IDENTIFICATION OF LABELED THYROXINE AND TRIIODOTHYRONINE IN AMPHIOXUS TREATED WITH ^{131}I

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SUMMARY

Specimens of the protochordate, amphioxus, were maintained in sea water containing ^{131}I for periods ranging from 1 h to 11 days. The utilization of the ^{131}I was studied with the aid of paper-chromatographic techniques.

The highest concentration of ^{131}I was found in the endostylar region of these animals, but ^{131}I protein was also found in the notochord, the hepatic cecum, and carcass tissues.

[^{131}I]thyroxine and [^{131}I]triiodothyronine were found in this thyroidless organism and clearly identified by co-chromatography with authentic compounds. In contrast to observations made in mammalian thyroid glands, [^{131}I]triiodothyronine was formed in proportions equal to or greater than those of [^{131}I]thyroxine.

INTRODUCTION

Studies of iodine metabolism in animals transitional between vertebrates and invertebrates are of particular value in tracing the evolutionary development of the thyroid gland. Such studies have been made, with the aid of ^{131}I, in a number of Protochordates[1,2] and in the two cyclostomes, the hagfish[3] and the lamprey[4,5]. In the latter, formation of organically-bound ^{131}I in the endostyle of larval specimens was demonstrated by the use of radioautographic procedures[6]. Formation of labeled MIT, DIT, T_4, and T_3 in this organ has also been reported[5,7]. The evidence obtained has been interpreted as showing that the endostyle of larval lampreys represents the most primitive functional form of the thyroid gland[8]. In the even more primitive organism, amphioxus, the ability to form organically-bound iodine has been shown, by radioautographic procedures, to be localized in the organ homologous with the endostyle of larval lampreys[1,2]. COVELLI *et al.*[9] have recently reported the presence of labeled T_4 and T_3 in extracts of whole specimens of ^{131}I-treated amphioxus. In the present investigation we have examined the products of iodine metabolism in amphioxus by the techniques of ^{131}I-labeling and paper chromatography. Various tissues were isolated and their ^{131}I-labeled constituents were compared with those of the endostyle. It is shown that, although the highest concentration of ^{131}I is found in the endostylar

Abbreviations: MIT, monoiodotyrosine; DIT, diiodotyrosine; T_4 thyroxine; T_3, 3′,3,5-triiodothyronine.

region, ^{131}I-labeled protein, iodotyrosines and iodothyronines, can be demonstrated in similar proportions in all tissues examined.

EXPERIMENTAL

Specimens of amphioxus (*Branchiostoma lanceolatum*) were collected in southern California and shipped by air express to Berkeley where they were maintained in sea water at 10–12°. A total of 94 specimens, each weighing from 30 to 325 mg, was immersed in sea water containing ^{131}I (3 to 8 μC/ml sea water) for periods ranging from 1 h to 11 days.

Because of the small size of the animals and the softness of their tissues, we were unable to obtain discrete endostylar issue, and had to take much of the branchial cage along with it. Samples of the hepatic cecum, the notochord, and either the entire carcass or a portion of the tail posterior to the anus were also taken for study. Tissues from 2 to 9 animals were collected and pooled on a watch glass which was kept in a moist chamber. The tissues were quickly weighed on an analytical balance. The actual quantities of tissue analysed varied from 20 mg to 200 mg, and the ^{131}I contained in these tissues was at least $2.4 \cdot 10^5$ counts/min/g (Table I). The smallest portion of tissue analysed contained not less than $5 \cdot 10^3$ counts/min.

For chromatographic analysis, tissues were ground in all-glass homogenizing tubes with 4 or 5 volumes of a solution containing 0.9% NaCl, 0.002 *M* 1-methyl, 2-mercaptoimidazole, 0.001 *M* $MnSO_4$, and buffered to pH 8.5 with 0.03 *M* Tris[10]. Portions of these tissue suspensions were hydrolyzed with pancreatin (50 mg/ml of tissue suspension) at 37° under toluene for one day. Hydrolyzed and unhydrolyzed tissue samples were delivered directly (without butanol extraction) onto strips of filter paper and chromatographed with the following solvent mixtures: (a) collidine–3 *N* NH_4OH (100:35) (b) butanol–ethanol–3 *N* NH_4OH (100:20:40) (c) butanol–ethanol–water, (100:20:40) (d) *tert.*-pentanol saturated with 4 *N* NH_4OH. In order to insure maximum quantities of radioactivity on chromatograms for radioautographic and assay purposes, Whatman 3 MM filter paper was used. With such thick paper, as many as 500 μl of tissue homogenate (equivalent to 100 mg of original tissue) were chromatographed on one 10-cm wide strip of paper. All chromatograms were unidimensional, and developed only in the ascending fashion. The completed chromatograms were dried, and autographed with Kodak Blue Brand X-ray film. The ^{131}I contents of chromatogram components were determined by assay in a well-type NaI scintillation detector.

