Table 1. Incorporation of ^{3}H-thymidine after nuclear transplantation in *Amoeba proteus*.

Nuclei (No.) having			
5–25 grains	26–45 grains	46–75 grains	>75 grains
G-2 nuclei transplanted to G-2 cytoplasm			
11	4	0	0
G-2 nuclei transplanted to S cytoplasm			
1	2	3	2
S nuclei transplanted to S cytoplasm			
0	0	1	12
S nuclei transplanted to G-2 cytoplasm			
4	4	1	1

cultivation of amoebae (*3*) and transplantation of nuclei (*4*) have been described.

Four types of transplants were used: (i) an S nucleus into an S cell (a control); (ii) a G_2 nucleus into a G_2 cell (a control); (iii) an S nucleus into a late G_2 cell; and (iv) a late G_2 nucleus into a S-phase cell. From one experiment to the next, the S and G_2 cells were roughly the same ages within their respective subsections of the cell cycle. Immediately after nuclear transplantation, each cell was placed in a medium containing ^{3}H-thymidine (25 μc/ml, 15 c/mole) for 5 hours, after which the nuclei were isolated and assayed by autoradiography.

Incorporation of ^{3}H-thymidine by an S nucleus transplanted into an S-phase cell is not detectably different from that by a normal, nontransplanted S-phase nucleus (grain counts above 75 per nucleus) indicating that disturbances engendered by the operation do not affect DNA synthesis to a measurable extent. When an S nucleus is transplanted into a G_2 cell, the amount of ^{3}H-thymidine incorporated is clearly reduced (Table 1). When nuclei have 0 to 45 grains, it is doubtful that such counts can be accepted as a demonstration of DNA synthesis. Grain counts of this magnitude, as some of our other experiments have shown, are found frequently for nonsynthesizing nuclei, and such activity is largely or probably entirely insensitive to deoxyribonuclease treatment. A nucleus synthesizing DNA is almost always easily recognized because its incorporated activity is so much greater than the nonspecific labeling.

In the case of the ten nuclei transplanted into G_2 cells, eight do not show significant labeling, and two are not different from controls (an S nucleus into an S cell). Obviously the new environment (a G_2 cell) for the S nucleus has a marked effect on the incorporation of ^{3}H-thymidine.

When G_2 nuclei are transplanted into G_2 cells, all have counts of 0 to 45 grains. This amount of labeling cannot be considered significant. When G_2 nuclei were transplanted into S-phase cells the results showed wide variation, but clearly two and probably five out of eight G_2 nuclei had incorporated ^{3}H-thymidine in their new environment.

Thus, an S nucleus placed in a G_2 cell slows or stops its DNA synthesis, and a G_2 nucleus placed in an S-phase cell may recommence DNA synthesis. These results could be explained if only S cells are capable of conversion of ^{3}H-thymidine to ^{3}H-thymidine triphosphate, but there is much evidence (*1*) that nucleoside triphosphates, while necessary for DNA synthesis, do not act in the control or regulation of synthesis. Alternatively, the results are interpretable either in terms of cytoplasmic inhibitors of DNA synthesis (present in G_2 cells but absent in S cells) or cytoplasmic initiators (present in S cells and absent in G_2 cells). Although the experiments summarized in Table 1 do not allow a choice between an inhibitor or an initiator situation, we favor the initiator hypothesis of Jacob, Brenner, and Cuzin (*2*), not only on the basis of their work, but also because the sequential pattern for DNA synthesis in mammalian chromosomes seems more reasonably explained by a hypothesis that assumes sequential appearance of initiators.

Finally, the conclusion that the cytoplasm has an important influence on nuclear DNA synthesis may surprise no one, but there is perhaps merit in proving the existence of a phenomenon, the "evidence" for which has been primarily assumption.

