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# Antithyroxine Properties of *N*-(2,5 Dihydroxyphenyl) Pyridinium Acetate.* (19232)

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(Introduced by Joseph L. Lilienthal, Jr.)

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With the increasing evidence that the circulating thyroid hormone is free thyroxine(1-3) an added importance is afforded recent studies (4-6) directed at determining agents which may possibly inhibit the action of thyroxine. Such research may elucidate the little understood mechanisms whereby the thyroid hormone acts to stimulate the metabolism of body cells as well as to regulate thyrotropin production by the anterior hypophysis. As Harington(7) has indicated, structural analogues of thyroxine possessing properties metabolically competitive to thyroxine are of physiological interest and potential clinical application. The observations by Woolley(4) that several structural analogues of thyroxine inhibit thyroxine-induced metamorphosis of tadpoles have been confirmed and extended by Williams *et al.*(8), by Frieden and Winzler (9.10). and in our laboratory(11). However, several investigators(8,11-13) have been unable to demonstrate that these analogues inhibit to any noteworthy degree the effect of thyroxine when tested in animals of higher forms.

In 1948, while the tadpole metamorphosis test was being used as a screening method to determine the antithyroxine potentialities of a large number of compounds, it was found in this laboratory that *n*-(2,5-dihydroxyphenyl) pyridinium acetate† (DHPPA) appeared to have such properties. Subsequently, the effects of this compound on the oxygen consumption of normal rats and of normal rats receiving thyroxine were observed. These studies comprise this report.

*Methods and results.* A. *Metamorphosis of tadpoles. Xenopus laevis* tadpoles, bred and raised according to the method of Deansley and Parkes(14), were kept in "livered" tap water‡ and fed a mixture of dried powdered liver and flour until they reached 18-26 mm in length. The tadpoles were transferred to "livered" water in beakers of 250 ml capacity, 5 tadpoles to each beaker; graded doses of thyroxine and/or DHPPA were added, and the final volume of each beaker was brought up to 200 ml. All beakers were placed in a water bath at room temperature. At the end of the third day the tadpoles in each beaker, after a brief rinsing to remove any residual drug, were transferred into clean beakers, each containing 200 ml of "livered" water. The beakers were returned to the water bath until the seventh day when the test was terminated. The animals received no food during the experimental period. Each tadpole was observed daily for evidence of metamorphosis.

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‡ "Livered" tap water was prepared by adding 100 mg dried powdered liver to each gallon of tap water and then filtering after 24 hours. This procedure removes excess chlorine.

TABLE I. Effect of *n*-(2,5-dihydroxyphenyl) Pyridinium Acetate (DHPPA), With and Without *dl*-thyroxine, on the Metamorphosis of *Xenopus Laevis* Tadpoles.

| | Thyroxine* | DHPPA* | No. of tadpoles | Tadpoles positive on day: 4 No. | 4 % | 5 No. | 5 % | 6 No. | 6 % | 7 No. | 7 % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A. | .05 | — | 14 | 2 | 14 | 12 | 86 | 14 | 100 | 14 | 100 |
| | | .5 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 5 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | .05 | .5 | 9 | 0 | 0 | 6 | 66 | 8 | 89 | 9 | 100 |
| | .05 | 5 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 55 |
| B. | .05 | — | 14 | 11 | 79 | 12 | 86 | 14 | 100 | 14 | 100 |
| | | 5 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 20 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | .05 | 5 | 14 | 2 | 14 | 7 | 50 | 9 | 64 | 10 | 71 |
| | .05 | 20 | 15 | 0 | 0 | 3 | 20 | 4 | 27 | 5 | 33 |

In A tadpoles varied from 20 to 26 mm in body length; temp. averaged 22.2°C (range 20-23.5), and pH of bathing medium at termination of experiment varied from 5.5 to 5.7. In B tadpole length varied from 18 to 22 mm; temp. averaged 24°C (range 23-24.6), and pH of medium at termination of experiment varied from 6 to 6.5. pH was determined with nitrazine paper. Twenty control tadpoles receiving no drugs during each experiment did not show metamorphosis.

* Dosage of thyroxine and DHPPA is expressed in mg per 100 ml environmental water.

