Glucagon Treatment of Muscular Dystrophic Mice: A Lack of Effect

POPE[1] reported that glucagon, at doses from 2 to 20 μg/mouse/day, increased the mean survival time, the mean maximum weight and the age at maximum weight of genetic muscular dystrophic mice. These treatments also improved the clinical condition of the mice. Based on POPE'S initial report, BRADLEY et al.[2] attempted to confirm POPE'S observations but did not succeed. In this communication, we wish to report that our results support the conclusions of BRADLEY et al.[2]. At the concentrations prescribed by POPE, glucagon had no observable effect on genetic muscular dystrophic mice.

Thirty-seven male and female muscular dystrophic mice (*dy/dy*) of the 129/ReJ strain were purchased from the Jackson Laboratory, Bar Harbor, Maine. They were divided into 3 treatment groups as shown in Table I. To avoid a cage to cage variation, 3 mice, 1 from each treatment group identified by ear mark, were housed in 1 transparent plastic cage. The extra mice on 10 μg glucagon were also housed 3 to a cage. Purina Laboratory Chow and water were available ad libitum. Lights in the mouse room were kept on from 06.00 to 18.00 h.

Glucagon (Lilly) was given s.c. once a day in a volume of 0.1 ml diluent at 2 or 10 μg per mouse, 7 days a week. The experiment was started when the mice were 52–56 days of age and was terminated 100 days later. At that time, the dystrophy of the surviving mice was quite severe and they had not been gaining weight for several weeks. The dystrophic condition of all the mice was confirmed by pathological examination upon autopsy.

Table I presents the mean maximum weight attained by each group of mice. Male mice were significantly heavier than female mice although the difference was only 2 g. Our mice, both male and female, were lighter than POPE'S[1] probably because of the difference in diets. POPE used Purina Mouse Breeder Chow which has a higher energy content and fat content than the Purina Laboratory Chow we used. In our experiment, there was no difference in body weight within each sex between either of the glucagon-treated groups and the diluent group.

Table II presents the number of experimental days survived by each mouse. The female mice as a group survived longer than the male mice, a finding also reflected in POPE'S data[1]. In our study, no difference was observed within each sex between either of the glucagon-treated groups and the diluent group. We, therefore, cannot substantiate POPE'S claim that glucagon treatment is beneficial to the muscular dystrophic mice. Other means must be devised for the treatment of this disease.

Table I. Maximum weight[a] of dystrophic mice during the experiment

	Female (*N*)	Male (*N*)
Diluent[b]	14.0 ± 2.6 (5)	15.6 ± 1.7 (5)
2 μg glucagon	13.0 ± 1.9 (5)	15.6 ± 3.0 (4)
10 μg glucagon	14.0 ± 1.3 (10)	15.8 ± 1.9 (8)
Combined	13.7 ± 1.5 (20)	15.7 ± 2.0 (17)[c]

[a] Mean ± S.D.(g). [b] The diluent (Lilly) contained 1.6% glycerine and 0.2% phenol, pH 2.5–3. [c] Significantly different from the combined female group at $p < 0.005$.

Table II. Number of experimental days survived by each dystrophic mouse

	Survival (days)	$\bar{X}$ ± S.D.
Female mice		
Diluent	46, 100, 100, 100, 100	89.2 ± 24.1
2 μg glucagon	38, 39, 100, 100, 100	75.4 ± 33.7
10 μg glucagon	51, 54, 70, 100, 100 100, 100, 100, 100, 100	87.5 ± 20.7
Combined		84.9 ± 24.4
Male mice		
Diluent	23, 52, 66, 66, 100	61.4 ± 27.8
2 μg glucagon	3, 3, 55, 81	35.5 ± 39.0
10 μg glucagon	10, 19, 37, 62, 95 98, 100, 100	65.1 ± 38.5
Combined		57.1 ± 35.8[a]

The experiment was terminated after 100 days
[a] Significantly different from the combined female group at $p < 0.01$.

Zusammenfassung. Nachweis, dass Körpergewicht, Dystrophie und Sterblichkeit bei männlichen und weiblichen muskeldystrophischen Mäusen (*dy/dy*) nach 100tägiger Behandlung mit Glukagon (2 oder 10 μg pro Tag, s.c.) unverändert blieben.

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[1] R. S. POPE, Am. J. Physiol. *225*, 518 (1973).
[2] W. G. BRADLEY, J. G. POLGAR, M. H. WILLIAMS and H. G. BODDIE, Br. med. J. *3*, 699 (1972).

Presence of Resilin in a Scorpion *Palamnaeus swammerdami* and its Role in the Food-Capturing and Sound-Producing Mechanisms

It is known that the pedipalp of scorpions ends in a chela formed of an immovable tibia and a movable tarsus. The tarsus moves against the strong processes of the tibia for grasping the food[1]. CLOUDSLEY-THOMPSON[2] reports that the scorpions produce sound by briskly drawing the tip of the tarsus backwards and forwards against the teeth of the tibia. In a recent study on the musculature of pedipalp of a scorpion *Heterometrus scaber*, MATHEWS[3] reports that there are only retractor muscles for closing the tibia and extensors are totally lacking, in contrast to the chela of crabs where both types of muscles occur. Inspite of the absence of extensors, the chela in

scorpions can be opened or closed. The author also reports that the cuticle at the hinge of the tarsus shows elastic property which may be responsible for the opening of the chela. But little is known of the nature of this elastic cuticular hinge. An attempt has been made in the present investigation to throw some light on this problem.

