females may keep the last inseminated egg in the uterus. Such an egg begins the embryonic development within the mother and is generally laid as an 'overaged' egg during the 7 min period. (2) Our experience has shown that females are too much disturbed by changing the dishes every 3 min and egg-laying activity decreases rapidly after about 1 h. With the alternative schedule egg collection can be extended for 6 h or more. If for a particular experiment the extreme short periods are not needed, regular changes every 5 min (or longer) are possible. Some tests showed that the collection period could not be reduced to less than 3 min without a drastic reduction in the number of eggs deposited.

To keep the disturbance of the flies to a minimum, the change of the dishes is done as follows: the egg-laying apparatus stands on a solid, vibration-free table. The lid of the wooden box is carefully lowered. An automatic switch (14) turns off the room light at the same time in order to prevent attraction of the flies by light. Now, in the dark, the spring is removed and the dish is gently rotated back and forth during about 10 sec. This causes the flies (which do not fly in the dark) to walk off the surface of the dish. Thereby they cross the ring of plaster which absorbs any fluid on the animals' legs. Thus neither the blotting paper nor the bell-glass ever get wet. A new dish is now fixed to the plate. Upon closing the lid of the box, the light in the room is turned on automatically.

The special temperature and humidity conditions are based on preliminary measurements of the dew-point temperature on the surface of a medium on which females have been laying eggs. Using these conditions in the whole experimental room has 2 advantages: (1) a dish brought into the apparatus has already the optimal temperature and (2) eggs collected from the apparatus remain under constant conditions[12].

Zusammenfassung. Es wird eine verbesserte Methode beschrieben, mit der innerhalb einer Sammelperiode von 3 min etwa 50–100 frisch besamte Eier von *Drosophila melanogaster* gewonnen werden können. Verglichen mit den bisher üblichen Sammelperioden von 10, 30 oder mehr min erhält man wesentlich stadienhomogenere Gelege. Eine weitere Verkürzung der Sammelperiode unter 3 min ist wegen der stark abnehmenden Anzahl Eier je Gelege nicht möglich. Vorausgesetzt, dass alle Störungen der Fliegen durch Erschütterungen, Licht, Temperaturschwankungen usw. ausgeschaltet werden, können z.B. für strahlenbiologische Experimente zahlreiche 3-min-Gelege im Laufe von 6 oder mehr Stunden gewonnen werden.

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Department of Zoology, Swiss Federal Institute of Technology, Zürich (Switzerland), 9 May 1968.

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STUDIORUM PROGRESSUS

Factors Influencing Rates of Tail Regeneration in the Lizard *Anolis carolinensis*

In an investigation of the somatotropic effects of certain hormones in the lizard *Anolis carolinensis*, tail regeneration was studied as one of a number of physiological variables related to growth[1]. In spite of a rigidly controlled experimental regime and use of only males of restricted age and size, considerable individual variation in tail regeneration was found. Although such variations have been reported[2,3] they have not been studied in detail. There are conflicting reports regarding certain possible regulatory factors in lacertilian tail regeneration, especially the role of the vertebral autotomy plane, and there have been speculations on largely uninvestigated factors such as epidermal involvement. We attempted to elucidate the basis for individual variation in the regenerative response in *A. carolinensis* by examining these and other factors, especially temperature.

Materials and methods. In early September, 60 adult male *A. carolinensis* (average snout-vent length 64.5 mm, body weight 5.0 g) were put at 32 ± 0.5 °C with 6 h light daily[4]. Some animals were injected with gonadotropins, or gonadotropins plus prolactin, but there were no significant differences in tail regeneration and the data were pooled for this analysis. Procedures for hand-feeding, assessing growth and autopsy are reported elsewhere[1].

The original tail (average length 124 mm, range 111–138) was amputated with a razor blade 18–21 mm behind the vent: amputated portions averaged 360 mg. None of the animals appeared to have had previously regenerated tails except at the very tip. The length of the regenerating tissue was measured weekly and after 6 weeks the newly regenerated portion was removed and weighed.

The epidermal condition at amputation and the position of amputation relative to the natural autotomy plane (Figure 1) was determined from histological preparations of the proximal 1.5 cm of the amputated portion: methods are described elsewhere[5].

