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Unresponsiveness of the Adult Toad to Thyroxine Administration.* (31081)

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An *in vitro* effect of thyroxine on vasopressin sensitive structures of the toad has been reported(1). We have confirmed the effect of thyroxine in water transfer and active sodium transport across the isolated toad bladder, and further demonstrated that the effects of thyroxine and vasopressin on water transfer but not on sodium transport, were synergistic (2).

Reports in the literature are contradictory as to the effects of thyroxine administration to the intact adult toad. Enzymes related to oxidative metabolic pathways, known to be altered in the thyroxinized mammal, have been reported to have normal activity in the adult toad after repeated injections of thyroxine(3).

In thyroxine treated mammals an increase in oxygen consumption has regularly been found in intact animals, tissue slices and tissue homogenates(3), whereas similar studies in the toad have been reported as showing either an increase in oxygen consumption(4, 5,6) or no demonstrable change(7). In the isolated bladder of normal toads Thornburn and Matty(8) have reported increased oxygen consumption after adding thyroxine *in vitro,* an observation which we have confirmed. Reports on the effect of thyroxine on water uptake and transport of electrolytes through the skin of the adult amphibian are also contradictory(9,10,11).

Our interest in the interrelation of thyroxine and vasopressin stimulated us to study the effects of thyroxine in *Bufo spinolosus,* the species in which our former studies were made.

In addition to studies on the action of thyroxine on vasopressin sensitive structures of toads given daily injections of thyroxine, determinations were made of the oxygen consumption by the isolated bladder and liver homogenates obtained from the same animals; and also of the cystathionase and glutamic pyruvic transaminase activity of these liver homogenates. The activity of these enzymes, unrelated to oxidative metabolism, has

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been reported to be decreased early in the thyroxine treatment in the rat(12).

Methods. Adult male toads, *Bufo spinolosus arencum,* weighing 30 to 80 g (average 55 g) were used to measure the water uptake by the method described by Sawyer(13). The toads were fasted 4 days previous to the experiments, and kept in water that was renewed daily. Tap water was used 2 days and distilled water the 2 days immediately preceding the experiment. On the day of experiment the bladder was emptied by abdominal pressure, and the cloaca was ligated. Under such experimental conditions we have found that water uptake is uniform in the first 6 hours.† Because of this finding, in all experiments in which the effect of an acute injection of hormone was studied, this injection was made after the first 3 control hours, and carried on for 3 more hours. In chronic experiments, thyroxine injections were given daily for 2, 4, 7 or 21 days previous to the experiments. In all experiments there was a control and an experimental group, the weight of toads in each group being similar. In water uptake experiments changes in weight were expressed in percentage change in initial weight.

All hormones were injected in the dorsal lymph sac through a long needle introduced through the skin of one of the hind limbs. Vasopressin (Pitressin, Parke Davis) was dissolved in redistilled water and given at a dose of 1 U/100 g body weight. Thyroxine was given as L-Thyroxine Sodium (L. Light & Co.) dissolved in phosphate buffer 0.1 M pH 8.5, and given at a dose of 0.8 mg/100 g body weight per injection. All control toads were injected with equal volume of the solvents used in preparation of the hormone solutions.

At the end of the balance experiment the spinal cord was pithed, the liver excised and homogenized in 0.25 M sucrose; the bladder was isolated and kept in Ringer's solution. In some experiments the isolated bladders were used to assay water permeability according to the method of Bentley(14). In others, oxygen consumption of the isolated bladder before and after addition of 100 mU of vasopressin to the bathing medium was measured. In the liver homogenates oxygen uptake was also measured, before and after adding sodium succinate by the conventional manometric technique of Warburg at 28°C.

In the liver homogenates the activity of the enzymes, glutamic-pyruvic transaminase and cystathionase, was also measured, according to the techniques used by Fernández and Horvath(12) and by Horvath(15).