RESULTS

^{131}I *content of various tissues*

The data in Table I show that, in every animal examined, the branchial tissue (which contained the endostyle) had higher concentrations of ^{131}I than did any other tissue studied. The concentrations of ^{131}I in the branchial tissue were 1.3 to 8 times those in the carcass It is very likely that, if purely endostylar tissue had been taken, the ratios for ^{131}I content of endostyle to ^{131}I in carcass would have been considerably higher. These findings are therefore in agreement with those obtained by radioautographic examination of tissue sections which show centers of ^{131}I accumulation in the endostyle of amphioxus[1,2].

TABLE I

^{131}I CONTENTS OF TISSUES DISSECTED FROM AMPHIOXUS MAINTAINED IN ^{131}I-SEA WATER

Expt.	*Number of animals*	*Period immersed in ^{131}I sea water*	*Tissue taken*	*Weight of tissue taken*	*Total ^{131}I in tissue taken*	*^{131}I/g of tissue*
				mg	*10^5 counts/min*	*10^6 counts/min*
3	8	1 h	Branchial cage	213	0.52	0.25
			Tail	162	0.31	0.19
			Sea water			1.3*
	9	4 h	Branchial cage	221	0.87	0.39
			Tail	287	0.42	0.15
			Sea water			1.3*
4	2	1 h	Branchial cage	20.7	0.40	1.93
			Notochord	42.7	0.10	0.24
			Tail	36.2	0.09	0.25
			Sea water			1.5*
	2	6 h	Branchial cage	22.1	0.61	2.76
			Notochord	50.0	0.16	0.33
			Tail	23.4	0.08	0.33
			Sea water			1.5*
	6	24 h	Branchial cage	39.5	0.85	2.31
			Notochord	70.2	0.20	0.32
			Tail	31.1	0.16	0.56
			Sea water			1.5*
5	7	2 h	Branchial cage	217	6.54	3.01
			Notochord	168	0.91	0.54
			Hepatic cecum	48	0.32	0.67
			Carcass	1080	6.19	0.57
			Sea water			5.3*
6	4	2 days	Branchial cage	100	7.92	7.92
			Carcass	180	5.23	2.91
			Sea water			3.27*
	4	4 days	Branchial cage	141	7.00	4.96
			Carcass	312	9.59	3.07
			Sea water			4.73*
	8	6 days	Branchial cage	100	19.1	19.1
			Carcass	400	23.2	5.80
			Sea water			5.01*
	4	8 days	Branchial cage	200	29.4	14.7
			Carcass	390	22.4	5.74
			Sea water			5.35*

* 10^6 counts/min ^{131}I per ml of sea water.

Chromatographic analysis of tissues

The results of the chromatographic examination of tissues from ^{131}I-treated amphioxus are shown in Figs. 1 and 2 and Tables II and III. It is clear from these results that amphioxus possesses the capacity to incorporate iodine into the thyroid hormones T_4 and T_3. An unusual feature was the large proportion of ^{131}I incorporated into T_3. By the end of the second day of immersion in ^{131}I sea water, labeled T_4, T_3, and the iodotyrosines, MIT and DIT, were present in sufficient quantities in hydrolysates of the labeled tissues to permit their identification by co-chromatography with authentic compounds. Expressed as percentages of the total ^{131}I of the tissues (Table III), the ^{131}I incorporated into iodothyronines appears small (5–20%) because of the large (44–71%) inorganic iodide component present in all tissues specimens. It

should be stressed, however, that these percentages were calculated from ^{131}I-assays for T_4 and T_3 bands that contained more than 10^3 counts/min in every case, and they are therefore significant. Moreover, the recoveries of ^{131}I in T_4 and T_3 bands of duplicate chromatograms developed in the three different solvent systems agreed quite well (Fig. 2).