In order to facilitate comparison between our results and those of previous workers who have used temperatures around 18–22 °C, a second experiment at 21 °C was conducted with 18 males in April. Ad libitum feeding maintained or increased the animals' weights. Severals were transferred to higher temperatures as described below.

Results. Tail regeneration at 32 °C (Figures 2 and 3). No detectable elongation occurred until 7–10 days after amputation, and then there was a period of rapid growth averaging 1.5 mm/day from the 14th to the 28th day. The average growth rate for the 10th to 42nd day after amputation was 0.98 mm/day. The mean length of the regenerated tail at the end of 6 weeks averaged 28.5 mm, representing 28% replacement of amputated tissue. Prominent

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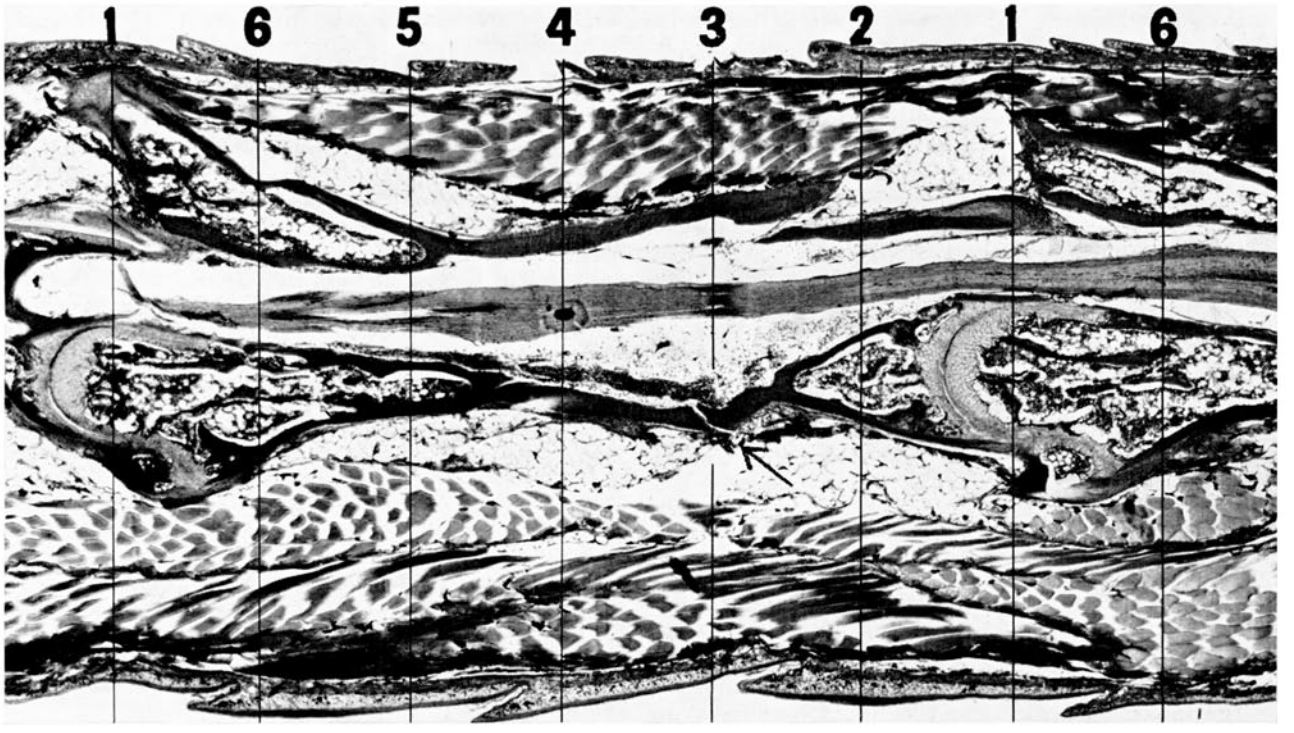

Fig. 1. Section of tail vertebrae of *A. carolinensis* showing the positions used to designate planes of amputation referred to in Figure 3. Although amputations occurred at all positions, when the cut passed through the soft tissues at positions 2 and 4, there was a tendency for the vertebrae to separate at the natural autotomy plane (position No. 3, at arrow).

scales and greatly reduced growth rates during the 6th week suggested that the regenerates were approaching their final length.

