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Wychwyt radiojodu u młodych karpi (*Cyprinus carpio* L.) wstrzymanych we wzroście

Radioiodine uptake in young carp *(Cyprinus carpio L.)* retarded in growth

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A b s t r a c t — In the light of results obtained in recent years, radioiodine disposition in young carp (*Cyprinus carpio* L.), retarded in growth is described. The pharynx centre accumulates most of the administered radioiodine. Activation of renal centre takes place already after 10 days of intensive feeding.

Thyroid gland in teleost fish is situated in the form of loosely scattered cell groups of secretory type in the pharyngeal part, around aorta and its branchial ramifications. Although thyroid gland of fish is of different anatomical structure from that found in higher vertebrates, it has the same physiological features. It possesses the characteristic ability of accumulating free iodine from blood stream circulation system in the opposite direction to concentration gradient — called "iodine pump".

Coefficient T/S (iodine level in thyroid tissue to iodine level in serum) according to G o r b m a n and B e r n (1964) in normal conditions reaches 25 in fish, and under the influence of TSH it can increase up to 500. The thyroid follicle containing colloid in its lumen is the basic element of iodine metabolism in fish. Accumulated iodine under the influence of enzymatic complex is transferred into molecular form and bound to aminoacids built in tyroglobulin structure.

By the transformation inside molecule from the simple aminoacids

containing iodine (mono- and di-iodotyrosines) hormonally active iodine compounds are produced (tri-iodothyronine and tyroxine), and later released into blood stream.

Not much is known as yet about the role of thyroid hormones on the cell level. Their physiological action in the organism can be divided into two groups:

a) metabolic action: stimulating oxydation on tissue level and so directing the metabolic rate,

b) morphogenetic action: stimulating, especially in lower vertebrates, metamorphosis and organ formation in individual development.

Thyroid specificity in carp

The phenomenon of heterotopic location of thyroid tissue in some teleost fish has been of late described in endocrynologic literature. So Hoper (1950), Vievien et al. (1954) described a not typical location of thyroid tissue in some specimens of *Cyprinodontidae* family, Baker et al. (1955), Baker-Cohen (1959) and MacIntyre (1960) in *Xiphophorinae* family. Single follicles or their whole formations were found in kidney, spleen, eye, heart muscle and pericardium cavity. Some authors tried to describe this phenomenon as pathologic; the majority, however, were of the opinion that tissue thus located performs its physiological function and its dispersion could be explained by the migration of tissue elements in developing embryo along blood vessels of body cavity.

Chavin (1956 a, b, c) observed also in goldfish (*Carassius auratus* L.) a considerable accumulation of heterotopic thyroid tissue in head kidney which in this species has only an erytropoetic function.

The phenomenon of a typical location of thyroid tissue in thoracic kidney of carp was later described (Łysak 1962, Sugiyana, Sato 1960) and in this species it has a purely physiological character. In the following years in the former Fishery Department (now the Institute of Applied Zoology of Agricultural Academy in Kraków) the series of experiments was carried out with the use of radioactive iodine, its aim being a closer examination of the phenomenon here described.

The following fish species of Southern Poland were tested on iodine location with the original method of in vivo measurement of radioiodine disposition in the whole body (Łysak 1964): crucian carp *(Carassius auratus gibelio* Bloch), its hybrid with carp, tench (*Tinca tinca* L.), orfe *(Leuciscus idus* var. *orphus),* chub (*Leuciscus cephalus* L.), gudgeon (*Gobio gobio* L.), stone loach (*Nemachilus barbatulus* L.), perch pike (*Lu-*

cioperca lucioperca L.), stone perch (*Acerina cernua* L.), largemouth bass (*Micropterus salmonides* Lacep.), rainbow trout (*Salmo gairdneri* Rich).

