mean that the phenomenon of ribosomal gene amplification is necessarily involved in the present material. The above mentioned findings in *Smittia* may be interpreted simply as indicating that the intranucleolar DNA constitutes units of replication which are autonomous of the chromosomes and behave quite asynchronously with the chromosomal cycles of duplication. An amplification of the ribosomal DNA within the nucleolus cannot, however, be ruled out in such cases since, in a recent molecular hybridization study at the chromosome level[17] in *D. hydei*, ribosomal RNA was seen to hybridize specifically and in considerable amounts with the DNA within the nucleolus and not at all with the DNA of any band in the chromosomes. This problem remains to be tested biochemically in dipteran material keeping in mind the possible variability of the genome fraction set apart for ribosomal RNA in the diploid cell[18] and also the repression of replication of certain chromosome segments of diploid tissue during development of polytenic nuclei[19,20].

Riassunto. Ghiandole salivari di larve di *Smittia* (Chironomidae) sono state incubate in vitro in presenza di timidina tritiata. Le modalità di marcatura dei cromosomi e dei nucleoli dimostrano che in questo materiale non esiste correlazione tra frequenza di marcatura del DNA intranucleolare e modalità o intensità di marcatura del DNA cromosomico. In particolare sono stati riscontrati casi in cui il DNA intranucleolare appare marcato mentre il DNA cromosomico non è in fase di replicazione. I risultati ottenuti sembrano indicare che il DNA intranucleolare in *Smittia* sia costituito da una o più unità di replicazione autonome.

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Indefinite in vivo Life Span of Serially Isografted Mouse Mammary Gland[1]

Whether mammary parenchymal tissue may survive in vivo indefinitely or not if environmental conditions are favourable[2] is controversial[3]. Mammary grafts from a quiescent gland of a 734-day-old virgin female mouse regenerated normal looking glands and secreted milk following parturition in host mice[4]. The quiescent status of mammary glands of old mice seems not to be due to the ageing of the glands themselves. Mammary tissues of the original donor CBA mouse were serially isografted by Hoshino's quantitative transplantation technique[5], and the longest in vivo life span of grafted mammary tissues observed was 1379 days[2], which is much longer than the life span of a mouse. From these results, we postulated in 1967 that the capability of mammary parenchymal tissue to survive in vivo is indefinite if the favourable environmental conditions were renewed. In 1968, Daniel et al.[3] made a contradictory report that the maximal time that any normal mammary transplant series could be carried was 24 months, which is a period within the life span of a mouse, and he concluded that normal mammary glands have a limited ability to proliferate in vivo even under favourable conditions. Recently, we obtained additional cases which seem to support our previous postulation and would like to report these results in this communication.

Materials and methods. The 3 new lines of serial isografts of mammary glands (designated as Lines 3, 4, and 5) were established and the data obtained from them were compared with the 2 lines previously reported by us[2] (referred to as Lines 1 and 2). All transplanted tissues were excized from the 3rd pair of mammary glands of the donor mice. For the 1st generation, 0.6 mm mammary duct-segments were isografted from virgin female donors into the 4th mammary gland-free fat pads[6,7] of the virgin female hosts by Hoshino's quantitative transplantation technique[5]. Identical techniques were used for transplantation of successfully grafted mammary tissues to succeeding generations from the preceeding one. Serial transplantation of the mammary tissues derived from the original donors were undertaken: from a 135-day-old CBA mouse to (♀ BC_B × ♂ CBA)F-1 mice (Line 1), and to (♀ CBA × ♂ BC_B)F-1 mice (Line 2), from a 268-day-old C3H mouse to (♀ C57BL × ♂ C3H)F-1 mice (Line 3) and to (♀ C3H × ♂ C57BL)F-1 mice (Line 4), and from a 206-day-old (♀CBA × ♂ C57BL)F-1 mouse to (♀CBA × ♂C57BL)-F-1 mice (Line 5). The interval between the serial transplantation ranged from 34 to 222 days. Following mammary transplantation, the hosts were neither mated nor given any other treatment. Throughout the experiments, all animals were maintained under uniformly controlled environment and provided with Purina Lab Chow and water ad libitum. The BC_B, CBA, C3H, and C57BL mice are all pedigreed inbred strains which have been raised by sister-to-brother mating and maintained in our laboratory[8,9].

