Viability was estimated by determining the percentage of the IVC that excluded 0.07 percent trypan blue dye from their nuclei. The number of cells with stained nuclei progressively decreased from 12 percent in those cells initially dispersed to less than 4 percent in cells dispersed at the end of 60 minutes of incubation with the enzymes. Individual cells with active cilia were seen in the counting chambers; these were the only cells in the dispersed population which could be positively identified by light microscopy. Scanning electron microscopy showed the surface character of the dispersed cells (Fig. 1).

Granular pneumonocytes or type II alveolar cells (Fig. 2) were frequently present among the cells identified by transmission electron microscopy. The type II alveolar cell is thought to be the source of the alveolar surface active material or surfactant (*5*). We also identified the following types of IVC: ciliated, goblet, Clara, argentaffin, mucous, type I alveolar, capillary endothelial, fibroblast, smooth muscle, macrophage, and mast cells.

The yield of IVC from rabbit lung by this method is greater than 50 percent by volume of the original lung weight. Yields of this magnitude equal or exceed those obtained by most techniques for the dispersal of other tissues. This technique has also been successfully applied to the dispersal of cat, rat, and dog lungs.

The total yield of IVC from a single rabbit lung was combined in 70 ml of KSSS, 30 ml of fetal calf serum, 3 mg of deoxyribonuclease, and 0.5 ml of crude catalase. Oxygen consumption (Q_{O_2}) was determined on samples of mixed IVC in a thermostatically controlled (38°C) glass incubation chamber, and the P_{O_2} was kept at 130 ± 10 torr by an oxygen stat delivering 0.022*M* H_2O_2 (*4*). The *p*H was maintained at 7.4 ± 0.02 with a *p*H stat delivering 0.02*M* NaOH. Cells were gently agitated with a magnetic stirrer. The Q_{O_2} for the IVC averaged 6.2 μl (standard deviation 1.3) of oxygen per hour per milligram, dry weight, in ten animals. By comparison the Q_{O_2} for rabbit lung slices was reported to be 6.7 by Barron and colleagues (*6*) and 8.0 (S.D. 1.0) by Krebs (*2*). We found that IVC from rabbit lung were capable of incorporating 3 percent of added [1-^{14}C]palmitate into saturated lecithin when maintained for 90 minutes in this incubation system—a process that would correspond to formation of 5 to 10 percent of the pool of saturated lecithin if one considered it to be de novo synthesis or a deacylation-reacylation reaction utilizing unsaturated lecithin. Incorporation of labeled precursor into this major component of pulmonary surfactant (*7*) shows that the IVC can carry out an energy-requiring series of reactions at a significant rate.

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Reversed Development and Cellular Aging in an Insect

Abstract. *Larvae of the beetle* Trogoderma glabrum *undergo partially reversed development when deprived of food and water. Retrogressive larval ecdyses occur, and the larvae become diminished in size and weight. Given food, the larvae regrow, ecdyze, and regain their previous degree of maturity. Repeated cycles of retrogression and regrowth are possible. Although larval in form and organ differentiation, repeatedly retrogressed insects display a physiological deterioration suggestive of aging. A deterioration of the ability to regrow is accompanied by increasing fat body polyploidy.*

Experimentally reversed development has never been accomplished with a metazoan animal, in the sense of the animal's becoming progressively "younger" from the standpoint of physiological state or tissue differentiation. Were the genetically controlled developmental program reversed, there might also be the possibility of reversing genetically controlled aging processes. However, to the degree that aging effects are probability-related accidental damage inflicted on cell systems, they could not be forestalled by a reversal of the developmental program (*1*).

Insects are good experimental animals for research on these relationships, because their postembryonic development is divided quite sharply into larval and adult developmental sequences, with a corresponding division of the total genetic complement into larval and adult genomes (*2*). In a small number of holometabolous species, adult development can be prevented under conditions that prevent the "turning on" of the adult genome (*3*). Genetic control of aging processes should be a function of the adult genome, and if so, any aging manifested in long-lived larvae would be attributable only to time-related tissue damage. We have investigated some aspects of the problem, and have found that larval development may be reversed, at least to a limited degree. The superannuated larvae produced eventually displayed physiological dysfunctions suggestive of senescence.

Larvae of a dermestid beetle, *Trogoderma glabrum* (Herbst) were used, being reared in stock culture as previously described (*3*). Nearly mature "last" instar (sixth) larvae were removed from stock culture and isolated in small test tubes (10 by 75 mm) without food, granular substrate, or water. Under these conditions, the larvae underwent an apparent reversal of larval development. Larvae so treated did not

pupate, but retrogressed via molting cycles in which the postecdysial larvae were smaller than were the preecdysial forms. The first retrogressive ecdysis occurred within 10 days after isolation, and subsequent retrogressive ecdyses occurred at about 4-week intervals thereafter. After either 20 or 36 weeks of retrogression, the larvae were given food; they then regained the weight and size they had lost. Regrowth also involved two or three molting cycles. If again isolated prior to their being committed to pupation, the larvae would again retrogress. By successive cycles of retrogression and regrowth, we have produced larvae more than 2 years of age, which is in sharp contrast to the insect's normal life span of 8 weeks (from newly laid egg to death of spent adult) under our culturing conditions.