A response was considered as "positive for metamorphosis" when one or both forelimb buds erupted through the skin. In preliminary experiments 0.05 mg % *dl*-thyroxine in the tadpoles' environmental water for 3 days uniformly produced metamorphosis of all tadpoles within 7 days, and was not lethal. Since the rate of metamorphosis was affected by environmental temperature, control groups receiving only thyroxine were run with each experiment. Animals receiving no drugs never showed metamorphosis during the experimental periods. The tadpole offspring from separate matings of different *Xenopus* frogs were used in 2 successive experiments. Results are shown in Table I. An antagonism to thyroxine apparently proportional to the concentration of DHPPA was noted. DHPPA appeared to be non-toxic to tadpoles in the concentrations used. In a later experiment involving the use of 40 tadpoles it was found that concentrations of DHPPA, 35.0 or 50.0 mg %, with or without *dl*-thyroxine, 0.05 mg %, were lethal to the tadpoles within 48 hours.

B. *Oxygen consumption of rats.* Eight male Sprague-Dawley rats, weighing about 100 g each, were maintained on Purina dog chow and water. Over a period of 34 days the oxygen consumption of each animal was determined at intervals of 4 to 8 days; this served to condition the animals to the apparatus. The animals were fasted 16 to 24 hours prior to testing. The oxygen consumption of each animal was determined in a closed circuit volumeter at constant temperature and pressure. The original apparatus described by Lilienthal, Zierler and Folk(15) was used. When the 8 rats attained a weight of about 250 g each, they were divided into 4 groups of 2 animals each, and the experiment recorded in Table II A was conducted. The values of oxygen consumption represent the average of the 2 animals in each group. A progressive decrease of basal metabolism of growing rats is well recognized(16), and was noted in these 8 rats in the preliminary period. The rather marked reduction in oxygen consumption seen in Table II A was probably due to the fact that these animals were still in the growth phase. The marked decrease of oxygen consumption by the control animals 4 days after injection may also have been due to the fact that on the day prior to this determination the weather was exceptionally hot and humid. The results show that DHPPA did not significantly alter oxygen consumption nor prevent the rise induced by thyroxine.

To determine if time and mode of administration of DHPPA were important, an additional experiment was conducted as recorded in Table II B. Animals of Groups II, III, and IV were employed, using the final de-

TABLE II. Effect of *n*-(2,5 Dihydroxyphenyl) Pyridinium Acetate (DHPPA), With and Without *dl*-thyroxine, on the Oxygen Consumption of Adult Rats.

| Group | | | Oxygen consumption, cc/min/100 g body wt | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before drug | | | After administration of drug | | | | | |
| | | | 15 days | 8 days | Avg | 1 day | % change* | 4 days | % change* | 13 days | % change* |
| A. | I | Saline—1 cc s.c.† | 1.84 | 1.78 | 1.81 | 1.74 | — 3.9 | 1.57 | —13.8 | — | — |
| | II | Thyroxine—2 mg/kg s.c. | 1.71 | 1.75 | 1.73 | 1.92 | +11 | 1.64 | — 5.2 | 1.55 | —10.4 |
| | III | DHPPA—200 " " | 1.67 | 1.63 | 1.65 | 1.61 | — 3 | 1.58 | — 4.9 | 1.45 | —12.1 |
| | IV | Thyroxine—2 " " DHPPA—200 " " | 1.62 | 1.58 | 1.60 | 1.80 | +12.4 | 1.52 | — 4.8 | 1.41 | —11.9 |
| | | | | | 7 days | 1 day | % change | 6 days | % change | | |
| B. | V | Thyroxine—2 mg/kg s.c. DHPPA—200 " " | | | 1.55 | 1.92 | +23.9 | 1.59 | +2.6 | | |
| | VI | DHPPA—200 mg/kg i.p.‡ (immediately before thyroxine) Thyroxine—2 mg/kg s.c. | | | 1.41 | 1.66 | +17.7 | 1.39 | —1.4 | | |
| | VII | DHPPA—200 mg/kg i.p. (24 hr before thyroxine) DHPPA—200 mg/kg i.p. (immediately before thyroxine) Thyroxine—2 mg/kg s.c. | | | 1.45 | 1.73 | +19.3 | 1.39 | —4.1 | | |

* Change in % as compared with avg reading before drug.
† s.c. = subcutaneously. ‡ i.p. = intraperitoneally.
In Groups IV and V injections were made at separate sites.

termination of oxygen consumption of the first experiment as the base-line oxygen consumption of the second experiment. Again, DHPPA did not prevent the increase in oxygen consumption induced by thyroxine. The differences in the magnitude of the increases are considered of no significance.

