Reports and Letters

Pronuclear Fusion as Affected by X-rays and by Postirradiation Anaerobiosis

In studies on the modification of the mitotic time schedule in the eggs of *Arbacia punctulata* by exposure of the gametes to x-rays, Henshaw (*1*) made the remarkable observation that x-irradiation caused no retardation from the time of entrance of the sperm head into the egg through fusion of the pronuclei, although it did affect mitotic processes. Since virtually all biological processes have been shown to be sensitive to radiations to a greater or lesser degree, it seems a priori that x-irradiation should also affect the process of pronuclear fusion, for this is a dynamic process and is not merely the result of chance collision of pronuclei. Even though this situation holds for *Arbacia,* it appears doubtful that it should be general for other organisms.

A test of this was made for eggs of *Ascaris lumbricoides suum* (*2*). These eggs were chosen because of their highly desirable characteristics for the study of pronuclear fusion phenomena. Cytological observations in this laboratory show that all fertilized eggs taken from the terminal 25 mm of the uteri of *Ascaris* are in the pronuclear stage. On incubation at optimal temperatures, pronuclear fusion begins slowly and continues for 48 hours. The eggs of *Ascaris* thus provide an excellent opportunity for study of the effect of x-rays on the rate of pronuclear fusion. These nuclear events differ greatly from those of the more extensively studied eggs of *Ascaris magalocephala.*

Pronuclei are obscured in the cell by large amounts of dense yolk and other granular material. Visibility in the living eggs was enhanced by two methods, namely, compression of the cells and centrifugation of the cells. Of the various stages observed in pronuclear fusion, the one designated ¼-fused was used as the criterion of fusion. The sum ¼-fused is used to designate that stage in which the male and female pronuclei show early signs of union and in which they begin to lose their isodiametric form; the joining process is detectable along an area of the nuclear membranes of approximately one-fourth the diameter of the pronuclei. The importance assigned to ¼-fusion is the fact that, once this dynamic process has begun in the normal cell, it proceeds to completion with amazing regularity. If the cell or its constituents have been damaged, one of the earliest and most reliable signs of nuclear damage, which is ultimately reflected in aberrations in cleavage and embryogenesis, is the delay in the early stages of pronuclear fusion.

The cleaning and preparation of the egg stocks, as well as the irradiation procedures, have been described previously (*3*). Irradiations were carried out with unfiltered 100 kv (peak) x-rays; the dose rate was 12 kr/min.

Figure 1 shows the effect on pronuclear fusion of 24 kr and 240 kr of x-rays. The retardation of pronuclear fusion is evident and parallels the retardation of cell cleavage that has previously been observed (*3*). Interest in recovery mechanisms in radiation effects has prompted a study of postirradiation treatments calculated to bring about recovery from radiation damage. The effect of one successful type of postirradiation treatment, anaerobiosis, on fusion of pronuclei is reported here. Since *Ascaris* is a facultative anaerobe, it is possible to hold eggs for long periods of time under anaerobiosis without detectable harm to the eggs, even at optimal incubation temperatures. Pronuclear fusion is arrested during anaerobiosis; on aerobic incubation, these processes continue undisturbed in normal, unirradiated cells at the same rate as they do in eggs that have not been subjected to anaerobiosis. Eggs that have been x-irradiated and held anaerobically for a 24-hour period immediately following irradiation, however, show an increased rate of pronuclear fusion when they are subsequently incubated aerobically under optimal conditions. This recovery from radiation damage is shown by a comparison of the two pronuclear-fusion curves for eggs that were treated with x-rays only with the curves for eggs that were treated with x-rays and anaerobiosis (Fig. 1).

Pronuclear fusion in *Ascaris* is, therefore, susceptible to x-irradiation damage, as evidenced by a retardation of the fusion process; and recovery, which is evidenced by an acceleration of the fusion process in the injured cell, can be brought about by anaerobic treatment following irradiation. Since the pronuclear fusion rate in *Arbacia* is very rapid in comparison with that in *Ascaris,* two possibilities may be suggested for failure to observe retardation from x-irradiation in *Arbacia*: (i) the rapid rate of fusion makes observations of delay difficult at the doses used; (ii) the much longer period required for pronuclear fusion in *Ascaris* would permit a greater opportunity for the effects of x-rays to express themselves.

The mechanism(s) whereby this recovery is achieved are at present not clear, but several observations may be made as follows.