DAVID M. PRESCOTT
Institute for Developmental Biology, University of Colorado, Boulder
LESTER GOLDSTEIN
Department of Biology, University of Pennsylvania, Philadelphia

References and Notes

1. K. G. Lark, in *Molecular Genetics*, J. H. Taylor, Ed. (Academic Press, New York, 1963), part 1, pp. 153–206.
2. F. Jacob, S. Brenner, F. Cuzin, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 329 (1963).
3. D. M. Prescott and R. F. Carrier, in *Methods in Cell Physiology*, D. M. Prescott, Ed. (Academic Press, New York, 1964), vol. 1, pp. 85–95.
4. L. Goldstein, *ibid.*, pp. 97–108.

9 November 1966

Dopamine Protects Mice against Whole-Body Irradiation

Abstract. *Injection of dopamine before whole-body x-irradiation of mice resulted in 80 percent survivors whereas no irradiated controls survived; injection after exposure had no effect.* D,L-*Dihydroxyphenylalanine, the precursor of dopamine, had no effect on survival when injected either before or after irradiation.*

Epinephrine protects mice against whole-body x-irradiation whereas norepinephrine does not (*1*). These two catecholamines are physiologically active substances in both vertebrates and invertebrates. In 1951 a third catecholamine, dopamine, was identified in mammalian heart tissue (*2*). More recent reports are that dopamine is the only catecholamine detectable in certain tissues such as lung, liver, and intestine; it accounts for as much as 50 percent of the catecholamine content in certain other organs (*3*). In view of its similarity in structure to epinephrine and its higher concentration in certain radiosensitive tissues, we investigated its efficacy as a radioprotective agent.

Male albino mice (strain BNL, aged 5 weeks, weighing about 15 g) were x-irradiated at 250 kv (peak), 30 ma, with added filtration: 0.5 m Cu, 1 mm Al (half-value layer, 1.25 mm Cu; homogeneity coefficient, 0.50). The exposure rate was 121 r/min; whole-body exposure varied from 700 to 800 r. Immediately before exposure, one group of mice were each injected intraperitoneally with 10 mg of dopamine in saline; another group were similarly dosed immediately after exposure. The time between injection and irradiation was 3 minutes. Controls were irradiated after receiving an equal volume of saline. Sham-irradiated controls were injected with dopamine or saline.

Administration of dopamine before 700-r exposure produced 80-percent survival; no irradiated controls survived (Fig. 1). Against 800 r, dopamine is

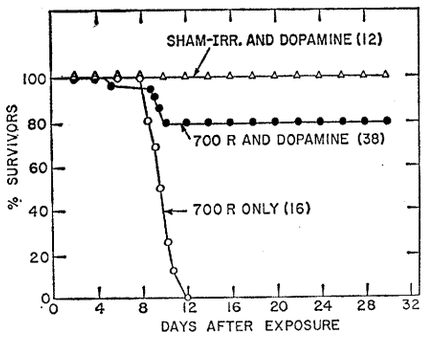

Fig. 1. Effect of dopamine on whole-body x-irradiation of mice; each mouse was injected intraperitoneally with 10 mg immediately before exposure. Number of animals appear in parentheses. The animals were observed for 30 days.

much less protective (Table 1). Administration after irradiation was not protective. The radioprotective efficacy of the same dose of dopamine was further determined on the spleens of x-irradiated mice. On day 7 after exposure, the average spleen weight of irradiated mice, pretreated or untreated with dopamine, was considerably less than that of the sham-irradiated controls (Fig. 2); but by day 20 it was greater in the recipients of 700 r and dopamine than in the sham-irradiated controls. Histology of the spleen at day 7 in the animals that received dopamine plus irradiation showed a marked decrease in number of lymphocytes, in which the animals resembled the irradiated controls; however, by day 20 there was an increase in the lymphocytic series. In animals whose spleens were shielded with lead during whole-body irradiation, Jacobson (*4*) observed an erythropoeitic hyperplasia of the spleen. Thus radioprotection by dopamine differs from lead shielding with regard to its effect on the spleen.

