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Effect of thyroxine and thiourea on liver and muscle energy stores in the freshwater catfish, *Heteropneustes fossillis*.

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Summary. The hepato-somatic index (HSI) and the lipid, glycogen and protein contents of liver and muscle were studied in *Heteropneustes fossilis* in response to various doses of thyroxine, thiourea, and thyroxine in combination with thiourea. These treatments did not change the HSI of *H. fossilis*. A low dose (5 μg/fish) of thyroxine had no effect on the lipid content of liver and muscle or on the glycogen content of liver, but it increased liver and muscle protein contents significantly. Mid (10 μg/fish) and high (50 μg/fish) doses of thyroxine decreased the lipid and glycogen levels of the liver and the lipid content of muscle whereas the same doses did not alter liver and muscle proteins. All the tested doses of thyroxine, thiourea, and thyroxine in combination with thiourea did not affect the glycogen level of muscle. This experiment showed that thiourea treatment failed to alter the lipid and glycogen levels of tissues, but was very effective in depleting the protein content of liver and muscle. The influence of thyroxine in combination with thiourea on the lipid and glycogen content of liver and on muscle lipid was similar to that of thyroxine alone. Only a low dose of thyroxine with thiourea restored liver protein level. A high dose of thyroxine with thiourea did not check the decline of liver protein. Thiourea blockade in muscle was cancelled by both doses of thyroxine when administered along with thiourea, and the protein content restored was significantly higher than in the controls. The present findings demonstrate that the influence of thyroxine on body energy reserves (lipid, glycogen and protein) is tissue-specific and dose-dependent.

Introduction.

Thyroid regulation of metabolism in fish has been well documented (Gorbman, 1969). Investigations on the role of the thyroid hormone in lipid, glycogen, and protein regulation have yielded conflicting information. Increased abdominal fat content in rainbow trout (La Roche *et al.*, 1963, 1966) and liver lipid content in platyfish (Baker-Cohen, 1961) have been observed in response to radio-thyroidectomy. Narayansingh and Eales (1975*b*) have shown that thyroxine treatment lowered liver and visceral lipid in brook trout. On the other hand, an increase in muscle lipid was noticed after the injection of thyroxine in coho salmon (Higgs *et al.*, 1976, 1977). Thyroxine hormone administration has also been reported to increase the hepatosomatic index

(HSI) in hypophysectomized goldfish (Hurlburt, 1977). Thiourea has been seen to have varied effects in fish. Data analysis revealed that thiourea exerts inhibitory (Chambers, 1951, 1953) as well as stimulatory (Hopper, 1965) effects on liver lipid stores in *Fundulus*. Thiourea has already been demonstrated to depress both the synthesis and the release of thyroid hormone in different species of fish (Singh, 1970 ; Singh *et al.*, 1977 ; Milne and Leatherland, 1978).

There is scant information regarding the influence of thyroxine on protein and glycogen content in fish. The intramuscular injection of thyroxine decreased muscle protein significantly in underyearling coho salmon (Higgs *et al.*, 1976), whereas it failed to bring about any change in yearling coho salmon (Higgs *et al.*, 1977). On the basis of *in vitro* experiments, Hochachka (1962) proposed the catabolic effect of thyroid on the carbohydrate reserves of brook trout.

The present study tried to determine the effect of administering thyroxine, thiourea, and thyroxine combined with thiourea on the HSI and on lipid, glycogen, and protein contents in the freshwater catfish, *Heteropneustes fossilis*.

Materials and methods.

Adult *H. fossilis* females having a mean weight of 45.5 g (41.5 to 47 g) and a mean length of 18 cm (16 to 20 cm) were collected from the Ramgarh Lake near Gorakhpur in october 1978, and were used in this experiment the same month. The fish were in post-spawning phase at that time (Sundararaj and Sehgal, 1970). They were fed macerated liver and dried shrimp throughout the experimental period. The temperature of the aquaria was not controlled, but the variation was similar in all the aquaria, ranging from 24 to 26 °C. The natural photoperiod was measured as 11.5L/12.5D.

Thyroxine and thiourea administration.