Results. The incidence of successful transplants at each transplantation generation in all the 5 lines is shown in the Figure. Except for Line 5, serial transplantation was discontinued after varying periods of transplantation instead of carrying on indefinitely. The longest periods of in vivo life span of the mammary glands derived from the respective original donors within the experimental limitation were 1414 days (6 generations) in Line 1, 1379 days (7 generations) in Line 2, 491 days (5 generations) in Line 3, 394 days (3 generations) in Line 4, and 748 days

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(10 generations) in Line 5. The incidence of successful transplants of approximately one-year-old mammary tissue in Line 1 to 5 was as follows: 9/14, 19/34, 4/40, 4/10 and 20/50 respectively. That of approximately 600-day-old tissue was 4/38 (3rd generation in Line 1), 3/40 (5th generation in Line 2) and 13/36 (7th generation in Line 5). However, that of 670- to 680-day-old tissue was 11/20 (Line 1), 20/42 (Line 2) and 14/50 (Line 5). In Line 1 and 2, where the serial transplantation intervals were 61 days or longer, 26/36 and 11/38, respectively, survived on transplantation for more than 3 years which is definitely longer than the ordinary life span of a mouse. In Line 5, where more frequent serial transplantation was performed at an average interval of 54 days, 30% of 48 grafts were recovered as regenerating single mammary glands at each transplantation site when the mammary tissues were 748 days of age at their 10th transplantation generation. These successfully grafted tissues were retransplanted into the hosts of the succeeding generation. Sometimes, at the time of serial transplantation, surviving and regenerating mammary graft tissues consisted of small glands with a few small and fragile duct systems. Stainability of these regressing grafts with trypan blue was usually poor. This type of weakly regenerating graft was found irrespective of either the age of the tissue or the number of transplantation generations.

Discussion. Although the two inbred strains of mice, CBA and C3H, were originally derived from the same ancestry[10], the incidence of their spontaneous mammary cancer is extremely different[11]. In the present study, transplantability of their mammary glands also appears to be different. Particularly in Line 4, transplantation rates were so low that we gave up further serial transplantation at the 3rd generation. However, this seems to be very unfortunate as in all of the other 4 lines, the transplantation rates became higher after being extremely low. Why such an extreme drop in the transplantation rate occurs is unknown. The conclusion that normal mammary glands have a limited ability to proliferate in vivo even under favourable conditions may have been reached by DANIEL et al.[3] when they encountered a very low incidence of the successful recovery of regenerating graft tissues. In their last transplantation generations they obtained from 2 to 38 successful transplants. They never lost successful grafts completely before giving up further serial grafting. It might be possible therefore to suspect that they may have been able to carry on their serial transplantation lines much longer if they had tried to retransplant the recovered graft tissues. In our study the weakly regenerating, regressing mammary grafts were found irrespective of age of the grafts and they could be found even in the first transplantation generation. Such grafts were not

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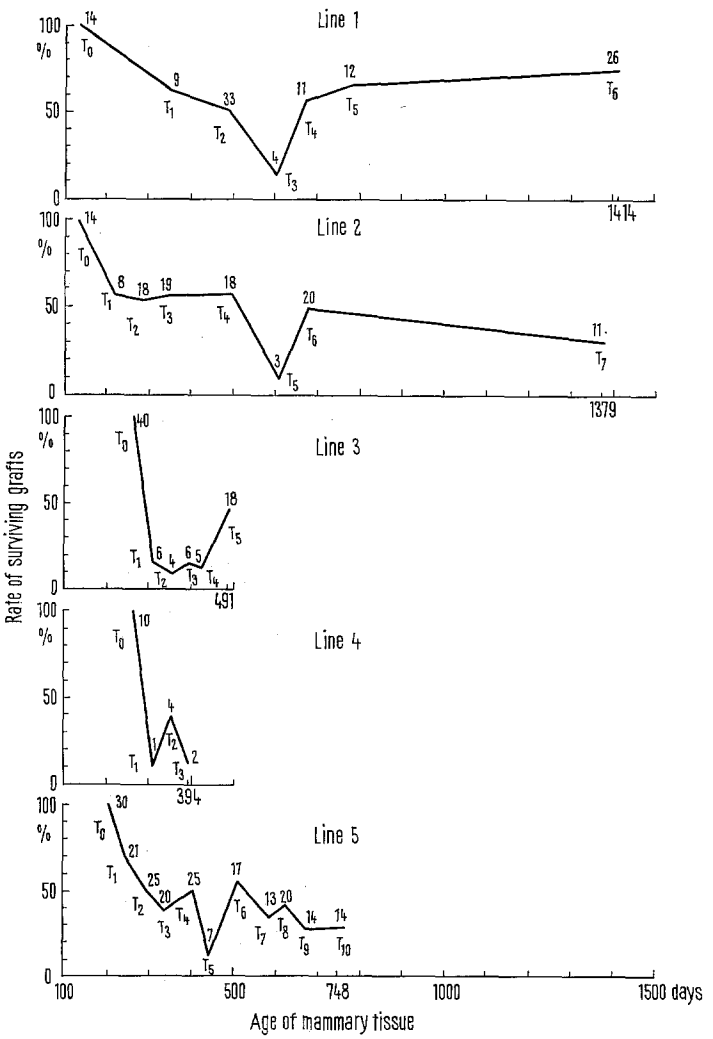

Rate of surviving grafts during serial transplantation of mammary glands in 5 lines. The number accompanying T represents the number of serial transplantation generation. The number above the line at each transplantation generation indicates the number of surviving mammary grafts.

used for subsequent serial transplantation in our studies. We are certainly agreeable to the comment made by DANIEL et al.[3] that epithelial cells of mammary glands may be heterogenous with respect to their proliferative potential. It is not unusual to see coexistence of prelactating lobuloalveolar growth and resting ductal branches in the same mammary gland during pregnancy in mice. Therefore, the capability of transplanted mammary parenchymal tissues to regenerate new mammary glands is not age-dependent. The present experiments apparently support our previous hypothesis that the capability of mammary parenchymal tissue to survive in vivo is indefinite if the environmental conditions are favourable.