When removed from stock culture at the beginning of the sixth larval stage, the insects were programmed to molt to the pupal stage (*3*), but pupal determination was revoked by the transfer to deprived isolation; subsequent molting cycles resulted in additional but diminished larval stages. Retrogressed larvae did not retain the ability to pupate, but could regain that capability only through regrowth. The process of regrowth included not only feeding and weight gain, but also the occurence of two (sometimes three) progressive larval ecdyses. By this process the larvae attained the developmental maturity required for the programming of pupation, a developmental stage they had previously reached just prior to the initial retrogression. In this sense a real reversal of development was demonstrated, although tissue differentiation was not altered by the process of retrogression. Because these insects may be maintained in a larval state for greatly prolonged periods, it is possible to investigate the question of aging in a juvenile (larval) system, uncomplicated by adult differentiation, reproduction, and (perhaps) genetically controlled senescence.

The number of days required for regrowth was used as one measure of physiological efficiency. The hypothesis tested was that any age-related deterioration of the system might be reflected by progressively longer periods of regrowth after successive retrogressions. The experimental results tended to support this hypothesis. Repeated cycles of 20 weeks of retrogresssion followed by regrowth to the original size and weight were carried out. The required regrowth period increased from 9 days in the first to 28 days in the fourth cycle. Two cycles of 36-week retrogressions have been completed; 19 days of regrowth were required after the first retrogression, and 29 days after the second. Obviously such repeated retrogressions were accompanied by a loss in the insect's capacity for rapid recovery.

Table 1. Larval retrogression and cell ploidy in *Trogoderma glabrum*.

Retrogressions		Cell ploidy distribution			
No.	Duration (wk)	Mean (*n*)	Mode		Range (*n*)
			n	%	
		(*A*) *Epidermis*			
0	0*	2.0	2	100	
4	20	2.4	2	70	2 to 6
2	36	2.7	2	60	2 to 6
		(*B*) *Fat body*			
0	0*	3.0	2	43	2 to 6
4	20	6.8	8	40	2 to 16
2	36	8.2	8	43	4 to 22

* Control.

Histological examination of larvae after different durations of retrogression and regrowth failed to disclose pathology in any tissue except that of the fat body. It is well known that the fat body cells of many insects tend to become polyploid as the insects mature and age (*4*). The relationship, if any, of ploidy to physiological function has not been elucidated. We have found the fat body cells of retrogressed *T. glabrum* larvae to be highly polyploid (Table 1). Chromosome counts at metaphase and anaphase in spermatocytes from normal nonretrogressed larvae showed five pairs of chromosomes. Acetocarmine staining was then applied to smears of larval tissues. No mitotic figures were observed in retrogressed larvae, but typically diploid cells (such as epidermis) displayed ten hererochromatic bodies per cell, and this was taken as an approximation of the $2n$ condition. As was expected, fat body cells from normal nonretrogressed sixth instar larvae (Table 1, B, controls) showed some polyploidy, with a range from 2 to $6n$; the mode (largest grouping) of the ploidy distribution was $2n$, however. Larvae that had been repeatedly retrogressed showed a much greater polyploidy in the fat cells, with up to $22n$ being observed. Many instances of apparent nuclear fusion were observed, in good agreement with the observations of Wigglesworth (*4*), who studied *Rhodnius prolixus* under starvation conditions. Epidermal cells, on the other hand (Table 1, A), showed only a slight tendency toward polyploidy during retrogression, although cells with as high as $6n$ ploidy were observed.

The central importance of the insect fat body in metabolism as well as a storage organ would suggest that the observed deterioration of the recuperative powers of repeatedly retrogressed larvae might logically be attributed to impaired fat body functions. A high degree of accumulative polyploidy may have been responsible for a progressive loss of metabolic efficiency, although this interpretation of our data is admittedly speculative. From the results of this study, we have concluded that some aging processes were not forestalled or prevented in the beetle larvae, even though developmental processes were repeatedly reversed and restored by retrogression and regrowth.

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Inhibition of Sperm-Egg Interaction by Specific Antibody

Abstract. *Treatment of hamster ova, in vitro, with ovary specific antibody interferes with sperm-egg interaction by inhibiting sperm attachment to the zona pellucida and subsequent zona penetration. This inhibition results from precipitation of the zona by the specific antibody.*

The possibility of utilizing immunological procedures for fertility control in mammals dates back to Metchnikoff's experiments at the turn of the century (*1*). This possibility has gained new impetus from increased knowledge in immunology and the production of specific antibodies against components of the reproductive system (*2*). Antibodies against testicular components and spermatozoa have received more attention in the last few years (*3*) as