physiological significance of these JH effects on mitochondrial metabolism cannot be fully assessed until further studies relate the observations in vitro to processes in vivo[21].

Zusammenfassung. Nachweis, dass das Juvenilhormon in isolierten Mitochondrien der Getreidemotte *Plodia interpunctella* die NAD-gekoppelte Oxydation hemmt und die Succinat-Oxydation stimulieren kann. JH scheint auf die Nichthaem-Eisenkomponente der Atmungskette einzuwirken.

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Finite Growth Span of Mouse Mammary Gland Serially Propagated in vivo

In several recent reports[1-4] the assertion was made that normal mouse mammary gland has a limited capacity for growth when repeatedly isografted into young hosts. This finding has attracted interest because it appears to furnish a useful in vivo comparison to the cell culture findings of HAYFLICK[5,6] and others[7], who report that non-transformed fibroblasts also display a limited number of potential cell doublings. The mammary transplant technique has thus become a useful model system for the study of cellular aging, when aging is defined in terms of a time-related decline in growth rate.

This reported inability of mammary tissue to grow without limit has been disputed[8], most recently in a 1970 report by HOSHINO[9]: In these studies HOSHINO measured the survival rate of grafts, and scored as successes any transplants that were able to reconstruct identifiable mammary outgrowths. Using this criterion of regeneration rather than growth, as used by DANIEL et al.[1-4], HOSHINO found that 2 of his transplant lines survived for nearly 4 years, a period well beyond the ordinary life span of the mice used by him. He concluded on the basis of these and similar experiments that the life span of mouse mammary gland is potentially indefinite[8,9]. By extension it could be argued that the use of the mammary transplant system as a model of cellular aging is inappropriate.

In the present paper I submit results indicating that this contradiction is more apparent than real, and that the data of both investigators are mutually consistent when differences in terminology and experimental design are taken into account.

Materials and methods. Mice of the Balb/cCrgl strain were used. Female hosts 3 weeks of age were prepared for transplantation by excising the small mammary rudiment from each inguinal (No. 4) mammary fat pad[10]. This operation makes available 2 mammary gland-free fat pads which provide a natural site for the growth of mammary transplants. Primary implants (consisting of 0.5 mm pieces of either primary or secondary duct) were taken from the No. 3 mammary fat pad of a single 10 week virgin. In the primary transplant, 48 grafts were made into 24 hosts; these animals were then randomly distributed into 2 experimental groups.

At each passage all hosts from the previous transplant received a single i.p. injection of 0.5 ml of a 0.5% suspension of trypan blue, which aids in visualizing the mammary outgrowths[11]. All previously transplanted mice were anesthetized, their inguinal fat pads exposed, and the extent of mammary outgrowth in each fat pad estimated. The most vigorously growing gland was always selected for subsequent propagation, and 24 pieces 0.5 mm in size were removed and transplanted into the cleared fat pads of hosts for the next transplant generation. At the end of each passage, all transplanted glands and a sample of host gland were removed, fixed, extracted in acetone, stained with hematoxylin, dehydrated in alcohols to xylene, and stored in methyl salicylate. In each successful transplant the extent of mammary outgrowth was measured and for each generation the mean percent fat pad filled was calculated; this mean was used as an indicator of growth. These methods have been described in detail elsewhere[3].

Results. The experiment was designed to permit study of the potential growth span of mammary tissue by choosing conditions of serial passage which either did or did not allow unrestricted growth to take place. This was accomplished by making use of the following facts regarding mammary morphogenesis. A transplant of young mammary gland placed in the center of the cleared fat pad regenerates recognizable gland within about 2 weeks, and grows to fill the available fat within 2 to 3 months[4]. Growth ceases when the elongating ducts reach the limits of the fat pads, and unless the gland is again transplanted and more vacant fat is made available for growth, the gland will, in the absence of pregnancy, remain mitotically inactive for the life of the host.

It is therefore possible to control the extent of cell proliferation in each transplant line by varying the duration of the transplant interval. In the present experiment two intervals were used. In one subline transplants were made at 3 month intervals, so that when the outgrowth filled the fat pad it was again transplanted and vacant fat was again available for continued growth. In the second subline a 12 month transplant interval was selected, in which growth took place only at the beginning of each generation. For most of the 12 month period the gland in this subline was mitotically quiescent, although the cells remained metabolically

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active. In order to eliminate possible effects of host age on growth, the gland-clearing operation was performed on all hosts to be used during a yearly period. In the short-interval subline, hosts were always used whose ages corresponded to those in the yearly transplanted group.

Using growth rate (percent fat pad filled) as an indicator of tissue viability (Figure 1), it is apparent that the short-interval line showed a progressive and approximately linear decline in growth until at 22 months (generation 7) it was able to fill only 10% of the available fat, and subsequent transplantation became impractical. The long-interval subline, while declining slightly in growth rate, is still able to fill 60% of the fat at the end of 5 years of serial transplantation. Another subline was split from this long-interval group at 2 years, and was carried for 3 passages at the short transplant interval of 3 months. This tissue again displayed a decline in growth rate similar to that seen in the first short-interval subline, and the slopes of the two lines are similar. These data indicate that decline in growth potential occurs most rapidly under those conditions of transplantation which permit unrestricted proliferation; one concludes that under these conditions it is not possible to transplant mammary gland indefinitely.