In the statistical analysis of the results, we used a common values of S

$$S_c \text{ (standard error of the observations)} = \sqrt{\frac{(N_1-1)\,S_1^2 + (N_2-1)\,S_2^2}{N_1+N_2-2}}$$

Results.‡ *Water transfer by the isolated toad bladder.* In 8 controls a water transfer of 4.5 ± 3.0 μl/cm^2/hr was observed before adding vasopressin to the medium, and 181.7 ± 41.0 μl/cm^2/hr after addition of this hormone. Under the same conditions values obtained in the isolated bladders of 14 toads treated 4 days with thyroxine, were 5.0 ±

TABLE I.* Water Balance Response of Thyroxine Treated Toads.

				Water balance response†	
Group	Season	n	Days of treatment	Before vasopressin	After vasopressin
C‡	cold	30		7.8 ± 2.2	16.1 ± 4.3
T_4		34	4	8.5 ± 2.2	17.6 ± 4.1
C	cold	16		5.6 ± 1.4	12.3 ± 1.3
T_4		18	21	6.3 ± 1.1	11.6 ± 2.8
C	warm	8		11.2 ± 1.2	18.2 ± 6.1
T_4		10	21	13.4 ± 3.5	16.5 ± 3.8

* In this and following Tables, mean and standard deviation given.

† % Gain in weight.

‡ C = Control.

TABLE II. Oxygen Consumption of Toad Bladder, μl O_2/mg Dry Weight/Hr.

	n	Control	Vasopressin*
Control	10	1.36 ± .37	2.6 ± .70
T_4, 4 days	16	1.59 ± .68	2.94 ± .99
Control	7	1.69 ± .29	2.61 ± .60
T_4, 21 days	15	1.96 ±1.00	2.96 ± .44

* Vasopressin = 100 mU/ml.

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† In 14 normal toads the per cent gain in weight was 9.0 ± 3.1 in the first 3 hours and 9.1 ± 3.3 in the next 3 hours.

‡ Unless otherwise stated results reported were obtained during the cold season.

TABLE III. Oxygen Consumption by Toad Liver Homogenates, μl O_2/mg Dry Weight/Hr.

	n	Control	Succinate*
Normal	15	.72 ± .35	4.36 ± .86
T_4, 2 days	5	.59 ± .11	4.07 ± .93
T_4, 4 "	5	.79 ± .28	4.81 ± 1.08
T_4, 7 "	5	.59 ± .16	3.99 ± .63
T_4, 21 "	14	.81 ± .38	4.21 ± 1.05

* Sodium succinate, 15 μM/ml.

TABLE IV. Water Balance Response of the Toad After Acute Injection of Thyroxine.

Group	n	Water balance*	
		0-3 hr	3-6 hr
Control, no hormone	14	9.0 ± 3.1	9.1 ± 3.3
T_4 at 3 hr	17	10.1 ± 2.7	12.1 ± 2.4
Control, vasopressin at 3 hr	14	7.6 ± 3.1	16.3 ± 4.5
Vasopressin + T_4	15	7.5 ± 2.4	18.4 ± 4.3

T_4 = Thyroxine, 0.8 mg/100 g.
Vasopressin = 1 U/100 g.
* % Gain in weight.

3.7 μl/cm^2/hr and 189.5 ± 59.8 μl/cm^2/hr, respectively. Thus in these experiments no difference was shown between normal and thyroxine-treated toads.

Water uptake by the intact toad. Results summarized in Table I show no difference in uptake of water both in basal conditions and after administration of vasopressin in normal adult toad and in toads treated 4 or 21 days with thyroxine, regardless of the season in which the study was made.

Oxygen consumption by the isolated bladder. No significant difference in oxygen uptake of isolated bladders of normal or thyroxine-treated toads was observed (Table II).

Oxygen consumption by liver homogenates. Table III summarizes a series of experiments in which QO_2 by liver homogenates of toads treated 2 to 21 days with thyroxine was studied. No difference between normal and treated toads was obtained, either before or after the stimulating action of succinate.

Cystathionase and glutamic-pyruvic transaminase activity of liver homogenates. Although the mean cystathionase activity was lower in the liver homogenates of toads treated 21 days with thyroxine than in those of normal controls, this difference was not statistically significant: 44.6 ± 19.1 μM/g/hr in 10 controls and 31.9 ± 18.3 μM/g/hr in 15 thyroxine-treated toads ($p>0.10$).

