wurden parallel zu den x- bzw. y-Achsen der Fasciculatasäulen gelegt. Zur eindeutigen Entdeckung der Mitochondrien durch das Quantimet 720 wurden diese schwarz markiert und zwischen benachbarte Mitochondrien weisse Grenzlinien gezogen (Figur 1).

Als Stichproben wurden 44 Bildausschnitte männlicher und 45 Bildausschnitte weiblicher Tiere ausgezählt. Es wurden 9 Grössenklassen gewählt, deren Breite 4 RP betrug. 10 RP stellten den unteren Grenzwert dar. Partikel mit einem kleineren Durchmesser wurden ausgeschlossen. 1 RP = 0,0353 µm. Aus den an den Grenzen anfallenden Einzelwerten wurden die Mittelwerte x_n bzw. y_n und die zugehörigen Standardabweichungen s_n errechnet und über die Klassengrenzwerte aufgetragen. Die Sicherheit, S' wurde mit dem Student-t-Test geprüft.

Ergebnisse. Insgesamt wurden 4593 Mitochondrienanschnitte erfasst. Die Mitochondrien sind ellipsoid deformiert. Bei Männchen überwiegen die Mitochondrien kleiner als 0,78 µm (22 RP), bei Weibchen hingegen die Partikel grösser als 0,78 µm (Figuren 2 und 3). $p = 0{,}0005$ für die x-Achse; $p = 0{,}01$–$0{,}0005$ für die y-Achse. 50% aller gemessenen männlichen Mitochondrien besitzen eine Einzelfläche $< 0{,}434$ µm², während 50% der weiblichen eine Einzelfläche $< 0{,}577$ µm² aufweisen.

Besprechung. Quantitative Bestimmungen der Grössenverhältnisse der Mitochondrien in der äusseren Zona fasciculata der Rattennebenniere zeigen eine statistisch gesicherte Geschlechtsdifferenz und bestätigen den visuellen Eindruck. Weiterhin zeigt das Zahlenmaterial, dass die Mitochondrien ellipsoid deformiert und der Achse der Fasciculatasäulen entsprechend ausgerichtet sind. Da die Mitochondrien in enger Beziehung zur Steroidsynthese stehen (MAREK et al.[4]), trägt ihr geschlechtsdifferentes Verhalten dazu bei, den Geschlechtsdimorphismus funktionell zu interpretieren.

Summary. Diameters of mitochondria of the external zona fasciculata of the adrenal cortex of 4 female and 4 male Sprague-Dawley-rats are determined with the image analyzing computer Quantimet 720. On the average females have significantly larger mitochondria. The mitochondria show an ellipsoid deformation, the long axes are orientated parallel to the columns of the zona fasciculata.

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18. November 1970.

[4] J. MAREK, W. THOENES und K. MOTLIK, Naturwissenschaften *57*, 250 (1970).

Lack of Pituitary Involvement in the Cold-Induced Hyperglycemia of the Killifish, *Fundulus heteroclitus*

When acclimated to temperatures near freezing, many, but not all, teleosts physiologically respond by elevating the concentration of glucose in their blood[1]. This cold-induced hyperglycemia has been most thoroughly studied in the killifish, *Fundulus heteroclitus*, and in the toadfish, *Opsanus tau*. Studies on intact killifish showed that acclimation to −1.5 °C in salt water increased the concentration of serum glucose by as much as sixfold and that this increase was due to a breakdown of hepatic glycogen[1,2]. Similarly, a hyperglycemic state developed in cold-acclimated toadfish at temperatures near freezing[3,4] and, although no data were available, the speculation was advanced that the pituitary gland might be responsible for the hyperglycemic response[4].

Indeed, there is evidence that the pituitary of teleosts is involved in blood glucose regulation. FALKMER and MATTY[5] observed a slight hypoglycemia in hypophysectomized *Cottus scorpius* and MATTY[6] found that injections of growth hormone elevated serum glucose in intact *Cottus*. Furthermore, pituitary extracts from the fish, *Luciopimelodus pati*, produced diabetogenic activity in fish, toads, chickens, dogs, cows and man[7]. In addition, even though both hypophysectomized and intact carp had the same concentrations of serum glucose, when injections of epinephrine were administered, serum glucose rose in intact carp but declined slightly in hypophysectomized fish[8].