The pedipalps of the scorpion *Palamnaeus swammerdami* were fixed in Zenker's fluid and sections of such material were prepared by celloidin method. MALLORY's triple stain[4] and the toluidine blue-light green stain at different pH were employed for staining sections following ANDERSON and WEIS-FOGH[5]. Fluorescence in the cuticle was examined in a VEB Carl Zeiss Jena NF pK research microscope equipped with high pressure mercury lamp HBO 50. An excitation light filter, UV-filter type UG 1/3.5 (366 μm wave length) was used. For barrier filters in fluorescent microscopy filters GG9/OG1 (both in mounts) were fixed to binocular tube while viewing for fluorescence. For chromatographic analysis of the amino acids, the cuticle from the base of the tarsus as well as that from the adjacent regions were hydrolysed in 6 *N* HCl and analysed by paper chromatography according to BAILEY and WEIS-FOGH[6]. The amino acids in the chromatogram were visualised by exposing to ultra violet light and identified by their Rf values as well as by comparison with authentic samples of amino acids run on standard chromatograms under similar conditions.

Sections, when examined under a light microscope, show that the cuticle in the region of the hinge of the tarsus differed from that in adjacent regions in the absence of the amber coloured epi- and exocuticle, the entire hinge cuticle being colourless and translucent. The whole width of the hinge cuticle took up a red colour with MALLOR's triple stain and a deep sapphire blue colour with toluidine blue-light green stain at pH 4.6–4.8. According to ANDERSON and WEIS-FOGH[5], these are properties specific for the structural protein resilin. Results of treatment with chemical reagents lend support to this inference. The cuticle in the hinge region showed marked swelling reaction when treated with formic acid, formamide, phenol, lithium thiocyanate and cupric ethylenediamine.

One other diagnostic character of resilin is autofluorescence in UV light. Examination of unstained sections of the cuticle from the region in question with the fluorescent microscope shows that the entire cuticle fluoresced bright glossy blue (Figure). The fluorescence property disappeared after treatment with N-bromosuccinamide[7,8]. A similar result was observed after incubation of the sections of the cuticle with pepsin. The cuticle from the adjoining regions never fluoresced under any condition.

It is known from the works of ANDERSON[9] that the fluorescent property of resilin is due to the presence of two amino acids di- and trityrosine. Chromatographic analysis of the amino acids from the cuticle of the hinge region showed the presence of 15 spots of which 13 corresponded to alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenyl alanine, proline, serine and valine. There are in addition two spots which fluoresced blue in UV-light. The Rf values of these two spots are 0.5 and 0.18 which correspond to the dityrosine and trityrosine respectively, of the standard chromatogram.

In the light of the results reported above, it may be inferred that the cuticle in the hinge region of the tarsus may have a protein resembling resilin. It is now well established that most elastic tendons and cuticular regions serving as springs in arthropods have resilin[5,10] whose inherent property is regaining its original condition after being pulled out or extended. In the absence of the extensor muscles in the pedipalp in scorpions, the occurrence of a protein resembling resilin in the hinge of tarsus may aid in the operation of the sound-producing and food-capturing mechanisms.

Zusammenfassung. Untersucht wurde die Beschaffenheit der für das Funktionieren des Beutefang- und Geräuscherzeugungs-Mechanismus zuständigen Oberhaut am Tibia-Tarsus-Übergang der Zangen der Skorpionart *Palamnaeus swammerdami*. Im UV-Licht zeigt diese Oberhaut Autofluoreszenz. In Hydrolysaten wurden Dityrosin und Trityrosin nachgewiesen, was die Vermutung nahelegt, dass Resilin vorhanden ist.

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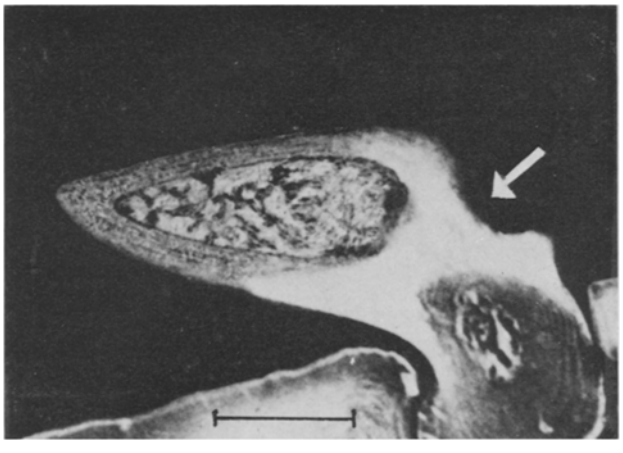

Tangential section through the tarsus of *Palamnaeus swammerdami*, viewed under fluorescence microscope. The arrow indicates the cuticle in the hinge region which fluoresces. (Scale line 1.0 mm.)

[1] R. F. LAWRENCE, *The Biology of the Cryptic Fauna of Forests* (A. A. Balkema, Capetown, Amsterdam 1952).
[2] J. L. CLOUDSLEY-THOMPSON, *Spiders, Scorpions, Centipedes and Mites* (Pergamon Press, New York, Paris, Los Angeles 1958).
[3] A. P. MATHEWS, J. Anim. Morph. Physiol. *12*, 271 (1965).
[4] F. B. MALLORY, *Pathological Technique* (Saunders, Philadelphia 1938).
[5] S. O. ANDERSON and T. WEIS-FOGH, Adv. Insect Physiol. *2*, 1 (1964).
[6] K. BAILEY and T. WEIS-FOGH, Biochim. biophys. Acta, *48*, 452 (1961).
[7] B. WITKOP, *Advances in Protein Chemistry* (Academic Press, New York 1961), vol. 8.
[8] K. T. JOSEPH and Z. GOWRI, J. scient. ind. Res. *29*, 504 (1970).
[9] S. O. ANDERSON, Acta physiol, scand., *66*, 9 (1966).
[10] T. WEIS-FOGH, J. exp. Biol. *37*, 889 (1960).
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