It should be noted that, although the ^{131}I content of the endostyle-containing tissue was greater than that of the other tissues examined, the same iodine-containing compounds were found in all tissues, and the distribution of ^{131}I among these com-

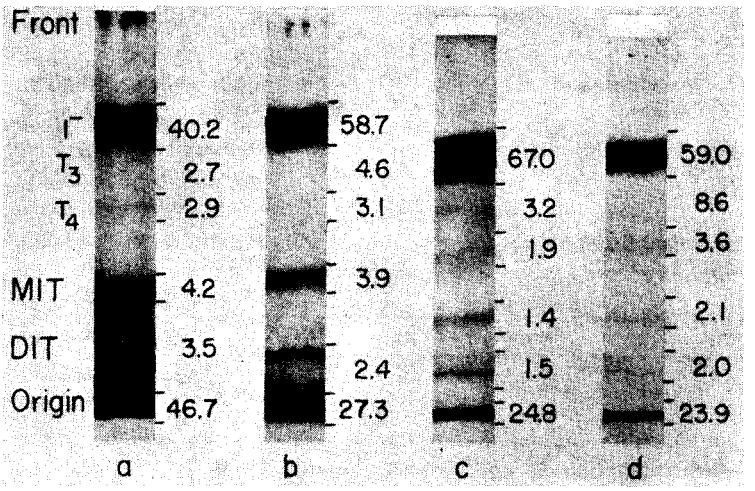

Fig. 1. Radioautographs of chromatograms prepared from suspensions (unhydrolyzed) of tissues from amphioxus immersed in ^{131}I sea water for 2 days (a, b) and 6 days (c, d). a, c, endostyle region; b, d, carcass. Chromatograms developed in collidine–3 N NH_4OH. Numbers to the right show percentages of total ^{131}I recovered in each band.

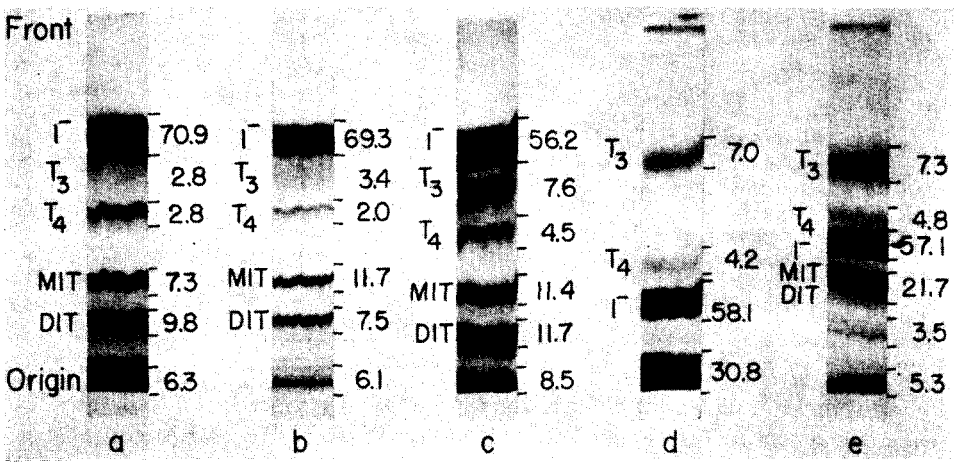

Fig. 2. Radioautographs of chromatograms prepared from hydrolysates of amphioxus tissues. a and b: endostyle region and carcass, respectively, from amphioxus in ^{131}I sea water for 4 days; chromatographed in collidine–3 N NH_4OH. c, d and e: endostyle region of amphioxus in ^{131}I sea water for 8 days; the same hydrolysate chromatographed in collidine–3 N NH_4OH (c), *tert*-pentanol–4 N NH_4OH (d), and butanol–ethanol–3 N NH_4OH (e). Numbers to the right show percentages of the total ^{131}I recovered in each band.

TABLE II

^{131}I DISTRIBUTION ON CHROMATOGRAMS OF UNHYDROLYZED AMPHIOXUS TISSUES

Days immersed in ^{131}I sea water	*Tissue taken*	*% of total ^{131}I recovered in:*					
		Origin	*DIT*	*MIT*	*T_4*	*T_3*	*I^-*
2	Branchial cage	46.7	3.5	4.2	2.9	2.7	40.2
	Carcass	27.3	2.4	3.9	3.1	4.6	58.7
6	Branchial cage	24.8	1.5	1.4	1.9	3.2	67.0
	Carcass	23.9	2.0	2.1	3.6	8.6	59.9
8	Branchial cage	27.4	4.4	3.2	3.2	8.8	52.9
	Carcass	22.2	4.1	3.1	4.5	18.5	47.9
11	Branchial cage	29.4	2.0	2.8	3.0	10.3	52.5
	Carcass	32.3	1.8	3.0	3.6	15.2	44.3