The regenerated tails averaged 109 mg (range 45–180 mg) at autopsy, an average replacement of 30%. The final length and weights of the regenerates were highly correlated ($r = 0.8$, $p < 0.001$). Short tails tended to be proportionally lighter (3 mg/mm) than the long ones (4.3 mg/mm). In general, individual differences in these final values reflected consistent differences in regeneration rates throughout the experimental period.

Despite the relatively uniform rate of blastema formation, rates and final extent of tail regeneration differed markedly among the animals studied.

The following factors were tested against final length and weight of the regenerates: initial and final body weight, change in body weight (all gained weight but this ranged from 1–25%), change in body (snout-vent) length, final weight of the liver and abdominal fat bodies, testicular weight and condition, and thyroid epithelial height at autopsy. None of these variables were significantly correlated ($p > 0.10$) with tail regeneration.

There was no correlation between tail regeneration and the position of amputation with respect to the autotomy plane nor the original epidermal condition (Figure 3).

Tail regeneration at 21 °C. At 21 °C, the time for the first external signs of blastema formation to become evident averaged 36 days, after 35 days in 8 individuals and on the 28th, 39th, 42nd and 45th day in 4 others. This contrasts with an average of about 8 days at 32 °C. There was only negligible tail growth during the 2 weeks following the appearance of the blastema. The regenerates grew to 4–7 mm within the next month, averaging 0.15 mm/day. Four animals had regenerated tails of lengths: 3, 11, 14 and 20 mm respectively after 6 months and no increase occurred during a further 4 months.

Three lizards were transferred from 21–32 °C 14 days after amputation. Blastema formation was observed 6 days after the transfer. Thus, there was apparently little progress toward blastema formation in the first 2 weeks at the lower temperature. 5 animals kept initially at 21 °C were transferred to 32 °C after blastema formation. In the

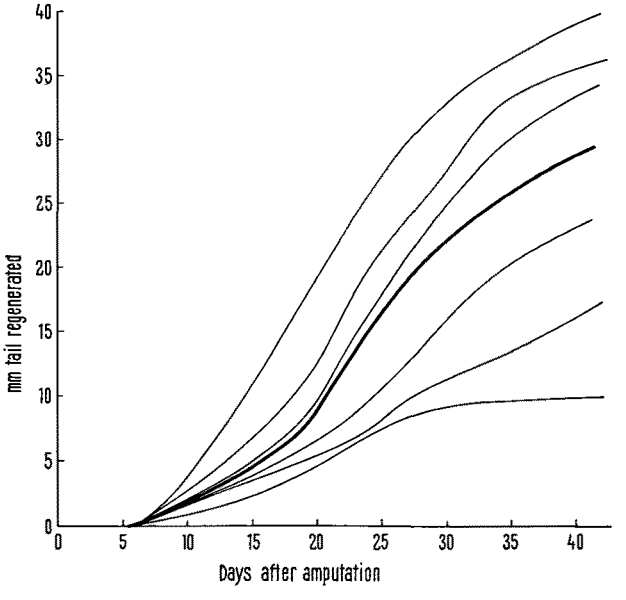

Fig. 2. Tail regeneration at 32 °C in adult male *A. carolinensis*. The central bold curve shows the mean weekly values of 60 animals. The thinner lines depict values for individuals selected to illustrate the variation in regeneration rates.

following 3 weeks at the higher temperature, tail regeneration proceeded at approximately the same rate – 0.92 mm per day – as in those maintained continuously at 32 °C (see above). In 6 animals transferred from 21–25 °C after blastema formation, the tails grew an average of 6.5 mm (range 2–14 mm) in 3 weeks, i.e. 0.3 mm/day. Tail regeneration effectively ceased when the lizards were returned to 21 °C after 3 weeks at the 2 higher temperatures.

Discussion. The effects of temperature on lizard tail regeneration have not previously been examined quantitatively. Our results indicate that temperature may influence at least 3 different aspects of the regenerative process (Table). Comparison of results obtained between 21 and 32 °C indicate that the higher temperature accelerates both rates of blastema formation and subsequent regeneration rates. However, the latter process is seen to be considerably more temperature dependent than the former when Q_{10} values are compared (Table). Finally, temperature influences the final form of the regenerated tail, a smaller proportion being replaced at the lower temperature. Similar variations in regeneration rates are, however, evident at both temperatures.

