variations occur in both species. From a comparison of the amino-terminal halves of the proteins shown in Fig. 1, it will be seen that there are more differences between the two mouse proteins than between MBJ 41 and Ag.

Table 1 contains a summary of the changes observed between the amino-terminal halves of MBJ 41 and MBJ 70, between the carboxyl-terminal halves of mouse and human K-chains, and also between the $\beta$- and $\gamma$-chains of human hemoglobin. All three cases show a similar pattern of changes. In view of this similarity, the size difference between MBJ 41 and MBJ 70, and the failure to find a single genetic mechanism to account for all of the observed changes, we feel that the evidence points overwhelmingly to the conclusion that the changes have arisen by the accumulation of random mutations of the various types which normally account for the evolution of protein molecules. There is much less variation expressed at individual positions (*1*) than would be expected to arise by any single mutation mechanism or by a random process, and it is clear that strong selective forces must operate to produce this restriction.

It is not yet possible to say whether the mutation and selection process take place over the normal evolutionary time scale, or during the somatic differentiation of the immune system in each individual. In either case, the available structural information places some remarkable constraints upon the genetic mechanisms involved in the production of light-chain molecules (*10*).

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## Protein Uptake in Multivesicular Bodies in the Molt-Intermolt Cycle of an Insect

Abstract. *Plant peroxidase injected into the hemocoel is taken up in granules by almost all tissues. These granules may become multivesicular bodies or isolation bodies which later breakdown. There is most uptake and breakdown at times in the molt-intermolt cycle when cells are engaged in active syntheses.*

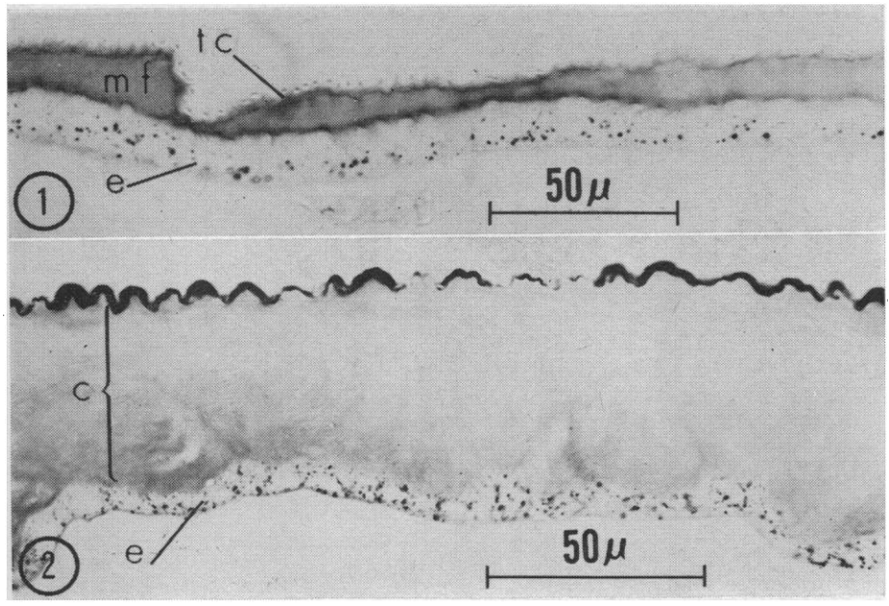
In the course of ultrastructural studies on changes during the molt-intermolt cycle it was found that almost all tissues contained dense, membrane-bound granules, varying in size from 0.1 to 10$\mu$ in diameter. Intensive work failed to show any gross connection with Golgi complexes. The granules did not contain acid phosphatase and could be distinguished from autophagic vacuoles and residual bodies (*1*) resulting from autophagy. In addition to the dense granules, the tissues also contained structures similar to multivesicular bodies (*2*) and to what are now recognized as a variety of intermediates. The origin of these granules and of the multivesicular bodies has been determined in studies on the fate of plant peroxidase injected into the hemocoel. The granules and multivesicular bodies contain protein taken up from the blood.

There is evidence for fairly specific uptake of blood proteins by developing oocytes to form yolk (*3*) and by salivary glands for the secretion that they extrude (*4*). The protein granules of the fat body which appear at metamorphosis are also sequestered blood protein (*5*). The visualization of plant peroxidase taken up by almost all tissues demonstrates the ubiquity of protein utilization in a most dramatic way.