L-thyroxine (Eltroxine, Glaxo, India) was dissolved in 0.6 p. 100 NaCl, and the control pH (0.6 p. 100 NaCl) and the thiourea solution were adjusted to 9 with NaOH. Prior to the start for the experiment, the fish were divided into 7 groups. The individuals of these groups were injected with thyroxine, thiourea, or thyroxine combined with thiourea. The details of the treatment, doses and number of fish in each group are shown in tables 1 and 2. The fish were injected daily at the same time between 3:00 and 3:30 p.m. to avoid a fluctuation in the lipid, glycogen and protein content due to the circadian rhythm of hormone administration (Meier and Burns, 1976). Before injection, the fish were lightly anesthetized (1:4000, MS 222) ; all the injections were given intraperitoneally unless otherwise noted. The volume of carrier liquid was always 0.2 ml.

Tissue analysis.

The fish were killed 24 hrs after the last injection. The liver was dissected out and weighed to the nearest mg using a single pan electric balance (Mettler). The HSI (g liver weight $\times$ 100/g body weight) of individual fish was recorded. Each liver lobe was divided into two pieces. One piece was utilized for lipid estimation and the other

for glycogen and protein estimation. A transverse slice of muscle was cut just behind the dorsal fin. This slice was divided into two equal halves by cutting it dorsoventrally. One lateral half was used for lipid estimation, whereas the other half was utilized for glycogen and protein estimation. Tissues samples were stored in deep-freeze until assayed. Lipid was estimated by the method of Folch *et al.* (1957). Details of the procedure have already been described (Singh and Singh, 1979). For glycogen and protein estimation, a weighed amount of tissue was homogenized in 10 p. 100 TCA (trichloroacetic acid). After centrifugation, the precipitate was washed with 5 p. 100 TCA. The supernatants obtained after centrifugation of the homogenate and washing of the precipitate were pooled for glycogen estimation. Glycogen was assayed by the procedure described by Dubois *et al.* (1956). The precipitate was dissolved in 1N NaOH, and the protein was estimated using the method of Lowry *et al.* (1951). Optical density of the color developed for the glycogen and the protein was measured at 490 mμ and 600 mμ, respectively, by a Spekol spectrocolorimeter. The significance of difference between the two sets of observations was calculated by Student's t-test (Garret, 1966).

Results.

Treatments with a low (5 μg/fish), mid (10 μg/fish) or high (50 μg/fish) dose of thyroxine for 15 days did not change the HSI (table 1). Thyroxine at a low dose (5 μg/fish) did not affect liver and muscle lipid contents (batch 2, tables 1 and 2), whereas higher doses (10 and 50 μg/fish) decreased the liver and muscle lipid significantly (batches 3, 4, tables 1 and 2). Thiourea alone (50 μg/fish) or in combination with a low dose of thyroxine (5 μg thyroxine + 50 μg thiourea/fish) failed to change the lipid levels of liver and muscle significantly (batches 5, 6, tables 1 and 2) when compared with the saline-treated controls. However, a higher dose of thyroxine with thiourea (10 μg thyroxine + 50 μg thiourea/fish) was effective in bringing down liver and muscle lipid levels (batch 7, tables 1 and 2). All the tested doses of thyroxine (5, 10 and 50 μg/fish) lowered the liver glycogen (batches 2-4, table 1), but thiourea treatment was without effect (batch 5, table 1). Both the doses of thyroxine along with thiourea (5 or 10μg thyroxine + 50 μg/fish) exerted a depressive effect on liver glycogen content similar to that recorded after treatment with the same doses of thyroxine alone (batches 6, 7, table 1). All the doses of thyroxine, thiourea, or thyroxine combined with thiourea failed to alter the muscle glycogen content (batches 2-7, table 2). An increase in liver and muscle protein was observed after the injection of 5 μg/fish thyroxine for 15 days (batch 2, tables 1 and 2). However, there was no significant change in liver and muscle protein levels after the administration of a thyroxine dose level of 10 or 50 μg/fish (batches 3, 4, tables 1 and 2). A very significant reduction in liver and muscle protein was observed in response to the injection of thiourea (batch 5, tables 1 and 2). Simultaneous injection of 5 μg thyroxine + 50 μg thiourea/fish restored the liver protein content to almost the level of the saline-treated controls (batch 6, table 1). A higher dose of thyroxine combined with thiourea (50 μg thyroxine + 50 μg thiourea/fish) did not prevent the decline of liver protein. Both the doses of thyroxine when administered along with thiourea (5 or 10 μg thyroxine + 50 μg thiourea/fish) increased the

TABLE 1

Effect of thyroxine, thiourea and thyroxine + thiourea on HSI, and liver lipid, glycogen and protein content in H. fossilis