The rates of regeneration observed at 21 °C in this study are similar to those previously reported for *A. carolinensis* at this temperature[6–8]. TASSAVA and GOSS[8] reported a replacement of about 23% by length of the amputated tissue after 133 days. The discrepancy between this value and the smaller value observed in our study (17%) may be due to the small sample sizes involved here and to slight differences in the amount of tail amputated (see below). They also used smaller (younger ?) individuals and did not define the sexes. Nevertheless, comparison of their value with that observed at 32 °C further confirms our conclusion that the final length regenerated is temperature dependent.

Possible effects of the individuals' nutritional state and its capacity for tail regeneration were presumably minimized in our study through the use of isocaloric feeding, sufficient to promote some weight gain. Nevertheless, we observed no influence of body growth on regeneration rates despite variations in weight gain between 1% and 25% of initial body weight and linear increases of 0–6 mm. Thus, there appears to be little influence of the nutritional state as long as body weight is maintained; i.e. in the absence of starvation.

Some workers[2,7,9] have reported apparent inhibition or retardation of regeneration following intervertebral amputation. Although inter-familial differences in the importance of this factor may exist, we note that in all 3 studies, the sample sizes were very small. Our data support previous conclusions[3,10,11] that there is no correlation between the position of vertebral breakage and the subsequent rate or extent of regeneration.

The possible role of the epidermis as a modifying influence in tail regeneration is suggested by work attempting to explain the lack of regenerative response in anuran limbs by rapid overgrowth of the epidermis preventing blastema formation[12]. Recent studies of the stratum germinativum of the squamate epidermis throughout the sloughing cycle (PANG, MADERSON and ROTH, unpublished data) have shown peaks of mitotic activity at 3 points in the cycle. However, the observations presented here suggest that the stages with which these peaks are associated[5] do not appear to have any effect on regenerative capacity.

A variety of factors other than those discussed above have been suggested as modifying influences in lacertilian tail regeneration. BRYANT and BELLAIRS[2] and TASSAVA and GOSS[8] demonstrated that rates of regeneration (but not the final proportion regenerated) increased in proportion to the amount of tail removed. HUGHES and NEW[13]

Effects of temperature on tail regeneration in *Anolis carolinensis*[a]

Regenerative process	Body temperature 21 °C	Body temperature 32 °C	Q_{10}
(1) Time to blastema appearance (days)	36.2	8	3.9
(2) Rate of tail elongation:			
(a) during first month after blastema formation (mm/day)	0.15	0.98	5.5
(b) during 10 days of maximum growth[b] (mm/day)	0.23	1.50	5.4
(3) Final proportion of tail replaced (% tail removed)	17	28	

[a] All values are means for 60 animals. [b] This period begins 20 days after amputation at 32 °C and 76 days after amputation at 21 °C.

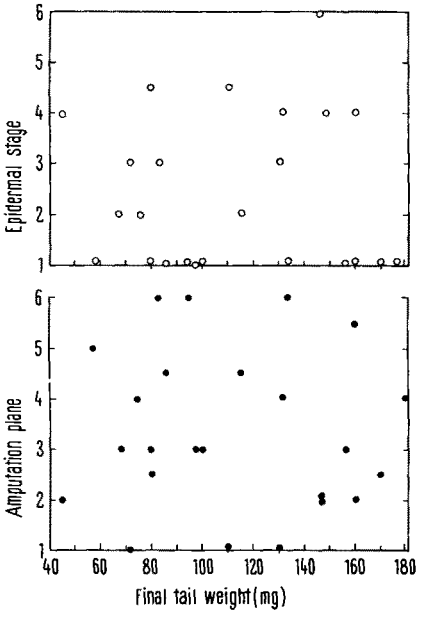

Fig. 3. Relation between final tail weight and epidermal stage at time of amputation (top) and amputation plane within tail (bottom) for 24 of the 60 lizards studied representing the range of regenerative response. The same relationship is seen if final tail length is used. Epidermal stages are based on those described for *A. carolinensis*[5]: stage 1 is a 'resting' stage that lasts 9–10 days following sloughing and stages 2–6 represent the 'proliferative' phase leading to the next molt after another 9–11 days at 32°C. Amputation planes correspond to the positions designated in Figure 1.

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and BRYANT and BELLAIRS[2] showed that repeated autotomies lowered regeneration rates. Neither of these factors were involved in the present study, though failure to control them would presumably give rise to variation beyond that reported here.

