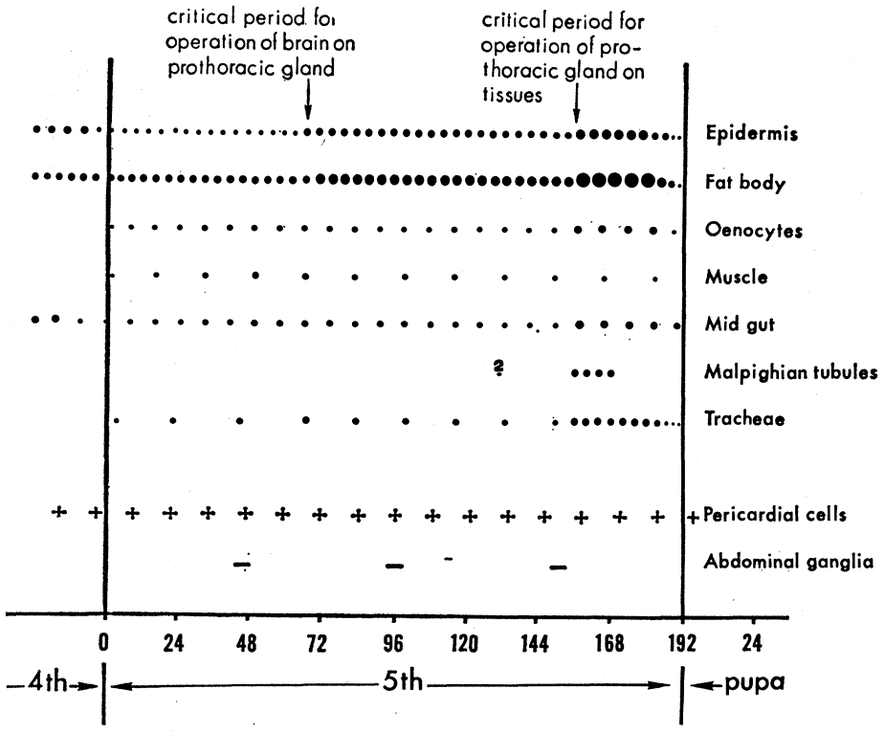

Fig. 4. Diagrammatic representation of the degree of protein (peroxidase) sequestration in several different tissues during the molt-intermolt cycle in *Calpodes ethlius* larvae. Protein sequestration cannot be distinguished in the silk glands, dermal glands, and oenocytoids, which have a natural peroxidase. The pericardial cells take up protein throughout the cycle. The ganglia may have very small granules but are negative compared to most tissues. Peroxidase is concentrated between the fat-body cells before sequestration within the cells. In the midgut and muscle there is concentration in folds of the plasma membrane.

in the fat body and epidermis almost as much as in the unligated, control larvae. Since uptake in these nonfeeding larvae does not result in the lowering of blood volume or protein concentration, there must be turnover. Thus the fat body not only synthesizes blood proteins (*10*) but also sequesters and hydrolyzes them. Other evidence for this comes from experiments upon *Calliphora* (*11*), in which amino acids labeled with C^{14} were used. These indicate that there may be an equilibrium in about 3 hours after injection between proteins synthesized and released into the blood and protein taken up from the blood. We have confirmed that the fat body of late third instar *Calliphora* larvae takes up peroxidase as it does in fifth instar *Calpodes* (the epidermis of *Calliphora* cannot be studied in this way, as it has a strong natural peroxidase). The continued sequestration by the epidermis in ligated larvae may be related to the finding (*12*) that the diffuse incorporation of amino acids into the endocuticle is not stopped by ligation. Sequestered blood protein may serve as a cuticle precursor. The sequestration and hydrolysis of blood proteins by several tissues that are actively carrying out syntheses demonstrates that in *Calpodes* proteins are being transported for use as a source of amino acids.

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Nuclear-Cytoplasmic Interaction in DNA Synthesis

Abstract. *In* Amoeba proteus *the transplantation of a nucleus engaged in DNA synthesis into a G_2-phase (after DNA synthesis) cell results in inhibition of such synthesis. When the nucleus of a G_2 cell is transplanted into an S-phase (period of DNA synthesis) cell, such a nucleus may begin to synthesize DNA.*

Little is known about the cellular mechanisms that regulate the initiation of DNA synthesis. The problem is a major one in cell biology since control over DNA synthesis appears to be central in the regulation of cell proliferation. Protein synthesis is probably required for the initiation of replication (*1*), and there is one piece of evidence that a replicating unit of DNA may control its own replication through a DNA product (*2*). However, there is no reason a priori to suppose that the regulation of DNA replication is any less precise or strict in biochemical-genetic terms than is the regulation of DNA transcription; in fact, there are reasons to expect that regulation of replication may be more precise.

As a physiological approach to defining necessary conditions in vivo for DNA synthesis, we have begun the study of recombinations of nuclei and cytoplasms derived from cells in different stages of the life cycle in *Amoeba proteus* using the technique of nuclear transplantation. This report deals briefly with DNA synthesis following transplantation of an S-phase (period of DNA synthesis) nucleus into a late G_2 cell (when DNA synthesis has stopped) and a late G_2 nucleus into an S-phase cell.

We determined the period of DNA synthesis in *A. proteus* at 23°C by measuring incorporation of ^{3}H-thymidine during various intervals of the interphase period. So that cells would be in synchrony dividing cells were selected with a micropipette. The S period lasts 3 to 6 hours, beginning in late telophase. No G_1 period is detectable in the normal cell cycle. The G_2 period is 30 or more hours long, and the total generation time is roughly 36 hours. Autoradiographic assessment of ^{3}H-thymidine incorporation was made on nuclei that had been isolated individually in a nonionic detergent, treated with 1*N* HCl at 23°C for 5 minutes, and washed with water. Procedures for

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.

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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