cortisone was accentuated by the addition of the cobalt atom to the C-20 carbonyl function (11). The observation of Mancini *et al.* (12) that cortisone administration significantly delays the onset of carcinogenesis in response to the MCD_{50} of methylcholanthrene in mice, may further emphasize the importance of anti-inflammatory reactions in retarding the cancerization process.

SUMMARY

The biweekly intraperitoneal administration of cobaltous chloride in doses of 10 and 25 mg./Kg. in mice subjected to the MCD_{50} of methylcholanthrene resulted in a per cent tumor inhibition of 49 and 77, respectively. Cobaltous chloride, at a dose of 50 mg./Kg., employed over a 6-week period during Trial *1* (total dose 600 mg.), eventually killed 46 of the 60 animals. Toxic symptoms were observed in all groups in Trials *1* and *2*, except for the 10-mg./Kg. group and the controls.

A repetition of the experiment with cobaltous chloride, at dosage levels of 25, 30, and 40 mg./Kg., resulted in percentage tumor inhibitions of 42, 58, and 71, respectively.

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Thyroxinlike Activity of Several Thyroxin Analogs

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Five analogs of thyroxin were tested for thyroxinlike activity on the basis of the *Rana catesbiana* tail fin assay procedure. Two of them, 3,5-diiodo-4-(3′,5′-diiodo-4-hydroxyphenoxy)-phenylpropionic acid and 3,5-diiodo-4-(3′,5′-diiodo-4-hydroxyphenoxy)-phenylbutyric acid, were by this test many times more potent than L-thyroxin; one of them, 3,5-diiodo-4-(3′,5′-dimethyl-4-hydroxyphenyl)-phenylalanine, was slightly more potent than L-thyroxin.

IN AN EARLIER publication Bruice *et al.* (1) reported upon the thyroxinlike activity of a number of analogs of thyroxin when tested for their ability to influence the rate of metamorphosis of the bullfrog *Rana catesbiana*. This paper describes similar studies on additional analogs.

EXPERIMENTAL

The assay method employed was based upon the same principle as that of the earlier investigators, involving the measurement of the rate of tail resorption of the larva of *R. catesbiana*. The validity of the method, with ample literature citation, is discussed in detail in their paper, and will not be repeated here. However, for reasons to be outlined, some deviation from their protocol was introduced.

Assay Method.—Solutions of L-thyroxin and of the compounds to be tested were prepared, and the pH was adjusted to 8.0 to 8.5 by the addition of potassium carbonate and hydrochloric acid. Groups of tadpoles, whose tail widths ranged from 1.2 to 2.0 cm. at the widest point, were placed in tap water, fasted, and observed for 48 hr., at the end of which time tadpoles not completely healthy were discarded. Those selected for testing procedures were placed in individual shallow, transparent flat-bottomed vessels, each containing 200 ml. of the appropriate solution. Eight animals were used at each level of dosage, and a control group of eight others was employed in each determination. If, in any test series, a majority of animals failed to survive, the test was repeated to yield data from a total of at least eight animals. The animals were placed in a large air thermostat at a constant 30° and maintained in their respective solutions for 6 days. Zero time in the measurements was taken as the time at which the tadpoles were placed in test solution. Width of the tail fin at its widest point was measured every 24 hr. with a celluloid ruler held vertically at the side of the resting animal.

Treatment of Data.—Because the decrease in tail fin width of all control animals was reasonably constant, these were combined. For a total of 27 such animals, the average decrease after 6 days was 5.5 ± 0.94 mm. The average decrease for each group of test animals at each dosage level after 6 days' exposure was computed. The value for the control groups was subtracted in each case, yielding a corrected decrease in tail fin width that can be attributed to the influence of the test substance.

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TABLE I.—ACTIVITY OF THYROXIN ANALOGS DETERMINED BY *R. catesbiana* TAIL FIN ASSAY