Endocrine glands under the direct control of the pituitary are also known to affect blood sugar levels in teleosts. For example, injections of hydrocortisone or ACTH are reported to elevate blood glucose levels in a variety of fishes[9–12], thus implicating the pituitary-interrenal axis in blood glucose regulation. The thyroid, however, which is also under pituitary control, is probably not important in blood sugar regulation in teleosts since thyroxine injections had no effect on blood sugar levels in *Cyprinus* and *Tinca*[13] and had little effect on liver glycogen levels in *Fundulus heteroclitus*[14]. Therefore, since the pituitary and some glands under the direct control of the pituitary are known to be involved in blood glucose regulation in teleosts, it is reasonable to suspect an involvement of the pituitary in the cold-induced hyperglycemia of fish.

To determine the role of the pituitary in the cold-induced hyperglycemia of *Fundulus heteroclitus*, serum glucose concentrations were compared in hypophysectomized and intact adult, male killifish acclimated to 20 °C and −1.5 °C in salt water. Fish hypophysectomized according to the procedure of PICKFORD[15] were kindly supplied by Prof. G. E. PICKFORD. These fish were

[1] B. L. UMMINGER, J. exp. Zool. *172*, 409 (1969).
[2] B. L. UMMINGER, J. exp. Zool. *173*, 159 (1970).
[3] P. F. NACE, M. L. MOULE and J. E. SCHUH, Can. J. Physiol. Pharmac. *42*, 225 (1964).
[4] M. A. SILVER and W. D. SHENK, Chesapeake Sci. *9*, 1 (1968).
[5] S. FALKMER and A. J. MATTY, Acta Soc. Med. upsal. *71*, 156 (1966).
[6] A. J. MATTY, Nature, Lond. *195*, 506 (1962).
[7] B. A. HOUSSAY and A. BIASOTTI, Compt. r. Soc. Biol., Paris *107*, 733 (1931).
[8] F. MAZEAUD, Compt. r. Soc. Biol., Paris *163*, 24 (1969).
[9] P. F. NACE, Biol. Bull. *109*, 366 (1955).
[10] M. OGURI and P. F. NACE, Chesapeake Sci. *7*, 198 (1966).
[11] T. FARKAS, Prog. Biochem. Pharmac. *3*, 314 (1967).
[12] D. G. BUTLER, Gen. comp. Endocrin. *10*, 85 (1968).
[13] H. VORHAUER, Biochem. Z. *296*, 90 (1938).
[14] G. E. PICKFORD, Anat. Rec. *112*, 429 (1952).
[15] G. E. PICKFORD, Bull. Bingham Oceanogr. Coll. *14* (2), 5 (1953).

captured in April, 1967, and maintained at 20 °C for 8 weeks prior to hypophysectomy in late June. After hypophysectomy, the fish were kept at 20 °C for 15 weeks and half were then gradually transferred to water at −1.5 °C after acclimation to intermediate temperatures for various lengths of time (Table I). The remaining half were kept at 20 °C until the experiment was terminated. Control groups of intact fish were also maintained at 20 °C and −1.5 °C. At the time of autopsy, no hypophysectomized fish had increased significantly in length since the date of hypophysectomy and none showed any significant trace of nuptial coloration. These indications, together with an overall pallor and complete sexual regression, provided adequate evidence of the absence of a pituitary remnant. Serum glucose was determined the day following autopsy using an ultramicro adaptation of the Glucostat (Worthington Biochemical Corporation) enzymatic method, following the procedure of SAIFER and GERSTENFELD[16]. The results are presented in Table II.

At 20 °C, serum glucose levels were significantly lower in hypophysectomized fish than in intact controls. However, after living for 10 days at −1.5 °C, hyperglycemia developed in both intact and hypophysectomized killifish. These results indicate that even though the pituitary is involved in serum glucose regulation at 20 °C, the pituitary and the glands under its direct control are not responsible for eliciting the hyperglycemia observed at subzero temperatures.