1) There is a limited parallel between the present results and those reported for postirradiation anaerobiosis and KCN treatment of irradiated *Vicia* seeds (*4*). The work on *Vicia* indicates that oxidative metabolism is necessary for rejoining of radiation-induced chromosome breaks. In the present study, no observable development took place during anaerobiosis either, but afterward, under aerobic incubation, recovery was evident and is attributable to the period of anaerobiosis (or KCN treatment as noted in observation 3). The present study, therefore, carries the problem a step further.

2) The recovery is not due simply to the delay in vital processes that is caused by anaerobiosis, for these same processes can be delayed by lowered postirradiation incubation temperatures. Low temperatures, however, do not foster recovery (*3*) but actually decrease survival and prolong the net time required for cell cleavage when the eggs are subsequently incubated at optimal tmperatures.

3) Evidence from other experimentation (*5*) indicates that the cytochrome

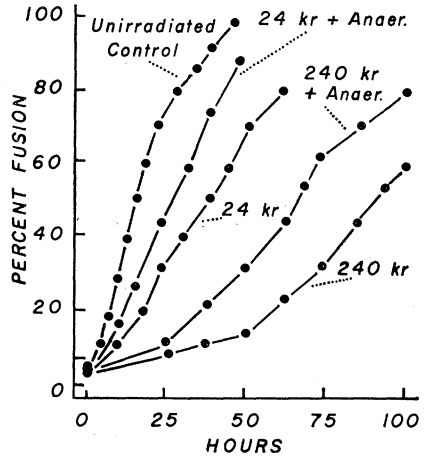

Fig. 1. Effect of x-rays alone and of x-rays followed by 24-hour anaerobiosis on the pronuclear fusion time of the eggs of *Ascaris lumbricoides suum.*

system may be involved, for essentially the same results can be secured by post-irradiation exposure to KCN before the incubation period at optimal temperatures.

4) Since anaerobic recovery is greatest when the deoxygenated eggs are held at optimal temperatures, it appears that anaerobiosis, as well as KCN treatment, functions in some other way than merely by preventing the cytochromes from carrying out oxidative processes that are necessary for the expression of the latent damage in the irradiated cell. While the cytochromes are being held in abeyance, a positive function, probably the anaerobic synthesis of proteins and other substances essential for recovery of the damaged cell, is taking place. The exact nature of these anaerobic recovery processes must await further experimentation.

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References and Notes

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2. This research was performed under contract No. AT(11-1)-205 between the U.S. Atomic Energy Commission and the University of Notre Dame.
3. C. S. Bachofer and G. Pahl, *Radiation Research* 2, 50 (1955).
4. S. Wolff and H. E. Luippold, *Science* 122, 231 (1955).
5. G. Pahl and C. S. Bachofer, unpublished results.

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Effect of DDD and Some of Its Derivatives on Plasma 17-OH-Corticosteroids in the Dog

In 1949, Nelson and Woodard (*1*) reported that 2,2-bis-(para-chlorophenyl)-1,1-dichloroethane (DDD) causes atrophy of the adrenal cortex in the dog. Other workers have confirmed this finding and have obtained indirect evidence of decreased adrenal function following DDD administration (*2–4*). Recently, Larson *et al.* (*5*) studied various DDD derivatives in order to determine the relationship of chemical structure to the production of adrenal cortical atrophy or hypertrophy. In the present investigation (*6*) the effect of DDD, of 2,2-bis-(para-ethylphenyl)-1,1-dichloroethane (Perthane), and of 2-hydroxy, 2,2-bis-(para-chlorophenyl)-1,1-dichloroethane (FW-152) on adrenal function in dogs has been followed as indicated by changes in the plasma 17-hydroxycorticosteroids after injection of ACTH, this response being determined before and after the administration of each of the three compounds. Compound FW-152 differs from DDD and Perthane in that it has been found to produce the histological appearance of adrenal cortical hypertrophy rather than atrophy (*5*).

Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels (*7*). Preliminary experiments revealed that the normal level of circulating 17-hydroxycorticosteroids in dogs is much lower than that found in human beings. In 32 determinations on 9 dogs, the mean level with S.D. was 2.6 ± 1.5 μg/100 ml of plasma. Since these values are at the lower limit of accuracy for the method, it was necessary to work with dogs whose adrenals had been stimulated with an intravenous injection of ACTH. Initially, time-response curves were run on several animals, and it was found that the plasma 17-hydroxycorticosteroids reached a maximum level between 1.5 and 2 hours after 20 I.U. of ACTH (*8*) had been given intravenously. In all subsequent experiments, a control blood sample was drawn, ACTH was given, and a second blood sample was taken at the end of 2 hours. In this way, a 2.5- to 10-fold increase in plasma 17-hydroxycorticosteroids over control levels was obtained; this permitted the measurement of a significant change after the administration of an atrophy-producing compound. Although the range of ACTH response varied among dogs, the response in the individual animal was relatively constant.

In Figs. 1 and 2, the control 17-hydroxycorticosteroid levels after injection of ACTH are represented by a vertical bar that gives the extremes and mean values for three to five tests on each animal. Fig. 1 shows the marked diminution of adrenal responsiveness that occurred after administration of various doses of DDD. Fig. 1*A* illustrates a fall in plasma levels from 20 to 0 μg/100 ml. This animal showed no signs of distress, and after discontinuance of the compound, normal adrenal response to ACTH gradually returned. On subsequent treatment with a

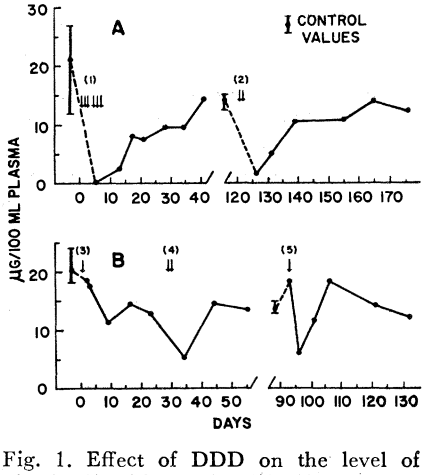

Fig. 1. Effect of DDD on the level of plasma 17-hydroxycorticosteroids 2 hours after injection of ACTH; ↓ indicates oral administration of DDD as a 20-percent solution in corn oil as follows: *1, 2, 3,* and *4*—100 mg/kg day; *5*—200 mg/kg day.

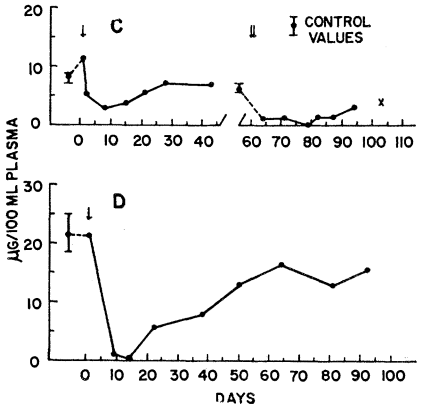

Fig. 2. Effect of Perthane on the level of plasma 17-hydroxycorticosteroids 2 hours after injection of ACTH; ↓ indicates oral administration of Perthane as a 20-percent solution in corn oil at 200 mg/kg day; *X,* animal sacrificed.

smaller dose, a similar fall and recovery were noted. A fall in plasma 17-hydroxycorticosteroids to zero was also obtained with several animals that were treated with Perthane, data on two of which are shown in Fig. 2. One animal (Fig. 2*C*) recovered so slowly after the second treatment with this compound that it was sacrificed at the end of 4 weeks for histopathological study of the adrenals, which showed the typical adrenal cortical atrophy that is produced by Perthane (*5*). The second dog (Fig. 2*D*) evidenced a marked sensitivity to Perthane—a single dose produced a fall in plasma 17-hydroxycorticosteroids to zero. Another dog, not shown, was given six doses of Perthane of 200 mg/kg day. On the fifth day, the plasma level was zero, and death occurred on the eighth day.

As is apparent in Figs. 1*B* and 2*C*, levels somewhat higher than normal were occasionally obtained 6 hours after the compounds were given. This effect was fleeting and was not consistently found.

Two dogs were treated daily with FW-152 at 50 mg/kg day. At the end of 1 week, one died suddenly of an unknown cause, having shown no significant change in the 17-hydroxycorticosteroid level. The other dog was sacrificed after 26 days, when it was in a moribund state. Because of poor fixation, histological examination was unsatisfactory. During the experimental period, the plasma 17-hydroxycorticosteroids fell slowly from 14 to < 2 μg/100 ml, a finding that is of interest in view of the microscopic adrenal changes indicative of hypertrophy that have been previously reported following administration of this compound (*5*).

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