Batch (1)	Treatment (daily for 15 days)	HSI	Lipid (mg/g wet weight) M ± SEM	Glycogen (mg/g wet weight) M ± SEM	Protein (mg/g wet weight) M ± SEM
1-(6)	6 p. 100 NaCl (Control)	1.05 ± 0.12	46.92 ± 5.56	131.12 ± 14.00	109.45 ± 14.48
2-(6)	Thyroxine 5 μg/fish	1.11 ± 0.09	35.64 ± 6.65	47.65 ± 1.94 (^{a})	189.21 ± 13.96 (^{b})
3-(5)	Thyroxine 10 μg/fish.....	1.18 ± 0.04	21.52 ± 1.15 (^{b})	53.48 ± 10.41 (^{a})	115.65 ± 4.48
4-(5)	Thyroxine 50 μg/fish	1.04 ± 0.05	20.96 ± 4.82 (^{b})	46.13 ± 2.51 (^{a})	107.92 ± 5.65
5-(5)	Thiourea 50 μg/fish	1.35 ± 0.09	46.96 ± 5.38	92.7 ± 8.58	58.32 ± 7.84 (^{b})
6-(5)	Thyroxine 5 μg + Thiourea 50 μg/fish	1.11 ± 0.03	53.20 ± 4.45	67.35 ± 4.45 (^{a})	97.71 ± 9.12 *
7-(5)	Thyroxine 10 μg + Thiourea 50 μg/fish	1.25 ± 0.11	22.81 ± 2.23 (^{b}) **	41.49 ± 6.49 (^{a}) *	44.57 ± 6.78 (^{b})

(1) No. of fish in each batch is given in parenthesis.
Significance of difference compared between control and each treated batch.
(^{a}) < 0.05, (^{b}) < 0.01.
Significance of difference compared between batch 5 *vs.* batch 6 and 7.
* < 0.02, ** < 0.001.

protein content of muscle (batch 6, 7, table 2) over that of fish receiving thiourea (batch 5, table 2) and of the controls.

TABLE 2

Muscle lipid, glycogen and protein content in response to the administration of thyroxine, thiourea and thyroxine + thiourea in H. fossilis

Batch (1)	Treatment (daily for 15 days)	Lipid (mg/g wet weihgt)	Glycogen (mg/g wet weight)	Protein (mg/g wet weight)
		M ± SEM	M ± SEM	M ± SEM
1-(6)	0.6 p. 100 NaCl (control).....	11.79 ± 0.79	5.42 ± 1.96	101.74 ± 6.72
2-(6)	Thyroxine 5 μg/fish	12.71 ± 0.92	3.86 ± 0.23	139.49 ± 6.65 (a)
3-(5)	Thyroxine 10 μg/fish	4.60 ± 0.45 (b)	2.81 ± 0.21	113.11 ± 2.15
4-(5)	Thyroxine 50 μg/fish	4.47 ± 0.31 (b)	2.96 ± 0.35	96.41 ± 4.38
5-(5)	Thiourea 50 μg/fish	9.71 ± 0.16	3.63 ± 0.63	33.81 ± 2.39 (b)
6-(5)	Thyroxine 5 μg + Thiourea 50 μg/fish	10.60 ± 0.56	4.47 ± 0.65	133.05 ± 9.97 (a)*
7-(5)	Thyroxine 10 μg + Thiourea 50 μg/fish	3.58 ± 1.61 (a)*	3.91 ± 0.45	134.24 ± 6.60 (a)*

(1) No. of fish in each batch is given in the parenthesis.
Significance of difference compared between control and each treated batch.
(a) < 0.05, (b) < 0.001.
Significance of difference between batch 5 vs. batches 6 and 7.
* < 0.001.

Discussion.