Because the radioprotective effectiveness of epinephrine or serotonin is significantly reduced in splenectomized mice (*5*), the protective effect of dopamine also was investigated in splenectomized animals. Splenectomy was performed under ether anesthesia. Preliminary results indicate that the percentage survival of whole-body irradiated mice pretreated with dopamine is not changed by splenectomy. This finding suggests that the radioprotective mechanism of dopamine differs from that of the other amines.

Table 1. Percentages of mice surviving for 30 days whole-body irradiation following injection; numbers surviving are shown in parentheses.

N	Exposure (r)	Injection	Survival (%)
12	0	Dopamine	100 (12)
29	700	Saline	0 (0)
46	700	Dopamine	80 (37)
22	750	Saline	0 (0)
17	750	Dopamine	35 (6)
15	800	Saline	0 (0)
13	800	Dopamine	8 (1)

In order to determine whether the injected dopamine is present in the radiosensitive organs during exposure, 3,4-dihydroxyphenyl-ethyl-β-H^3 amine HCl (H^3-dopamine; specific activity, 21.6 mc/m*M*) was injected intraperitoneally into unirradiated mice at 1 μc per gram of body weight. The animals were killed 10 minutes later because irradiation was then complete. The tissues were rinsed twice in saline and blotted with gauze, and the wet weights were recorded. The tissues were then transferred to counting vials and were dissolved and digested in 5 ml of warm hyamine for 1 to 3 hours. When the digests had cooled, 10 ml of toluene, containing 40 mg 2,5-diphenyloxazule and 0.5 mg of 1,4-bis(2,5-phenyloxazolyl)benzene, was added to each vial and the H^3 activity was determined (*6*). Efficiency of the counting in the toluene-hyamine system was 3.2 percent. Since the sample color varied according to the source of the tissues, quenching of the samples was corrected for by use of H^3-toluene as an internal standard.

Figure 3 shows that the adrenals had the highest radioactivity, the brain the lowest. Considerable radioactivity was present in the radiosensitive organs: gonads, spleen, and small intestine; since dopamine is itself the end product of catecholamine biosynthesis in these organs, most of the radioactivity was probably present as dopamine. In the brain and adrenal gland, dopamine can be converted into norepinephrine and epinephrine, but the brevity of the interval between our injection of the drug and irradiation severely limited the conversion of dopamine to the other catecholamines.

The radioprotective action of D,L-dihydroxyphenylalanine (DOPA), the precursor of dopamine, was also investigated. Each mouse was injected intraperitoneally with 10 mg of DOPA immediately before irradiation, other mice immediately after exposure. The DOPA, being insoluble in saline, was injected as a suspension. Whether given before or after irradiation, this amino acid

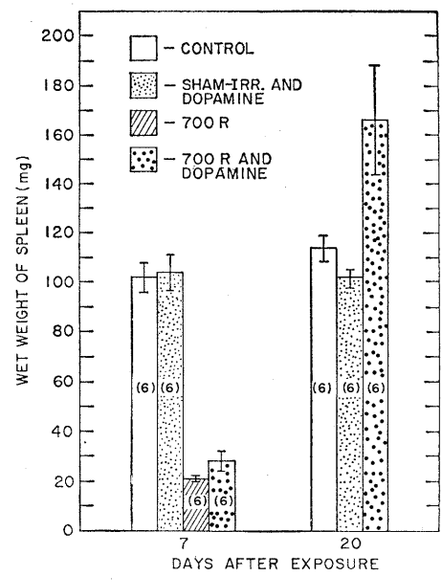

Fig. 2. Effect of dopamine on the wet weights of the spleens of whole-body x-irradiated mice. Each mouse was injected intraperitoneally with 10 mg immediately before exposure. Numbers in parentheses show the number of animals. Vertical bars, S.E. of the means.