Of the above species only the carp, crucian carp and their hybrids revealed the presence of radioiodine accumulating tissue in extra pharyngeal areas. The remaining fish species were characterized by normal, pharynx localization of this tissue. In order to describe more precisely the mechanism of accumulation and secretion of iodine by kidney thyroid centers 2-year old carps were placed under influence of thyroid stimulants (tyrothropine) and inhibitors (natrium salt of methylotiouracil).

These experiments showed that in carp centers located in thoracic kidney responded to tyrothropine and methylotiouracil by raising or lowering the uptake of previously administered radioiodine in a way characteristic of normal thyroid tissue. By using different amounts of tyrothropine it was noticed that thyroid kidney centers in carp have a double phase of stimulation state. In the first phase under TSH influence the iodine uptake of the tissue increases, while the second phase following immediately the first, brings a marked growth of the amount of hormonal iodine (tyroxine and triiodothyronine) secreted to blood (Łysak 1971 a, b).

Microautoradiographic tests

Using the stripping film technique of Doniach, Pelc (1950), Curran, Clark (1961) and Sehe 1960), microautoradiographic tests were made also with radioiodine, in order to trace in carp the anatomic structures taking part in radioiodine accumulation. As the result of these tests, centers accumulating radioactive iodine in thoracic kidney of carp were noticed in 1—2 year old specimens.

The structure of the centers loosely scattered in the whole kidney was the same, judging by the cell shape as well as by the presence of colloid, as the structures found in pharynx of other fish thyroid follicles (Łysak 1964).

Chromatographic tests

For biochemical identification of iodine compounds present in carp kidney thyroid centres various methods have been used thin-layer chromatography (Frey 1964, Hollingsworth et al. 1963), column chromatography (Jacobson, Widström 1962) and on ion-exchange paper (DEAE), Sleeman, Diggs (1964). Using those methods it was

possible to find iodine-compounds characteristic of thyroid tissue in digested homogenets of thoracic kidney. The number of those compounds clearly increased in fish previously treated with tyrothropine proving the thyroidal character of investigated tissue. The production of thyroid hormones also increased in kidney centers (triotyronine and tyroxine) at high temperature (Ł y s a k 1972).

Investigations of post-embryonal stages of carp development

A non-typical feature of kidney localization of thyroid centers in carp is not an old feature from the evolutional point of view (the remaining species of *Cyprinidae* family except carp and crucian carp have thyroid gland located in pharynx). Therefore if Haeckl's law (recapitulation principle) is to be considered as valid to thyroid gland development it should be expected that in early (embryonal or post-embryonal) stages of this species development, thyroid tissue should be localized only in pharynx, and kidney centers, genetically younger, one may say of secondary character, should appear much later.

This theoretical hypothesis was checked upon empirically by the incubation of young carp larvae in solutions containing radioactive iodine. Such larvae after washing, were decapitated and then in well scintillation counter the amount of this element accumulated by head and rest of the body were measured.

Such incubations were made successively with young carp of 7—160 days; the cumulative results of the measurements are given in the Table I.

Tabela I. Zmiany w akumulacji radiojodu w postembrionalnym okresie rozwoju

Table I. Changes in radioiodine accumulation in postembryonal development period

Test	Wiek (w dniach) Age (in days)	Ciężar ciała Weight of body	Procentowy udział w wychwycie radiojodu Percentage share in radioiodine uptaking	
			głowa head %	reszta ciała rest of the body %
Nr I Ryby normalne normal fish	36-39	0.540	69.3	30.7
	49	1.351	53.6	46.4
	68-74	8.350	12.7	87.3
	154-160	105.750	3.2	96.8
Nr II Ryby wstrzymane w rozwoju Retarded fish A	140	0.452	63.0	37.0
B	150	0.832	29.0	71.0

The data presented in Table I corroborate the hypothesis as stated at the beginning (Łysak 1972). In seven days old carp, most of radio-iodine is situated in the head, whereas in 49-th day of life iodine division between head and rest of the body is approximately 1 : 1. When the weight of tested fish is about 1.3 g in natural farming conditions they are transferred in appropriate density to special ponds (called the second intermediate ponds), where they stay till autumn.