*Discussion.* These experiments indicate that DHPPA inhibits thyroxine-induced metamorphosis of tadpoles but fails to prevent an increase in oxygen consumption of rats given thyroxine. However, certain major differences in the experiments are apparent. In the tadpole experiments, the thyroxine and DHPPA were mixed together in the animals' environmental water, whereas injections of these materials were made at separate sites in the rats. The possibility that DHPPA may alter thyroxine thereby diminishing or destroying its physiological activity must be considered. The compound may possibly alter the absorption of thyroxine by the tadpoles. Since DHPPA in the concentrations used did not appear toxic to the tadpoles, it does not seem likely that the failure of metamorphosis was due to the tadpoles' inability to respond to thyroxine. Finally, the discrepancy of results in the two experiments may have been due to inadequate dosage of DHPPA in the rats.

Barker *et al.*(12) have observed the effects of this compound on the oxygen consumption of rats. DHPPA, 9.5 mg/kg/day, for 6 days did not alter significantly the oxygen consumption of normal rats, and a sharp increase of oxygen consumption occurred following the injection of thyroxine. However, in rats receiving DHPPA, 95 mg/kg/day, there was, respectively, a 10.5 and 12.8% drop in oxygen consumption after 8 and 13 days of drug administration, but in these groups response to thyroxine was not tested. They also observed that in thyroidectomized rats receiving thyroxine, 12 μg/kg/day, no depression of oxygen consumption was produced when DHPPA was given at 100 and 500 times the molar concentration of thyroxine for 15 and 28 days, respectively. While these investigators did not demonstrate that DHPPA inhibited the action of administered thyroxine, they did observe that large doses of DHPPA reduced the oxygen consumption of normal rats. In these latter experiments the total doses of DHPPA were approximately 4 to 6 times greater than those used in our experiments.

*Conclusions.* (1) *N*-(2,5-dihydroxyphenyl)

pyridinium acetate inhibited the metamorphosis of tadpoles induced by thyroxine. The degree of inhibition of metamorphosis appeared proportional to the relative concentration of the drug. (2) *N*-(2,5-dihydroxyphenyl) pyridinium acetate, 200 mg/kg body weight, did not lower the rate of oxygen consumption of normal rats nor prevent the acceleration of oxygen consumption induced by *dl*-thyroxine, 2 mg/kg body weight.

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## The Potentiating Effect of Alcohol on Thiopental Induced Sleep.* (19233)

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Studies of the action of thiopental on rats (1) show that alcohol tolerant animals are much more resistant to thiopental than are normal rats. The induction time of alcohol anesthesia in mice is significantly reduced when the animals are premedicated with a barbiturate(2). Observations of a different nature show that the sleeping time of thiopental treated animals is greatly prolonged by premedication with Antabus(3). However no evidence is available concerning the effect of alcohol on thiopental sleeping time. The present report is concerned with the effect of alcohol in subanesthetic doses on the duration of sleep produced by thiopental. Attempts were made to determine an analgesic effect of alcohol and the influence of thiopental on alcohol analgesia.

*Methods and materials.* Initially an attempt was made to determine the effect of thiopental, alcohol and combined thiopental-alcohol administrations on the response of mice to a painful stimulus. The apparatus described by Chen and Beckman(4) was used for determining analgesic effect. This consisted of a metal chamber which was open to the atmosphere only through a reflux condensor. The chamber was partly filled with an azeotropic mixture of ethyl formate and acetone which has a boiling point of 55°. The chamber was heated with a hot plate so that the azeotropic mixture boiled continuously. The mice were dropped on the top of this container 2 minutes after intravenous injection of the drugs. The time required for the animal to respond by licking its hind foot was determined with a stop watch and was recorded as the reaction time.

Observations of the duration of sleep produced by the intravenous administration of thiopental, alcohol, and combined thiopental-alcohol were made. The thiopental dosage

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