Since the pituitary and the glands under its direct control are not responsible for the cold-induced hyperglycemia in killifish, further investigations are needed to establish the hormonal control of this response. Endocrine glands not under pituitary control that might be involved are the pancreatic islets and chromaffin tissue. Of these, the pancreatic islets seem to offer the most promise as controlling agents in the cold-induced hyperglycemic response since seasonal cycles in their histology are known. For example, PALLOT[17] examined the islet tissue from 8 species of fish to find increased numbers of α-cells during the winter; in the spring, the number of α-cells had diminished. In addition, SAID and AL-HUSSAINI[18] found that in the islets of *Tilapia* and *Mugil* there was almost no insulin present in the autumn and winter, but it was abundant in the summer; glucagon was present at all seasons without much difference. Either a decrease in insulin production or an increase in glucagon production, as reported for other fish, could account for the cold-induced hyperglycemia in *Fundulus heteroclitus*[19].

Table I. Period of acclimation of *Fundulus heteroclitus* to various temperatures

Group	Final temperature (°C)	Time at each temperature for acclimation 20 °C	10 °C	4 °C	−1.5 °C
Intact	20	51 weeks	–	–	–
	−1.5	23 weeks	22 weeks	4 weeks	10 days
Hypophysectomized	20	50 weeks	–	–	–
	−1.5	23 weeks	22 weeks	4 weeks	10 days

Table II. Serum glucose concentrations (mg/100 ml) of intact and hypophysectomized male *Fundulus heteroclitus* acclimated to various temperatures[a]

Group	Acclimation temperature 20 °C		−1.5 °C	
Intact	69.6 ± 3.2	(7)	230.4 ± 41.6	(5)[c]
Hypophysectomized	55.6 ± 4.0	(7)[b]	396.7 ± 51.4	(4)[b,c]

[a] Data expressed as mean ± standard error (sample size). [b] Significantly different from intact controls ($p < 0.05$) using Student's t-test. [c] Significantly different from 20 °C controls ($p < 0.05$) using Student's t-test.

Zusammenfassung. Glukose-Steigerung im Serum von *Fundulus heteroclitus* nach Akklimatisation an −1,5 °C wird nicht durch die Hypophyse verursacht. Hingegen ist die Hypophyse notwendig, um die Serumglukose bei 20 °C bei normaler Konzentration zu halten. Hypophysektomie bei 20 °C hat eine Abnahme der Serumglukose zur Folge.

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Department of Biology, Yale University, New Haven (Connecticut 06520, USA), 11 December 1970.

[16] A. SAIFER and S. GERSTENFELD, J. lab. clin. Med. *51*, 448 (1958).
[17] G. PALLOT, Bull. Histol. appl. Physiol. Path. *16*, 33 (1939).
[18] E. E. SAID and A. AL-HUSSAINI, Zool. Soc. Egypt Bull. *21*, 1 (1966/67).
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The Acidic Glycosaminoglycans in the Skin of Athyroid Rats: the Effects of L-Tri-Iodothyronine

Since the work of WATSON and PEARCE[1] it has been known that subnormal thyroid activity affects the glycosaminoglycans of the skin. The changes reported were an increse in the hyaluronic acid and a decrease in the dermatan sulfate content of the skin[2,3]. These results seem to be independent of thyrotrophin because the same changes are noted after hypophysectomy as in hypothyroidism[2].

This report presents data on the glycosaminoglycan content of the skin of normal, athyroid and athyroid-treated rats with L-tri-iodothyronine (L-T3), using the techniques now available for the quantitation of glycosaminoglycans in order to get further insight into the

[1] E. M. WATSON and R. D. PEARSE, Am. J. clin. Path. *19*, 442 (1949).
[2] S. SCHILLER, G. A. SLOVER and A. DORFMAN, Biochim. biophys. Acta *58*, 27 (1962).
[3] J. A. KOFOED and C. E. BOZZINI, Acta physiol. latinoam. *17*, 323 (1967).