Field studies have led to suggestions that the age and 'activity' of individuals may modify tail regeneration[14–16]. In view of the wide variations in regeneration obtained under relatively uniform experimental conditions, we suggest that conclusions based on field studies involving small sample sizes may be tenuous.

The peripheral innervation (see references in ZIKA and SINGER[17]) and the ependyma[18] have been shown to be important determinants of whether regeneration will occur, but their influence on the rates of tail regeneration has not been examined in detail. Such factors may underlie the large degree of variability in our study that was unaccounted for by factors generally thought to influence regeneration rates.

We emphasize the need for care in the selection of sample sizes in tail regeneration studies even when the conditions of the specimens and experimental regime are relatively uniform. The profound temperature effects on tail regeneration raise doubts regarding the validity of interspecific comparisons based on studies where 'comparable' temperatures were not used for all species. In this regard, we suggest that cognizance be taken of the species characteristic preferred body temperatures[4]. These considerations may also be useful in experimental design since regeneration rates most advantageous for study can be attained by selecting appropriate temperatures[19].

Résumé. L'étude du phénomène de régénération de la queue chez le mâle adulte du lézard *Anolis carolinensis*, à 32 °C, s'est montré extrèmement variable même dans des conditions uniformes. Ces variations n'étaient en relation ni avec les dimensions ni avec l'accroissement du corps, ni avec l'endroit de l'amputation dans la vertèbre, ni avec la condition de l'épiderme au moment de l'amputation. L'étude à 21 °C a montré que les effets de la température diffèrent selon les phases du processus régénératif. Le temps de formation du blastême a donné une moyenne de 36,2 jours, mais de 8 jours à 32 °C, $Q_{10} = 3,9$; pendant le mois suivant, la croissance fut à peu près de 0,15 mm par jour mais de 0,98 mm par jour à 32 °C, $Q_{10} = 5,5$. Au terme de sa régénération, la queue n'a atteint que le 17% de sa longueur, même 10 mois après l'opération, tandis qu'elle atteignait 28% au bout de 6 semaines à 32 °C.

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Avian Cardiovascular Parameters: Effect of Intravenous Osmotic Agents, Relation to Salt Gland Secretion

Interest in the avian (nasal) salt gland which serves as an extrarenal osmoregulatory system[1], led us to speculate what effects hypertonic i.v. osmotic agents, which cause the gland to secrete hypertonic sodium chloride, might have on representative cardiovascular and plasma electrolyte parameters. Also, we questioned what role these events might have in the initiation of the secretory response. Indirect evidence was obtained by measuring the following parameters in the pentobarbital anesthetized goose: blood pressure, heart rate, blood flow in both carotids and the right alar artery blood flow, blood volume, erythrocyte volume, and plasma sodium, potassium, and osmotic concentration values at various times before and after i.v. administration of 10 ml each of 10% sodium chloride and 20% sucrose. Analysis of the evidence suggests that the increases in blood volume may be the measurable initiating stimuli for salt gland secretion.

Two mongrel dogs and 7 Toulouse domestic geese were used in this study. The geese were maintained on 1.5% sodium chloride and 0.05% potassium chloride drinking water to hypertrophy the salt gland and make it functional. Purina pigeon chow was given ad libitum. Total carotid and alar blood flows were measured in the heparinized bird with precalibrated cannulating flow probes[2] (EMP-300 I.D. $^1/_{16}$ and $^1/_8$ inches) and a dual-channel flowmeter manufactured by Carolina Medical Electronics, Inc. Lateral blood pressure was recorded from a port in the blood flow probe on the alar artery using a Statham blood pressure transducer. Blood volume was determined by the RIHSA[3] isotope dilution method using a Volémetron[4] instrument set on the 0.5 l scale and using 2.0 ml specimen tubes. Preliminary experiments revealed that optimal mixing of RIHSA in the goose occurred by 4 min post injection time. Therefore, this dose-mixing time was considered as a minimum in all subsequent volume determinations. Erythrocyte volumes, at each blood volume sample time, were calculated from micro-hematocrit and blood volume determinations. Plasma sodium and potassium concentrations were determined at each blood volume sample time on freshly drawn plasma using an internal lithium standard flame photometer[5]. Plasma osmotic concentrations were determined using a microvolume Advanced Osmometer[6].

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[6] Advanced Osmometer, Type 9413. Advanced Instruments, Inc., Newton-Heights, Mass.