Events in the 8 days (192 hours, S.D. 13) of the last (fifth) larval stadium of *Calpodes ethlius* have been precisely timed and can be predicted to within a few hours. During the fifth stadium, larvae gain in weight from about 0.5 to 1.5 g. Batches of larvae were injected at various stages with peroxidase (0.5 mg/g, Worthington) freshly prepared in 0.1 ml insect Ringer, for incorporation periods of 2 to 24 hours. The technique is similar to that extensively developed for vertebrate studies by Straus (*6*). The larvae were fixed, by standard procedures, in glutaraldehyde as if for electron microscopy (*7*). Tissues were incubated at room temperature for 10 minutes in a mixture of diaminobenzidine (0.03 percent) and hydrogen peroxide (0.01 percent) in tris(hydroxymethyl)aminomethane buffer (*p*H 7.6) and 10 percent sucrose (*8*). They were then either mounted for light microscopy or treated with osmium tetroxide for electron microscopy.

After the benzidine reaction the granules of sequestered protein appear dark brown in light microscopy (Figs. 1 and 2). Unstained sections viewed with the electron microscope show the peroxidase with extra density from the deposition of osmium. The enzyme is localized in granules and multivesicular bodies. The peroxidase could itself stimulate the tissues to sequester it as part of a "cleaning up" process. However, stained sections of larvae without peroxidase show a complete sequence from newly formed protein granules to multivesicular bodies with dense contents (Fig. 3). The similarity of the ultrastructure in tissues exposed and not exposed to peroxidase suggests that the uptake of foreign protein is qualitatively similar to the natural event.

Using this technique, we have studied protein sequestration in a number of tissues during the molt-intermolt cycle (Fig. 4). In most of the epidermis, protein granules are minute or absent until 60 to 70 hours after ecdysis, when deposition of intermolt endocuticle is greatly accelerated. This corresponds to the time when the prothoracic gland has been activated by the brain for




Figs. 1 and 2 (left). Micrographs of tracheal epidermis and surface epidermis, respectively, stained only by the benzidine reaction for peroxidase. Peroxidase had been injected into the hemocoel 8 hours earlier. The cells have many granules containing peroxidase. Fifth instar *Calpodes* larva about 24 hours before pupation. *c*, Cuticle; *tc*, tracheal cuticle; *e*, epidermis; and *mf*, molting fluid.

molting. After this time the granules become progressively more obvious until 156 hours after ecdysis (the critical period for the activation of tissues for molting by the prothoracic gland), when protein uptake is probably near its peak. From this time on, the epidermis is most active in the formation of pupal cuticle. The granules become less obvious just before ecdysis, when only rather empty multivesicular bodies are observable with electron microscopy. Whenever the epidermis is most active (synthesis of the intermolt endocuticle or of the pupal cuticle) then also the uptake of protein is most obvious.

In the fat body and epidermis the injected peroxidase has been traced into granules that become multivesicular bodies. Rather little appears in 2 hours, but by 4 hours, peroxidase appears in granules around the nucleus in the fat body and in granules throughout the epidermal cell. The presence of sequestered protein in multivesicular bodies agrees with reports of protein uptake in these organelles in rat kidneys and toad spinal ganglia (*9*). In material incubated for the demonstration of acid phosphatase by the Gomori method there are deposits of lead in the multivesicular bodies. The deposits are absent in newly formed granules. We therefore believe that the protein is being broken down in the multivesicular bodies. Sequestered protein is not destined for digestion in multivesicular bodies in all tissues. In the oenocytes, for example, the protein granules are surrounded by isolation membranes derived from smooth endoplasmic reticulum (ER). This process is similar to that described for sequestering mitochondria and rough ER in the fat body (*7*). Nevertheless the multivesicular bodies are probably the main organelles concerned in protein degradation.

The sequestration of blood proteins and their hydrolysis in multivesicular bodies suggests that these organelles are part of a system concerned in protein turnover. This idea is supported by the fate of peroxidase injected into "dauer" larvae, which are larvae ligated behind the thorax before they are induced to molt. The concentration of blood protein was measured 4 to 6 days after ligation and was the same as that in control animals of the same age as the experimentals before ligation (experimental mean 113 mg/ml, S.D. 11; control mean 110 mg/ml, S.D. 21). These values correspond to those near the peak attained in normal animals, even though the ligated larvae were starved for 4 to 6 days. Peroxidase injected into these larvae was sequestered

