6476

Cornification and Molting in Triturus.

A. ELIZABETH ADAMS AND MARGARET C. GRIERSON.

From the Department of Zoology, Mount Holyoke College.

Marked variations in intervals between molts and in duration of single molts were observed in 33 normal adult *Triturus viridescens* kept for 16 weeks in individual finger bowls in the laboratory after a previous laboratory residence of 3½ months. One animal did not molt until the eleventh week of observation; another shed 3 times with an interval of 8 weeks between the first and second molts and 5 weeks between the second and third; another molted 5 times in 6 weeks and then not again for 4 weeks. The average number of molts for the 33 animals was 4.97 in the 16 weeks, ranging from one to 9. The average interval between successive molts (end of one molt to beginning of next) was 13.19 days, with a range of 0 to 59 days. Single molts lasted from one day (1/5 of all recorded molts) to 28 days, and averaged 7.8 days. During the 16 weeks, the group was molting about 34.8% of the time, a higher frequency than that recorded by Adolph and Collins,[1] whose animals molted 9% of the time after being in captivity for 3 months and 22% of the time after 5 months, but consistent with a longer laboratory confinement (7½ months).

These data seem to throw light on the variability in the degree of blackening of thyroidectomized animals which results from the continued cornification of epidermis in such animals without its being shed. Of a group of 362 thyroidectomized animals, 243 became very black and at autopsy all but 33 lacked thyroid tissue. These possessed a few follicles usually located in the median line. Among the 119 that did not blacken after thyroid removal, no thyroid tissue was demonstrable by dissection in 9, but the rest had from one to 10 follicles. Occasional animals that blackened and then molted some time after the operation invariably had thyroid tissue. These variations, in which some animals with small amounts of thyroid gland blackened and others with apparently none or very little blackened faintly or not at all, seem correlated with the above described differences in the molting records of normals. Both sets of data indicate that the rate of cornification is an individual matter. The data on the thyroidectomized group further indicate that cornifica-

[1] Adolph, E. F., and Collins, H. H., *J. Morph.*, 1925, **40**, 575.

tion proceeds independently of the amount of thyroid secretion. However, it appears that the thyroid hormone controls the molting mechanism since molting of thyroidectomized or hypophysectomized animals or ones lacking both glands can only be brought about by administering (or releasing in the case of hypophysectomized individuals) thyroid hormone or iodine.[2]

Possible factors in the molting mechanism were tested by using both normal and thyroidectomized animals. Thyroxin was used to induce the molt in thyroidectomized animals; pilocarpine and atropine to release and prevent the release, respectively, of cutaneous gland secretion; sodium nitrite and ephedrine or epinephrine to increase or decrease, respectively, the lymph supply to the skin.[3] Salt solution was injected into control animals or they were untreated.

1. *Histological study of skin.* No striking changes were observed in the skin of thyroidectomized animals killed during the latent period before the beginning of a molt induced by an injection of thyroxin (0.1 cc. of a 0.01% solution, Squibb). When the molting reaction is becoming evident (72 to 84 hours after the injection), there are a few more mitotic figures in the stratum germinativum, but the proliferation is so slight that it does not seem likely to play a significant rôle in shoving the cornified layers off or in severing them from the uncornified layers beneath. Further, the tardiness in the appearance of the mitoses suggests that the thyroid hormone does not stimulate the skin directly, but initiates a chain of reactions in the body that lead to molting (Speidel).[4]

2. *Animals in moist chambers.* Seven of 12 normal animals placed in moist chambers molted in 28 to 96 hours after confinement without any type of injection. Exposure to moist air was also followed by shedding in ½ to ⅔ of the normal animals injected with each solution. However, since uninjected ones molted with equal frequency, it is concluded that the shift from water to moist air rather than the type of injection was the chief factor in the molting of the injected animals. Further, since uninjected and injected thyroidless newts, *with the exception of thyroxin-injected ones,* failed to molt whether in water or moist air, it seems plausible that the thy-

[2] Adams, A. E., and Richards, L., *Anat. Rec.*, 1929, **44**, 222; Adams, A. E., Richards, L., and Kuder, A., *Science*, 1930, **72**, 323; Adams, A. E., *Anat. Rec.*, 1931, **51**, Suppl. 40; Adams, A. E., Kuder, A., and Richards, L., *J. Exp. Zool.*, 1932, **63**, 1.