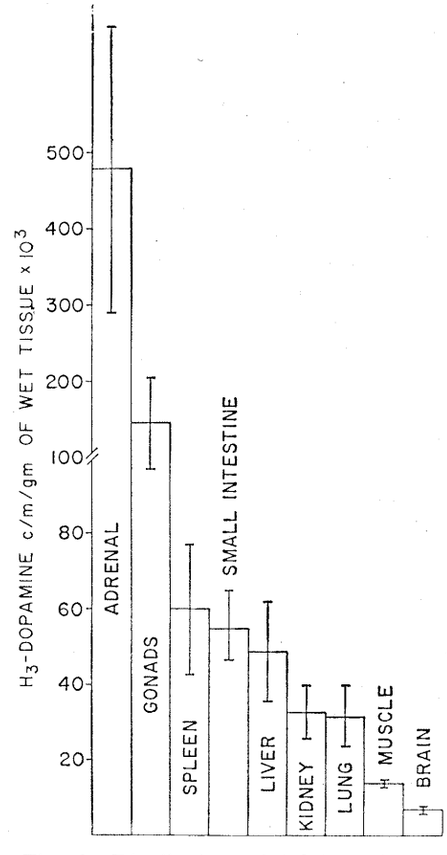

Fig. 3. Distribution of H^3-dopamine in unirradiated mice; it was given intraperitoneally at 1 μc per gram of body weight 10 minutes before the animals were killed. The radioactivity for each organ is the average for seven animals. Vertical bars, S.E. of the means.

had no effect on survival of the mice.

In the dosages used by us, both DOPA and dopamine produced increased motor activity and piloerection in mice; both are intermediary metabolites in the synthesis of norepinephrine and epinephrine:

phenylalanine → tyrosine → DOPA → dopamine → norepinephrine → epinephrine.

In addition to being a precursor of norepinephrine, dopamine apparently has physiologic actions of its own; its importance in brain function has recently been extensively reviewed (*7*). Thus endogenous dopamine may also be important to an animal's defense mechanisms against radiation damage.

The mode of action of dopamine in protecting against injury by radiation is obscure. Since norepinephrine, the precursor of epinephrine, is not a radioprotective agent, it is unlikely that the dopamine effect is by conversion to epinephrine. Our results in splenectomized mice also support the conclusion that it was dopamine itself, and not the metabolite epinephrine, that was the active agent in our experiments.

KEDAR N. PRASAD
MELVIN H. VAN WOERT
Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York, and *Medical Research Center, Brookhaven National Laboratory, Upton, New York*

References and Notes

1. Z. M. Bacq and A. Harvey, *Bull. Acad. Roy. Med. Belg.* **17**, 13 (1952); J. L. Gray, E. J. Moulden, J. T. Tew, H. Jensen, *Proc. Soc. Exp. Biol. Med.* **79**, 384 (1952).
2. M. Goodall, *Acta Physiol Scand.* **24**, suppl., 85 (1951).
3. H. J. Schumann, *Arch. Exp. Pathol. Pharmarkol.* **236**, 474 (1959); U. S. von Euler and F. Lishajko, *Acta Physiol. Pharmacol. Neerl.* **6**, 295 (1957).
4. L. O. Jacobson, *Bull. N.Y. Acad. Med.* **30**, 675 (1954).
5. H. J. Melching, C. Streffer, H. Sauer, *Strahlentherapie* **123**, 571 (1964).
6. With a Packard TRI-CARB liquid-scintillation counter.
7. O. Hornykiewics, *Pharmacol. Rev.* **18**, 925 (1966).
8. Supported by the AEC.