The difference in glutamic-pyruvic transaminase activity, however, was highly significant, being 164.2 ± 45.6 μM/g/hr in the 10 control toads and 112.3 ± 27.1 μM/g/hr in the 15 thyroxine-treated toads ($p<0.001$).

Water uptake in the toad under acute effects of thyroxine and/or vasopressin. Table IV shows the results of these experiments. There is no difference in uptake of water during the first 3 or last 3 hours of the experiment in control toads. In the 3 hours that follow the injection of thyroxine the increase in water uptake is of doubtful significance ($p>0.025$). The difference in uptake after injection of vasopressin with and without the simultaneous injection of thyroxine is not significant ($p>0.20$).

Discussion. Although we have previously shown a change of water permeability in toad bladders treated *in vitro* with thyroxine, we have been unable to show a change in permeability after thyroxine is given *in vivo,* either in the isolated bladder or through the skin of the intact toad. Consistent with these findings are our observations that there is no difference in oxygen consumption of bladders isolated from normal or thyroxine-treated toads, while Thornburn and Matty(8) have reported, and we have confirmed their observations, that addition of thyroxine to the fluid bathing isolated toad bladders is followed by an increase in their oxygen consumption. From the results obtained in these experiments, it may be concluded that there is a difference between the effects of thyroxine *in vitro* on tissues of the toads and effects of comparable doses *in vivo.* Circulating levels of thyroxine in the toad have not been determined and the doses used in our experiments are based on the results obtained on toads *in vitro,* and *in vivo* in mammals. They represent a dose comparable to those needed to produce metamorphosis in tadpoles. It seems possible that the tissues are responsive but the hormone is disposed of in such a fashion in the intact animal that the concentration of thyroxine at the tissue effector sites never reaches effective levels.

If this were the case, thyroxine might also be ineffective in systems other than those associated with alterations of oxygen uptake or water transport. This question was explored by studying the effect of the hormone, given *in vivo,* on the activity of liver glutamic-pyruvic transaminase. In rats, treatment with thyroxine reduces the activity of this enzyme. Similar results were obtained in the toad.

The discrepancy between the unresponsiveness of the toad skin and bladder and the responsiveness of the liver enzyme system may reflect differences in the sensitivity of the two systems to minimal concentrations of the hormone. Alternatively the disposal of thyroxine —probably by the liver—may remove the hormone from the membrane sites of action so rapidly that its effect is negligible, while permitting the hormone to accumulate in significant quantities in the liver to reduce the level of transaminase. It is also possible that the hormone itself is not responsible for the changes in the hepatic enzyme levels, but that its metabolic product is the functioning substance. Finally, the difference may be a result of differences in the time-course of action of the hormone; however, the fact that no effect on water transfer and oxygen consumption could be found even when several intervals were studied makes this unlikely.

Summary. Thyroxine injected into intact toads *(B. spinulosus arencum)* failed to increase the water permeability and oxygen uptake of the skin and bladder, although when added *in vitro* it caused clear cut increases in these parameters. The hormone did, however, reduce the activity of the liver glutamic-pyruvic transaminase. This suggests that the failure of thyroxine, *in vivo,* to affect the metabolism and function of toad bladder or skin is not a result of end-organ unresponsiveness, but reflects some peculiarity of the metabolism or disposal of the hormone *in vivo.*

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Efficacy of N^6-(2-Hydroxyethyl)Adenine Against Coe Virus Infection In Mice. (31082)

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Coe virus, first isolated by Lennette and co-workers from patients with mild respiratory illness(1), was later identified as Coxsackie A-21 virus(2). Investigators in various parts of the world found the virus to be responsible for significant numbers of "colds," especially among military recruits. Efforts to find a suitable animal host for laboratory investigation led to successful adaptation of Coe virus to weanling mice, where infection produced muscle degeneration and flaccid paralysis(3). Coe-infected mice have subse-