[3] Hatcher, R. A., and Eggleston, C., Useful Drugs, Eighth Edit., 1930, Am. Med. Assn., Chicago.

[4] Speidel, C. C., *J. Morph.*, 1926, **43**, 57.

roid gland is involved in the normal molting mechanism. Confinement of thyroxin-injected thyroidectomized individuals in moist chambers lengthened the latent period before the induced molt became evident (compare an average latent period of 72.5 hours for 7 such animals in water with one of 86.5 hours for 14 in moist chambers). These results show that a water environment is not necessary for molting, but probably facilitates the loosening of the cornified cells.

3. *Stimulating or depressing the cutaneous gland action.* It seems unlikely that the cutaneous glands have a rôle in molting because their ducts open to the surface of the epidermis, even in thyroidectomized animals with 4 or more cornified layers. Moreover, normal or thyroidless animals in moist chambers often show collapsed skin glands. Stimulation of the glands of 23 thyroidectomized newts by single injections (0.1 cc.) of a 1% pilocarpine hydrochloride solution produced no molting although within an hour the secretion was abundant on the surface of the skin. Ten of the animals were observed for 76 hours, 13 for 14 to 21 days. Pilocarpine also failed to speed up molting when it was given with thyroxin (0.01% solution, Squibb) to 5 athyroid newts. Furthermore, a 0.1% solution of atropine sulphate when administered with thyroxin did not delay the molt in 10 thyroidectomized animals, when compared with that occurring in animals injected with thyroxin only. This evidence, therefore, rules out the discharge of cutaneous gland secretion as involved in the chain of reactions causing molting.

4. *Varying the cutaneous lymph supply.* Since "red phase" (terrestrial) and "green phase" (aquatic) *Triturus* that molted in moist chambers had dry skin surfaces, but had fluid under the cornified layers, it seemed likely that the cutaneous lymph supply might be linked with the molting phenomena. Further, intercellular spaces in the lower epidermal layers are prominent and there is a marked capillary bed at the dermal-epidermal border. Therefore, sodium nitrite injections (a single injection, 0.1 cc., of a 1% or two injections of a 0.01% solution daily for 14 days) were used to relax the blood vessels and to allow more lymph to flow out into the skin.[3] However, this treatment failed to induce a molt in 25 thyroidectomized animals observed from 3 to 21 days. Although 10 of these were observed only 76 hours, the remaining 15 died or were killed from 5 to 21 days after treatment had begun, that is, well beyond the usual latent period for induced molts. Neither did sodium nitrite when given with thyroxin hasten the appearance of the induced molt in 5 thyroidless animals. Injections of ephedrine hydrochloride (a single injection, 0.1 cc., of a 1% solution or 2 injec-

tions of a 0.01% solution daily for 14 days) or of epinephrine (a single injection, 0.1 cc., of a 0.1% solution or 2 injections of a 0.1% solution daily for 8 days) were given to decrease the lymph supply to the skin. As was to be expected, no molts were induced in 19 thyroidectomized animals given the ephedrine or in 15 given epinephrine. Of 17 normals injected with these drugs, all but 4 individuals molted without any noticeable delay, but considering the variation of normal individuals in water and in moist chambers, these data do not give conclusive evidence that decreasing the lymph supply in normals retards their molting. On the other hand, when injections of ephedrine or epinephrine were given at the same time as thyroxin to thyroidless animals, the molt was retarded when compared with that of thyroidectomized animals receiving thyroxin only. For example, in one experiment, the average latent period for 4 thyroxin-injected animals was 115 hours, while for 9 of 15 animals receiving ephedrine or epinephrine with the thyroxin, it was 118 hours. Five of the remaining 6 molted much later (6, 7, 12, 14, 14 days after treatment had begun) and one did not molt although observed for 33 days. This failure to react after thyroxin is unique among large numbers of such tests carried out in this laboratory. In another experiment, the period before molting was observed in 10 animals receiving ephedrine with thyroxin was 14.5 days as compared with 78.8 hours for 5 animals receiving thyroxin only. These experiments seem to implicate the circulatory system in the chain of reactions leading to the actual molt (Speidel[4]).

Conclusion. The rate of cornification of the superficial epidermis of *Triturus* is markedly variable and probably independent of thyroid control, but the molting of the cornified layers is dependent on the thyroid. Experiments indicate that a change in cutaneous circulatory conditions is probably an essential factor in the molting mechanism.