Zusammenfassung. Durch wiederholte Transplantation liess sich Mammagewebe von Mäusen während einer Zeitspanne am Leben erhalten, welche die Lebensdauer von Mäusen wesentlich übertrifft.

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Abtransport von Schwefelverbindungen aus Bohnenprimärblättern (*Phaseolus vulgaris*) nach Begasung mit $H_2{}^{35}S$

Schwefelwasserstoff wird durch Blätter aufgenommen, oxidiert oder metabolisiert[1]. Neben dem überwiegenden Oxidationsprodukt $SO_4{}^{2-}$ können in den Blättern schwefelhaltige, primäre Amine nachgewiesen werden, die nicht den natürlichen Schwefelaminosäuren entsprechen[2]. Es stellt sich die Frage, ob die erwähnten Verbindungen mobilisiert und wohin sie allenfalls transportiert werden.

Figur 1 zeigt das typische Markierungsmuster in einer ganzen Pflanze nach einer Primärblattfütterung mit frisch zubereitetem $H_2{}^{35}S$-Gas. Die Darstellung erfolgte durch Reduktion von $^{35}SO_4{}^{2-}$ [3], Auffangen in 0,1 *N* NaOH und anschliessendem Austreiben mit 6 *N* HCl. Das Primärblatt wurde während 3 h in einer dichten Küvette begast (0,075 $mg^{H_2{}^{35}S}_{H_2{}^{35}S}$/100 ml). Nach 15 h wurde die ganze Pflanze abgetötet, autoradiographiert oder wie unten angegeben weiterbehandelt. Die halbseitige Markierung des ersten Trifoliums ergibt sich aus dem Leitbündelverlauf in der Pflanze, der andernorts beschrieben ist[4]. Augenfällig ist die Belieferung der wachsenden Sprossorgane.

Figur 2 gibt Aufschluss über den Ort des Transports im Leitbündel. Petiolastücke ausserhalb der Küvette wurden ohne Verlust der wasserlöslichen Substanzen durch Gefriersubstitution mit Äther präpariert[5] und nach der trockenen Methode mikroautoradiographiert[6–8]. Die Schwefelverbindungen werden vorwiegend im Phloem transportiert. Auf Mikroautoradiographien von Stengelstücken unter- und oberhalb des Petiolaknotens konnten ebenfalls Phloemmarkierungen festgestellt werden, wobei nur die Hälfte der Leitbündel im Stengelquerschnitt ^{35}S-Verbindungen führten. Daraus resultiert die halbseitige Markierung des ersten Trifoliums.

Die dünnschichtchromatographische Analyse der wasserlöslichen Substanzen[1,2] der Petiola ergab anorganische Schwefelverbindungen und die eingangs beschriebenen ^{35}S-haltigen, primären Amine. Figur 3 stellt die Autoradiographie eines Chromatogramms der Aminosäurenfraktion dar. Die numerierten Flecke entsprechen den ^{35}S-Aminen. Sie reagieren auf verschiedene Sprühreagenzien für Aminosäuren[9].

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Fig. 1. Autoradiographie einer Bohnenpflanze nach Primärblattfütterung mit $H_2{}^{35}S$. 2/3 natürliche Grösse.

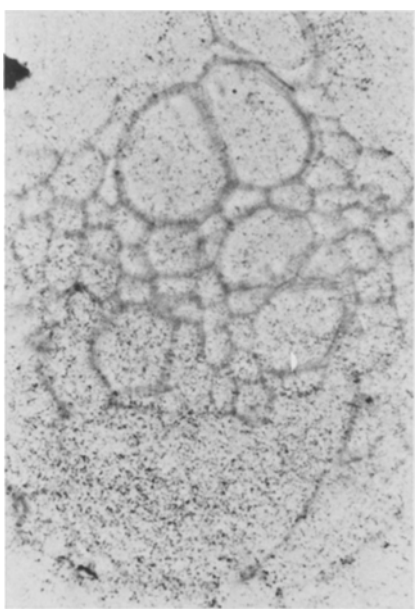

Fig. 2. Mikroautoradiographie eines Petiolaleitbündelquerschnitts nach $H_2{}^{35}S$-Fütterung des Blattes. ×2000.