10 November 1966

Hexagonal Pattern in Cell Walls of Escherichia coli B

Abstract. *Cell walls, isolated from* Escherichia coli *B, as examined by electron microscopy and optical diffraction contain a hexagonal lattice structure, the (1,0) planes of which are separated by 140 ± 8 angstroms. Unless the walls are briefly heated (10 minutes, 90°C) early in the isolation, the hexagonal array cannot always be observed. Enzymatic digestion with pancreatin and amylase improves visualization of the lattice; subsequent treatment with pepsin and sodium dodecylsulfate removes the hexagonal pattern. Protein or lipoprotein globular units within the wall may thus be arranged in a hexagonal array upon the mucopeptide layer.*

Hexagonal packing of subunits in cell walls of gram-negative bacteria has been observed in *Spirillum sp.*, *Rhodospirillum rubrum*, *Halobacterium halobium*, and *Lampropedia hyalina* (*1*). Although several investigators have studied the structure of the cell wall of *Escherichia coli* (*2*), there has been no evidence of periodic packing of constituent subunits (*3*). We now present electron micrographs and an optical diffraction pattern indicating a hexagonal structure in the wall of *E. coli*; the apparent lability of this structure may account for its not having been detected previously.

Escherichia coli B (*4*) was grown on synthetic medium (*5*); the cells were harvested in the late logarithmic growth phase. The intact cells were ruptured with glass beads (0.2 mm diameter) (*6*), and the cell envelopes (*7*) were separated by differential centrifugation and washed ten times with freshly distilled water. To preserve the hexagonal structure within the cell walls so that it may be observed, it is necessary to heat the suspension of cell envelopes for 10 minutes at 90°C; after this procedure the preparation can be examined directly by electron microscopy (Figs. 1 and 2) or subjected to enzymatic digestion prior to ultrastructural analysis (Figs. 3, 4, 5, and 6). For electron microscopy, preparations of cell envelopes, fixed in formalin and unfixed, were negatively stained with 1 percent aqueous potassium phosphotungstate (PTA) at *p*H 7.4. Fixed and unfixed preparations yielded identical results, and the same result was obtained after negative staining with 2 percent uranyl acetate.

Therefore only material stained with PTA is presented. Droplets of the envelope suspensions in PTA were placed on copper grids (200 mesh) covered with parlodion; the grids were dried and examined with an AEI EM6B electron microscope operating at 60 kv with double condensor alignment at a 50-μ objective aperture. The magnification was calibrated by including tobacco mosaic virus (outside diameter 180 Å) in some of the cell envelope suspensions (*8*).

Freshly isolated cell envelopes of *E. coli* B that were not heated, when examined in the electron microscope, show no reproducible hexagonal pattern (Fig. 1). In contrast, many of the heated envelopes show areas of the cell wall with small granules in a hexagonal distribution (Fig. 2). The cell membrane retracts from the cell wall in most of these preparations and forms the dark mass in the central region of the cell.

To improve the demonstration of this periodic structure associated with the cell wall, the heated envelopes were digested with pancreatin and amylase (*9*). As seen in Figs. 3, 4, and 6a, the hexagonal lattice structure is clarified considerably; it is present over the complete cell wall, and moire patterns (*10*) are produced by the overlay of two cell-wall surfaces. The measured distance between the (1,0) lattice planes is 140 ± 8 Å.

After digestion with pancreatin and amylase, the regular pattern associated with the cell wall can be removed by incubation of the cell envelopes in pepsin and subsequent treatment with sodium dodecylsulfate (SDS) (*11*). In such a preparation (Fig. 5) all hexagonal regularity has been lost, and the walls appear similar to the protein-free rigid substructure obtained by Weidel, Frank, and Martin (*2*) after SDS, phenol, and trypsin treatment.

An optical diffraction pattern (*12*) substantiates the hexagonal symmetry in the envelopes treated by heat and by pancreatin and amylase (Fig. 6b).

Our results demonstrate that a hexagonal structure is associated with the cell walls of *E. coli* B, and that the structure can be consistently observed if the walls are heated during their isolation. That the hexagonal pattern is a labile structure, preserved by heat treatment, rather than an artifact produced by high temperatures, is suggested by the fact that in a few cases a hexagonal array has been seen in walls which were not heated during isolation but which were subsequently treated with pancreatin and amylase. Although such observations are uncommon, the presence of the hexagon-