern of response to irradiation in embryonic rats undergoes a qualitative change when cellular differentiation begins within the embryo. A similar change in the pattern of response appears to have occurred in a series of irradiated mouse embryos studied by Russell(3), if due allowance is made for the relatively faster developmental schedule of the mouse. The data presented by Job, Leibold, and Fitzmaurice(4), on the irradiation of rat embryos over a wide range of ages, lack sufficient detail to support a positive conclusion, although the possibility of a change in the response to irradiation between the 8th and 9th days of gestation is not contradicted by their results.

Summary. X-irradiation of rat embryos on the 8th day of gestation had a more limited effect on subsequent development than did similar treatment on the 9th or 10th days. The only residual effect after doses of 12½ to 100r on the 8th day was retardation of growth. Exposure to comparable doses on the 9th and 10th days has been shown previously to result in the development of malformations and in an increase in mortality, as well as in retardation of growth. This difference in reactivity appears to be dependent upon whether cellular differentiation within the embryos has begun at the time of irradiation.

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Equal Effectiveness of L and D-Ethionine in Producing Tissue Damage in Rats and Mice. (20027)

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L-ethionine has been found to be as effective in producing fatty changes in the liver of female rats as the racemic dl-ethionine(1). Dl-ethionine produces renal changes in the rat similar to that produced by dl-serine(2). The nephrotoxic action of serine, however, is only caused by the d-isomer and not by the naturally occurring l-serine(3). It seemed therefore of interest to test both the l- and d-isomer of ethionine as to their possible nephrotoxic action as well as to their effect on the pancreas. In addition the influence of both isomers upon the liver and pancreas of male albino mice was tested. Changes induced by ethionine in the albino mouse will be briefly described since this species is apparently well suited for studies of this kind.

Material and method. The L- and D-ethionine were obtained from the California Foundation for Biochemical Research in Los Angeles. The L-ethionine had a specific rotation of $[\alpha]_D^{25} = +23.5°$ as a .85% solution in 0.2 normal HCl. The D-ethionine had a specific rotation of $[\alpha]_D^{25} = -23.6°$ as a .8% solution in .2 normal HCl. Dl-ethionine was obtained from the Nutritional Biochemical Corp., Cleveland, Ohio. Young male albino rats of the Wistar strain were given a protein free diet as previously described(4). After having been on this diet for 8 days, they received 1 mg ethionine per g body weight in 3 divided doses via the intraperitoneal route. The animals were killed 48 hours later. Male albino mice of the Swiss strain were fed a synthetic diet containing 16% casein. This diet was supplemented with either .5% of L- or D-ethionine. The animals were killed after 5 days. Total liver lipids of some of these mice as well as of controls were measured gravimetrically after extraction with chloroform. In order to study the influence of ethionine on the mouse, young albino mice of