These data do not give information regarding the rate of survival of the grafts, the parameter used in HOSHINO's studies[8,9]. In order to study the relationship between survival and growth, the percent survival in each generation of the various sublines was plotted in Figure 2, and in the short-interval sublines no correlation was found. As an example, in generation 6 of the first short-interval subline the percent fat pad filled was only 20%, yet the survivors numbered 85%. It is concluded from these data that many transplants are able to regenerate recognizable mammary gland which has, however, a severely restricted ability to grow.

Discussion. The finding reported herein, that mouse mammary epithelium has a limited potential for growth when conditions are suitable for expression of that potential, is highly reproducible, and has been confirmed in several reported[1-4] as well as in several unreported[13] instances. Mammary cells can acquire the potential for unlimited growth only if a profound alteration takes place, in which virus-induced transformation gives rise to phenotypic variants which display characteristics of either cancerous or precancerous cell types[1]. It is most unlikely that technical considerations can account for the difference in growth span between normal and transformed mammary cells; identical conditions of passage, which are judged to be optimal, are used for each.

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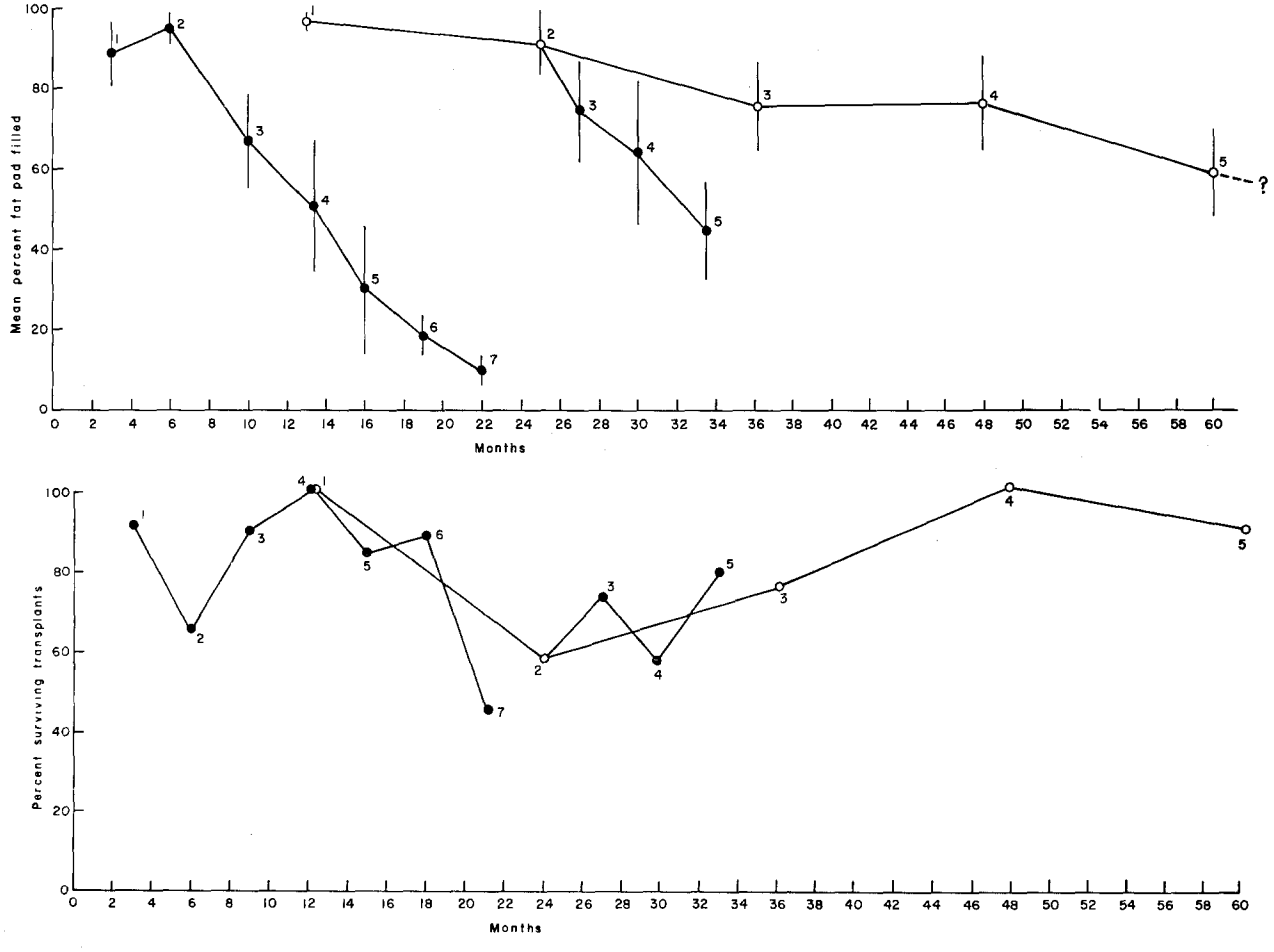

Serial passage of normal mouse mammary tissue. All transplant lines were initiated at time 0 by 48 transplants taken from a single donor. Each passage consisted of 24 transplants, and are indicated by circles and accompanying passage number. Tissue was transplanted at either short intervals of 3 months (●—●) or yearly (○—○). Vertical lines indicate 95% confidence intervals of the means. A portion of these results have been published previously[4], and are reproduced here by permission of the publisher.