In these specially fertilized ponds they find very good living conditions and begin to feed intensively.

During the following 100 days they increase their weight almost 100 times!

At the same time, as can be clearly seen from the quoted table, the thyroid center in thoracic kidney begins its activity.

However, the activization of these centers is not a function of fish age as one could judge after the test No I.

The last two horizontal columns of the discussed table present the results obtained in test No II A and B with carps experimentally delayed in growth in A. Krogh Institute in Copenhagen.

It has been found that in the age equal to the final age for carps tested by the first experiment, those specimens weighed only 0.4 g, and their kidney thyroid centers were not quite stimulated (A).

A ten-day period of intensive feeding (B) was enough for them to double their wight, which corresponds quite clearly with activization of accumulation activity of kidney centers.

The results of the 1973 tests will be in the nearest future repeated because of the small amount of the specially prepared material.

Conclusions

As the result of the presented above investigations the phenomenon of the localization of thyroid tissue in carp kidney have been described and explained. Conclusions could be condensed in the following points:

a) in carp (*Cyphinus carpio* L.) beside the little active pharynx center, there exist and act thyroid centers situated in thoracic kidney,

b) the presence of extra-pharyngeal thyroid centers in this fish species is physiologically normal. They are as sensitive as pharynx center's to thyroid stimulants and inhibitors, producing also the same thyroid hormones (tri-iodotyronine and tyroxine),

c) the presence of extra-pharyngeal thyroid centers in this species from the evolutionary points of view is of secondary character.

In the early stages of the post-embryonal development only the pharyngeal center accumulates iodine.

The kidney center becomes active later, when young carp begins to feed intensely.

STRESZCZENIE

Na podstawie dotychczas przeprowadzonych badań wiadomo, że karp jako jeden z nielicznych gatunków słodkowodnych ryb posiada dwa czynne ośrodki rozmieszczenia tkanki tarczycznej: gardzielowy i nerkowy. Oba te ośrodki posiadają normalnie rozwinięte pęcherzyki tarczyczne zawierające nabłonek kubiczny i koloid. Oba są podatne na środki pobudzające i hamujące akumulację jodową i produkcję tyroksyny i trój-jodotyroniny. Z ostatnich prac wiadomo również, że w naszym klimacie w naturalnym rozwoju karpia aktywizacja ośrodka nerkowego następuje bardzo późno, bo dopiero w 6 tygodniu życia i uzależniona jest w głównej mierze od dostępności pokarmu.

W badaniach niniejszych przeprowadzonych częściowo w Instytucie im. A. Krogha w Kopenhadze, stwierdzono, że karpie wstrzymane we wzroście (0,45 g wagi w 5 miesiącu życia) akumulują podany radiojod prawie wyłącznie przy pomocy ośrodka gardzielowego (63,0% całego jodu zakumulowanego). Intensywne żywienie tych ryb przez okres 10 dni spowodowało podwojenie ich wagi oraz przesunięcie intensywności procesów akumulacji jodowej na ośrodek nerkowy (71,0% całego jodu zakumulowanego).

REFERENCES

Baker K. F., O. Berg, A. Gorbman, R. F. Nigrelli, M. Gordon, 1955. Functional tumors in the kidneys of platyfish. Cancer Res., 15, 118—123.

Baker-Cohen K. F., 1959. Renal and other heterotopic thyroid tissue in fishes. (w — in: A. Gorbman ed.: Comparative endocrinology.) 283—301.

Chavin W., 1956 a. Thyroid distribution and function in the goldfish (*Carassius auratus* L.) as determined by uptake of tracer doses radioiodine. Anat. Rec., 124, 272.

Chavin W., 1956 b. Thyroid follicles in the head kidney of goldfish (*Carassius auratus* L.). Zool., 41, 101—104.