A decrease in the lipid content of liver and muscle in response to mid (10 μg/fish) and high (50 μg/fish) doses of thyroxine may be the result of the catabolic effect of this hormone on lipid reserves. This finding is consistent with the data reported in *Salmo gairdneri* (Barrington *et al.*, 1961) and in *Salvelinus fontinalis* (Narayansingh and Eales, 1975*b*). Increased adipose tissue lipase activity (Narayangingh and Eales, 1975*b*) and plasma free fatty acid levels (Murat and Serfaty, 1970) in response to the thyroxine treatment further substantiate the knowledge of fat mobilization from organ stores. However, the anabolic effect of thyroxine on lipid has been demonstrated in underyearling coho salmon (Higgs *et al.*, 1976) and yearling coho salmon (Higgs *et al.*, 1977). Unlike the findings in *Fundulus* (Chambers, 1951 ; Hopper, 1965), thiourea administration had no effect on the liver and muscle lipid content of *H. fossilis*. Le Ray *et al.* (1969) also noticed that the total lipid content of *Mugil auratus* was not influenced by a goitrogen, propylthiouracil. In this experiment, thyroxine combined with thiourea exerted the same action on the liver and muscle lipid levels as thyroxine would have alone.

The influence of thyroxine on glycogen content was tissue specific. Mid (10 μg/fish) and high (50 μg/fish) doses decreased the glycogen of the liver, whereas it had no effect on muscle. Thiourea was ineffective in changing the glycogen content of either the liver or the muscle. The depressive effect of thyroxine when administered with thiourea was observed only in liver glycogen. This is in agreement with the report of

Fontaine *et al.* (1953) who have demonstrated a drecrease in liver glycogen after thyroxine administration.

Protein synthesis in the liver and muscle was augmented by the low dose (5 μg/fish) of thyroxine ; mid (10 μg/fish) and high (50 μg/fish) doses had no effect in this experiment. Endogenous thyroxine blocked by thiourea resulted in decreased levels of liver and muscle protein. In thiourea-blocked fish, only one dose in the liver and both the doses in muscle (5 μg/fish and 10 μg/fish) restored the protein levels. Protein synthesis after thyroxine administration was observed in *Solvelinus fontinalis* by Narayansingh and Eales (1975*a*). A. dose-dependent response has been noted in the coho salmon, *Oncorhynchus kisutch* (Higgs *et al.*, 1976 ; Higgs *et al.*, 1977).

In the present experiment the HSI did not change in response to the various doses of thyroxine, thiourea, or thyroxine combined with thiourea. Contrary to this, several workers noted an increase in liver size after thyroxine (Takashima *et al.*, 1972 ; Hurlburt, 1978) and thiourea (Chambers, 1951, 1953) treatment, but thiourea treatment in carp decreased the liver size (Hatey, 1950). However, opinion is not unanimous concerning the mode of action by which the thyroid hormone affects liver size (Pickford, 1952, 1954 ; Pickford and Grant, 1968 ; Hopper and Yatvin, 1965).

It seems from the data on *H. fossilis* that different doses of thyroxine have different effects on lipid, glycogen, and protein levels of liver and muscle. The metabolic influence of thyroxine also varies in different tissues (liver and muscle).

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Résumé. On a étudié l'effet de doses variables de thyroxine, de thiourée et de thyroxine en combinaison avec la thiourée, sur l'index hépato-somatique et le contenu en lipides, glycogène et protéines du foie et du muscle chez *Heteropneustes fossilis*. Ces traitements n'affectent pas l'index hépato-somatique. Une faible dose (5 μg/animal) de thyroxine n'a pas d'effet sur le contenu en lipides du foie et du muscle ou en glycogène du foie, mais il augmente significativement les protéines du foie et du muscle. A moyenne (10 μg/animal) et forte (50 μg/animal) doses la thyroxine diminue les niveaux de lipides et de glycogène du foie et le contenu lipidique du muscle, tandis que les mêmes doses n'altèrent pas les protéines du foie et du muscle. Aucune des doses de thyroxine, thiourée et thyroxine + thiourée utilisées n'affectent le niveau du glycogène dans le muscle. Le traitement par la thiourée ne modifie pas les niveaux de lipides et de glycogène des tissus, mais se montre très efficace pour diminuer le contenu protéinique du foie et du muscle. En ce qui concerne les lipides et le glycogène du foie et les lipides du muscle, la thyroxine combinée à la thiourée a le même effet que la thyroxine seule. Une faible dose de thyroxine + thiourée rétablit le niveau protéinique du foie, mais à forte dose la chute des protéines du foie n'est pas arrêtée. Dans le muscle le blocage par la thiourée est annulé par toutes les doses de thyroxine administrée avec la thiourée : le contenu protéinique est alors significativement plus élevé que chez les animaux témoins. Ces résultats montrent que l'influence de la thyroxine sur les réserves énergétiques corporelles (lipides, glycogène, protéines) est spécifique suivant les tissus et dépend de la dose utilisée.

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