In contrast, the maximum time that mammary tissue can survive and remain in satisfactory condition is, at present, unknown. The small, slow-growing mammary tissues that result from several generations of short-interval passage are not discarded because they die, but because the amount of available tissue is not adequate for subsequent propagation. Indeed, these tissues which have lost their ability to proliferate appear to be in excellent condition as judged by light and electron microscopy[14]. In the present experiments, mammary tissue transplanted at yearly intervals was healthy at the end of 5 years, and it is not unreasonable to project that it will remain so for several more. This result may be interestingly compared to the data reported by HOSHINO[8,9], in which two important differences in method are apparent. First, HOSHINO plotted survivorship rather than growth rate against time. Second, very unequal transplant intervals were used in his lines. In one case for example[9], the interval between transplants ranged from 34 to 222 days, and the time from the final transplant to termination of the experiment was some 600 days. Because transplant intervals of this magnitude do not permit the tissue to express its potential for growth, these experiments are not formally comparable to the several reported instances in which mammary aging has been described in terms of limited growth potential under conditions of short, uniform transplant intervals. His results are, however, quite comparable to the long-interval line described in the present report, and both indicate that the ability of mammary cells to survive may be unlimited if environmental conditions are satisfactory. This conclusion is also supported by results showing that transplants derived from old donors are indistinguishable from those obtained from young donors[3].

Future misunderstandings might be avoided if terminology were more standardized (I have been particularly inconsistent in this regard), and it is suggested that the following terms be used to distinguish between two qualitatively different parameters. They may apply equally well to in vivo and in vitro studies of cell aging.

'Growth span' should refer to experiments in which proliferation is measured in reference to time, transfer generation, or some other temporally-related unit. The maximum potential growth span of a tissue or cell can be measured only under conditions which permit full expression of this proliferative potential.

'Life span' should indicate the ability of tissue of cells to remain living and in biologically satisfactory condition in respect to time or related units. Maximum potential life span may be measured only under conditions which best favor endurance, and which are designed to eliminate, for example, systematic effects of aging organisms upon constituent cells or tissue.

Résumé. Les glandes mammaires de la souris n'ont qu'une possibilité limitée de développement quand elles sont transplantées en série sous des conditions qui permettent une prolifération continue. Les conditions de transplantation qui maintiennent les greffes en phase statique, étendent notablement la période durant laquelle la glande peut être transplantée. Les résultats indiquent qu'en dépit de la durée limitée de croissance des cellules mammaires, la longueur de vie potentielle peut être illimitée sous des conditions environnantes optimum.

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On Some Interesting Features of the Hypothalamo-Hypophysial Vascularization in the Lizard *Hemidactylus flaviviridis*

Hypothalamic vascularization has been studied in a variety of reptilian species[1–5]. As in other reptiles, in *H. flaviviridis* also the primary capillary plexus of the median eminence gives rise to the portal vessels which irrigate the pars distalis (Figures 1, 2 and 4). The neural lobe receives an independent infundibular artery which is derived from the internal carotid. Apart from this, the median eminence also contributes vessels to the vasculature of the neural lobe. They are comparable to the 'portal vessels' in their formation (Figures 1 and 2). It is well established that the hypothalamic hormones conducted through the portal vessels control the adenohypophysial functions. In this species, the blood that flows into the neural lobe from the median eminence might also be exposed to some of the releasing hormones. An uninterrupted vascular septum demarcates the boundary between the neural lobe and pars intermedia (Figures 5 and 6). The neurosecretory axons of the paraventricular (PVN) and supraoptic (SON) nuclei ramify extensively and have perivascular endings in the neural lobe (Figure 3). Prominent Herring bodies were seen overlying the blood vessels of the vascular septum (Figure 6). Apparently this blood contains the neurohormones secreted by the SON and PVN and some releasing hormones which are conducted through the draining vessels from the median eminence to the neural lobe.

Meagre vascularization of the pars intermedia has been reported in several vertebrates, including reptiles[6,7]. However, in the lizard *Calotes versicolor*, an extensive vascular connection between the neural lobe and pars intermedia is reported[8]. In *H. flaviviridis*, as the pars intermedia is least vascularized (Figures 5 and 7) and is also totally devoid of neurosecretory axons, the only pathway of conduction of active principles into this component is through the blood vessels of the vascular septum. Thus, the pars intermedia is controlled by a neurovascular mechanism. The hitherto unknown vascu-

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