Chavin W., 1956 c. Thyroid distribution and function in the goldfish (*Carassius auratus* L.). J. Exp. Zool., 133, 219—221.

Curran R. C., E. B. Clark, 1961. A reliable autoradiographique technique. J. Path. a. Bact., 82, 219—221.

Doniach L., S. R. Pelc, 1950. Autoradiograph technique. Brit. J. Radiol., 25, 184—192.

Frey H., 1964. Application of TL chromatography to the quantitative determination of thyroid gland products. Scand. J. Clin. Lab. Inv., 16, 470—472.

Gorbman A., H. A. Bern, 1964. A textbook of comparative endocrinology. London-Sydney, J. Willey Co.

Hollingsworth D. R., M. Dillard, P. K. Bondy, 1963. Separation of iodamino acids and related compounds by thin-layer chromatography. J. Lab. Clin. Med., 62, 346—350.

Hoper A. F., 1950. The effect of mammalian thyroid powder and thiouracil on growth rates and on the differentiation of the gonopod in *Lebistes reticulatus* Peters. Anat. Rec., 108, 554.

Jacobson W., F. Widström, 1962. Separation of iodoamino acids by column chromatography with Sepadex G-25. Scand. J. Clin. Lab. Inv., 14, 258—288.

Łysak A., 1962. Thyroid secretory centres in the carp (*Cyprinus carpio* L.) revealed by means of iodine I^{131}. Bull. Ac. Pol. Scie., Biol., 10, 549—554.

Łysak A., 1964. Badania izotopowe nad ośrodkami wydzielniczymi u karpia i innych ryb kostnoszkieletowych z zastosowaniem jodu I^{131} — Thyroid centres in carp and some teleost fishes revealed by iodine I^{131}. Acta Biol. Crac., Zool., 7, 21—46.

Łysak A., 1971 a. Influence of the environment temperature and thyrotropine on the iodine metabolism in carp. Part 2. Results of chromatographic investigations. Pol. Hydrobiol., 18, 409—418.

Łysak A., 1971 b. Influence of the environment temperature and thyrotropine on the iodine metabolism in carp. Part 2. Results of chromatographic investigations. Pol. Arch. Hydrobiol., 18, 419—423.

Łysak A., 1972. Funkcja gardzielowych i pozagardzielowych ośrodków tarczycznych u karpia (*Cyprinus carpio* L.) we wczesnych stadiach rozwojowych. Zesz. Nauk. WSR w Krakowie, 73.

MacIntyre P. A., 1960. Tumors of the thyroid gland in teleost fishes. Zool. N. Y., 45, 161—172.

Sehe C. T., 1960. Radioautographic studies on the ultimobranchial body and thyroid gland in vertebrates: fishes and amphibians. Endocrinol., 67, 674—684.

Sleeman H. K., J. W. Diggs, 1964. Two-dimensional chromatography of iodoamino acids on DEAE-cellulose paper. Anat. Biochem., 8, 532—535.

Sugiyama S., T. Sato, 1960. Histological studies of the renal thyroid of the body fish in comparison with the pharyngeal thyroid. Folia Anat. Jap., 36. 385—393.

Vivien J., M. Ruhland-Geiser, 1954. Étude préliminaire de goitres envahisantes spontanés, rencontrés chez un cyprinodonte (*Lebistes reticulatus* Peters). P. Endocrin., 585—594.

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ERRATA

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321	43			Vollenweider R. A., 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. DAS (CSI), 68, 27. Vollenweider R. A., 1969. Primary production in aquatic environments. IBP Handbook, 12. Wróbel S., 1962. Wpływ nawożenia azotowo-fosforowego na skład chemiczny wody, produkcję pierwotną fitoplanktonu i przyrosty ryb w stawach — Einfluss der Stickstoff-Phosphordüngung auf die chemische Zusammensetzung des Wassers, auf die Primärproduktion des Phytoplanktons und auf den Fischzuwachs in Teichen. Acta Hydrobiol., 4, 151—204.