TABLE III

^{131}I DISTRIBUTION ON CHROMATOGRAMS OF HYDROLYZED AMPHIOXUS TISSUES

Expt.	*Period immersed in ^{131}I sea water*	*Tissue taken*	*Total ^{131}I on chromatogram (10^3 counts/min)*	*% of total ^{131} recovered in:*					
				Origin	*DIT*	*MIT*	*T_4*	*T_3*	*I^-*
1	2 h	Branchial cage	76.3	2.99	2.95	5.90			88.2
		Notochord	13.4	0.57	0.48	2.14			97.0
		Carcass	17.1	1.97	1.66	8.31			88.3
3	4 h	Branchial cage	4.5	6.9	10.8	11.4	Traces		70.8
		Tail	2.14	7.0	2.3	5.6	Traces		85.0
4	1 h	Branchial cage	16.3	0.23	0.26	0.52			98.7
		Tail	3.4	1.35	0.65	1.21			96.7
	6 h	Branchial cage	25.6	0.47	1.17	1.38	Traces		97.0
		Notochord	6.83	0.60		0.85			98.3
		Tail	3.13	1.40	0.99	1.66	Traces		
	24 h	Branchial cage	37.6	1.26	2.95	4.52	Traces		88.0
		Notochord	8.57	1.87	0.94	7.10	Traces		90.3
		Tail	6.96	4.15	0.11	7.65	Traces		87.4
6	2 days	Branchial cage	163	10.0	13.5	27.0	2.92	2.68	43.6
		Carcass	51.0	7.50	8.56	15.1	3.38	4.54	61.0
	4 days	Branchial cage	90.1	6.25	9.79	7.31	2.84	2.82	70.9
		Carcass	61.5	6.06	7.54	11.7	1.97	3.43	69.3
	6 days	Branchial cage	443	7.39	9.30	6.86	3.25	1.93	71.4
		Carcass	110	7.58	8.01	7.20	4.47	7.79	64.5
	8 days	Branchial cage	175	8.46	11.7	11.4	4.52	7.55	56.2
		Carcass	73.1	8.47	8.00	10.6	5.50	14.5	52.9
	11 days	Branchial cage	121	10.8	9.58	16.6	2.77	6.29	54.6
		Carcass	56.8	7.48	9.59	19.9	2.16	6.14	54.7

pounds in the endostyle-containing tissue was not greatly different from that in other tissues. As shown in Fig. 1 and Table II, inorganic iodide ^{131}I and origin (protein-bound) ^{131}I were the main labeled products observed in all unhydrolyzed tissues. Also noted in the unhydrolyzed specimens were definite bands for MIT, DIT, T_4, and T_3 in nonpeptide-bound form. Chromatograms of tissue hydrolysates showed decreased ^{131}I in the origin components, increased ^{131}I in the iodotyrosine bands, but little change in $[^{131}I]T_4$ and $[^{131}I]T_3$.

DISCUSSION

The present findings indicate that the formation of thyroid hormone can occur in amphioxus. However, as in the case of formation of iodotyrosine by gorgonid corals, no conclusion can be drawn as to the physiological significance of this mode of utilizing iodide. The slow rate at which T_4 and T_3 appeared, the small proportions of ^{131}I incorporated into them, and the large proportions of inorganic iodide ^{131}I present in all tissue specimens suggest a very low rate of iodine utilization. However, our radioactivity measurements may not reflect the actual amounts of stable iodine utilized, since specific activity measurements were not made. If we take into account the fairly high iodine content of sea water in which the animals were kept, it is possible that production of T_4 and T_3 might have been quite appreciable in magnitude.

Although the endostyle-containing portions of tissue consistently showed higher ^{131}I concentrations than did other tissues, there is no evidence that the capacity for producing iodoprotein and thyroid hormone is localized exclusively in this tissue. Indeed, the fact that iodoprotein was found in all tissues studied suggests that—unless the iodoprotein formed in the endostyle penetrates readily into other tissues—the ability to iodinate protein is widespread among tissues of amphioxus.

Another unusual finding was the high proportion of [^{131}I]T_3 formed. The formation of [^{131}I]T_3 in greater abundance than [^{131}I]T_4 is the reverse of the situation observed in higher animal forms. For example, values for the ratio [^{131}I]T_4/[^{131}I]T_3 ranged from 0.4 to 1.7 in amphioxus (Table III), whereas this ratio was recently reported to be about 5 in hydrolysates of rat thyroid tissue[11]. The significance of this tendency toward T_3 formation in amphioxus remains to be clarified in future studies.

The demonstration of thyroid hormone synthesis in the protochordate, amphioxus, lends some support to the view that the ability of tissues to synthesize the thyroid hormone preceded the appearance of the thyroid gland in the evolutionary process[12]. It would be of interest now to study the effect of thyroid hormone administration on this organism in order to determine whether an endocrine function for this hormone might also arise before the occurrence of a discrete thyroid gland.

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