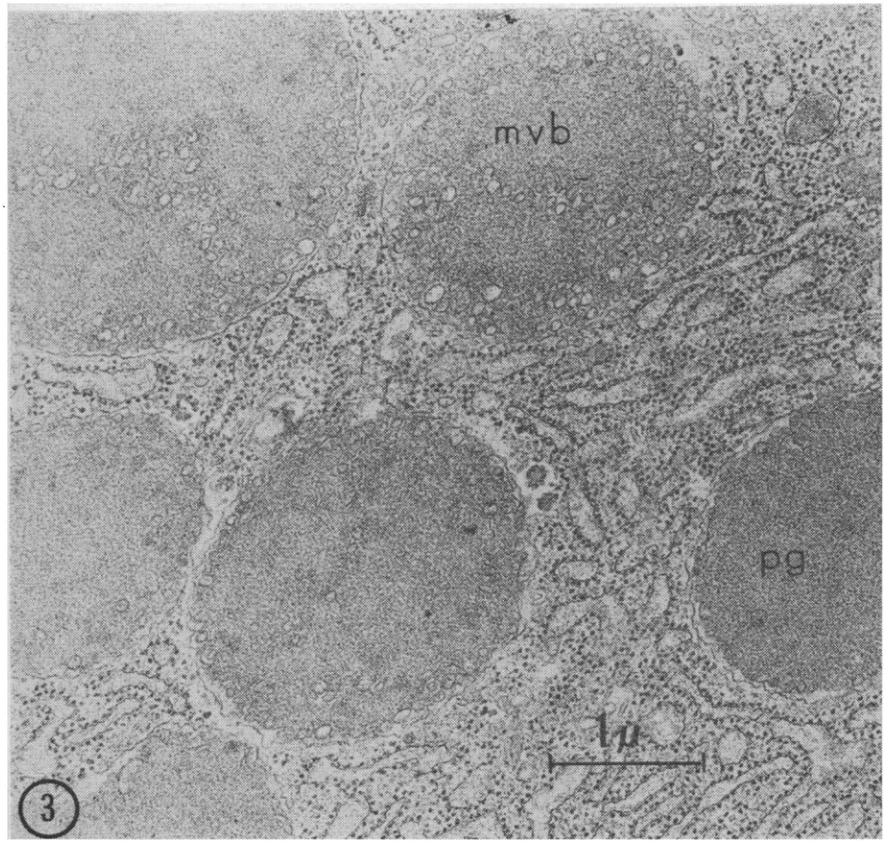



Fig. 3 (left). Electron micrograph of the perinuclear, peroxide-containing granules from fat-body cells. The granules are giant multivesicular bodies. Fifth instar *Calpodes* larva about 36 hours before pupation. *mvb*, Multivesicular body; *pg*, protein granule.

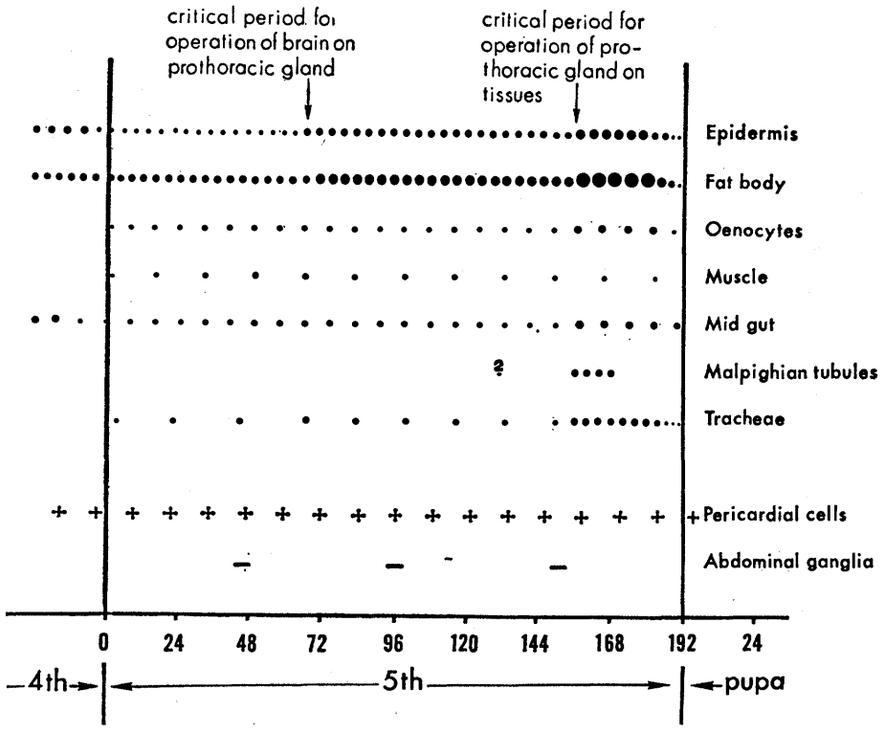



Fig. 4. Diagrammatic representation of the degree of protein (peroxidase) sequestration in several different tissues during the molt-intermolt cycle in *Calpodes ethlius* larvae. Protein sequestration cannot be distinguished in the silk glands, dermal glands, and oenocytoids, which have a natural peroxidase. 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