Compd.	Dosage, mcg./ml.	% Decrease	% Survival	Activity[a]
L-Thyroxin	1.0	20.97 ± 2.39	75.0	100
	2.0	41.69 ± 1.97	75.0	99.5
	5.0	50.33 ± 3.32	87.5	48.2
3,5-Diiodo-4-(3′5′-dimethyl-4-hydroxyphenyl)-phenylalanine	0.5	22.63 ± 4.71	50.0	149.7
	1.0	44.36 ± 4.10	75.0	146.7
	3.0	51.19 ± 3.69	100.0	56.4
	5.0	50.72 ± 4.59	50.0	33.5
3,5-Diiodo-4-(3′5′-diiodo-4-hydroxyphenoxy-)-phenyl propionic acid	0.0015	0.02 ± 1.30	62.5	62.5
	0.01	4.49 ± 1.45	87.5	2108.5
	0.02	10.88 ± 4.02	62.5	2554.6
	0.03	13.58 ± 2.98	75.0	2125.7
3,5-Diiodo-4-(3′5′-diiodo-4-hydroxyphenoxy)-phenyl butyric acid	0.02	24.72 ± 3.58	87.5	5917.1
	0.04	28.79 ± 3.89	75.0	3445.7
	0.06	32.57 ± 3.57	87.5	2598.7
3,5-Dimethyl-4-(3′5′-dimethyl-4-hydroxyphenoxy)-aniline	0.1	3.59 ± 2.80	87.5	56.8
3,5,3′5′-Tetranitrothyronine	0.1	7.65 ± 1.41	25.0	212.9

[a] Activity is per cent activity expressed relative to 1.0 mcg./ml. L-thyroxin as standard = 100% on an equimolar basis.

These, in turn, are expressed in Table I as percentage decreases.

To establish relative activity relationships, the activity of L-thyroxin at a concentration of 1.0 mcg./ml. was taken to be 100%. All others were converted to an equimolar basis and multiplied by 100 to show the per cent thyroxinlike activity.

DISCUSSION

The procedure reported here differed from that of Bruice *et al.* (1) in the following respects and for the following reasons. A temperature of 30° was maintained throughout in an attempt to eliminate temperature changes as a possible modifying variable. The animals remained in test substance for the complete test period rather than being removed after 48 hr. to test the reported linearity of the relationship between time and effect and to yield some indication of the possible toxicity of the compounds. Also, evaluations were based only on those measurements of animals surviving for the full 6 days. Even though measurements were made every 24 hr., it is possible that these might be invalid due to the influence of whatever conditions were responsible for the failure to survive. Finally, comparisons of potency were made against L-thyroxin, while those of the quoted investigators were against DL-thyroxin; the former having twice the potency of the latter on an equal weight basis.

It is indeed correct that response rate is linear, following exposure to any one dosage of each compound. However, this relationship is in reality a time–response comparison. As shown by the activity column in Table I, when different dosage levels are reduced to equimolar status, the dose–effect relationship is not linear but becomes actually the more typical hyperbolic curve. In other words, higher drug concentrations do not show proportionately greater effects.

3,5-Diiodo-4-(3′5′-dimethyl-4-hydroxyphenyl)-phenylalanine, in concentrations of 0.5 and 1.0 mcg./ml., exhibited a potency almost identical to that reported by Bruice *et al.* (1) for comparable equimolar concentrations of 3,5-diiodo-4-(3′,5′-dimethyl-4-hydroxyphenoxy)-phenylalanine, an indication that the ether linkage in the latter is not significant.

Bruice *et al.* found 3,5,3′5′-tetraiodo-desaminothyronine (the propionic acid analog) to be 65 times as active as L-thyroxin. Data in this report would indicate the ratio to be more on the order of 25 times as active. It has been reported that the method used has an accuracy of ± 20% for any single series (2). It is possible that the degree of accuracy is something less.

Rather surprising activity is shown by the butyric acid analog, particularly if one compares it to the propionic acid derivative. The latter bears closer chemical resemblance to thyroxin, yet the former is considerably more active.

3,5-Dimethyl-4-(3′5′-dimethyl-4-hydroxyphenoxy)-aniline appeared in these tests to have greater activity than that reported by the earlier investigators (1), but its level of activity is still not particularly great.

Finally, Bruice and co-workers found tetranitrothyronine to possess activity as long as contact was maintained with the animals. Lipman and Dutoit (3) found the compound to have slight activity in thyroidectomized rats. In the investigation reported here, the compound had reasonable activity, but its toxicity, indicated by a 25% survival rate, is too great to warrant biological significance.

Under normal circumstances, the death rate of *R. catesbiana* larvae during metamorphosis varies up to about 25% (4). With this in mind, survival rates shown in Table I can give only an approximation of the toxicities of the various compounds.

CONCLUSIONS

Several thyroxin analogs were tested for their ability to influence tadpole metamorphosis. Three of these, 3,5-diiodo-4-(3′,5′-diiodo-4-hydroxyphenoxy)-phenylpropionic acid, 3,5-diiodo-4-(3′5′-diiodo-4-hydroxyphenoxy)-phenylbutyric acid, and 3,5-diiodo-4-(3′5′-dimethyl-4-hydroxyphenyl)-phenylalanine, showed appreciable activity consistent with a low order of toxicity.

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