2.2×10^{-6} M which agrees within a factor of 2 with the theoretical amount (1×10^{-6} M) present; this appears to be the practical limit of sensitivity of this kinetic analytical system. While the method is semiquantitative, it will detect the presence of many organic compounds in very dilute (10^{-8} to 10^{-6} M) solutions and establish concentrations within an order of magnitude of the true value.

CONCLUSIONS

Radical processes such as oxidizing sulfurous acid salt systems are sensitive to many compounds which act to inhibit or accelerate the chain reaction. Characteristically these compounds are effective in significantly modifying the rate of the oxidation even in extremely high dilutions (10^{-6} M). Rate determinations of uncatalyzed reactions can be made in a matter of minutes with the automated apparatus; rates observed in air oxidized systems can be increased by a factor of 5 by using pure oxygen (Henry's law).

Establishment of a reproducible sulfite-bisulfite system in a standard automated reactor such as described in this report offers a unique detecting system. A wide variety of compounds including acids, alcohols, glycols, polysaccharides, amines, aldehydes, alkaloids, ketones, indoles, phenols and quinones, and *possibly many other classes of organic compounds will measurably inhibit the rate of oxidation in very low concentration.* Likewise, heavy metal ions in parts per million can be detected by observing their catalytic effect on the rate of oxidation. Presence of these various compounds in water or air samples (after bubbling through water) can be readily detected. Semiquantitative relationships between concentration of inhibitor and oxidation rate constant may be established with an empirical equation of the form (Eq. 2).

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Role of Serotonin in the Thyroid Action of Reserpine

By B. B. WILLIAMS and S. T. COKER

The possible role of serotonin in the thyroid action of reserpine was studied in intact rats. Thyroid alteration by chronic reserpine medication and by chronic pharmacological treatment designed to alter serotonin levels and activity were compared with controls by three parameters. Direct effects of reserpine and serotonin were also investigated. Some evidence for reduced thyroid activity by chronic systemic reserpine administration was provided. It was found that reserpine directly *in vitro* slightly increased I^{131} uptake, and that serotonin in direct *in vitro* tests significantly reduced it.

REPORTS of alteration of thyroid function by reserpine have been numerous. Contradictory results, however, in different experimental subjects and in use of different parameters are unexplained, and much information on mechanism is yet to be provided.

Mayer, Kelly, and Morton (1) found that a concentration of 0.083 mg./ml. of reserpine reduced the uptake of I^{131} by calf thyroid slices. Their analysis of slices following incubation indicated that the inhibition of thyroid activity was predominantly one of interference with organic binding of iodine. Ershoff (2) reported that doses of desiccated thyroid and reserpine which were nonlethal when given separately to immature rats resulted in 100% mortality within 2 weeks when given concurrently. DeFelice, *et al.* (3), using oxygen consumption as a parameter of thyroid activity, showed that the administration of reserpine for 5 days to a hyperthyroid guinea pig would lower the oxygen consumption to normal. This group also found that the administration of reserpine to euthyroid animals would lower oxygen

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consumption. Bierwagon (4) reported that a combination of reserpine and thyroxin lowered dehydrogenase activity in thyroid tissue, but that thyrotropin in combination with reserpine prevented this dehydrogenase inhibition. Taylor (5) found that the elevation of cooling rate by reserpine in rats exposed to low temperatures was lowered by prior administration of thyroxin but not by thyrotropin. They believe this confirms that the antithyroid effect is due in part to decreased response of the gland to thyrotropin. Pittman, *et al.* (6), in clinical studies using the thyroid secretion rate as a parameter, found that reserpine reduced thyroid activity in four of nine euthyroid patients. Since this inhibition could not be demonstrated in patients maintained on exogenous thyrotropin, they suggested that reserpine altered thyroid function by diminishing thyrotropin production.

Premachandra (7) reported that no level of reserpine dosage was found (5–100 mcg./100 Gm.) which depressed thyroid function in fowl without simultaneously reducing food intake and thus inferred that thyroid inhibition at high levels of reserpine administration was due to inanition. Uchida (8) concluded from growth studies in chick embryos that reserpine had practically no antithyroid action on the embryonic thyroid.

Elkes (9) found that reserpine counteracted the decrease in thyroid I^{131} uptake induced by LSD (*d*-lysergic acid diethylamide). Results with a similar implication were reported by Kar and Boscott (10) who found that LSD depressed I^{131} uptake in rats *in vivo* and that this depressed uptake was reversed by reserpine. Vogel and Tervooren (11) reported on the basis of oxygen consumption that reserpine reinforced the increased metabolic activity induced by thyroxin in rats. Johnson (12) found that reserpine in doses of 1–4 mg./Kg. increased oxygen consumption in rats.

It is generally recognized that the effects of reserpine are not due to the direct effect of the drug alone but also to release of certain physiological amines. The purpose of this study was to determine the possible role of serotonin in the thyroid action of reserpine by comparing thyroid function alteration induced by reserpine with that observed in animals with pharmacologically altered serotonin levels and activity.

EXPERIMENTAL

Systemic Administration.—Four groups of from 16 to 25 rats each were subjected to the following dosage regimens: (*a*), reserpine, 150 mcg./Kg. daily by intramuscular injection for 28 days; (*b*), LSD, 25 mcg./Kg. daily by subcutaneous injection for 14 days, followed by 50 mcg./Kg. daily by subcutaneous injection for 14 days (dose increased to compensate for tolerance); (*c*), iproniazid, initial single dose of 60 mg./Kg. by subcutaneous injection, followed by 5-hydroxytryptophan (5HTP) 50 mg./Kg. daily for 7 days, followed by 25 mg./Kg. daily for 21 days by intraperitoneal injection (toxicity of original dose level necessitated dosage reduction); (*d*), control (no drug). Rats were healthy Sprague-Dawley males ranging in weight from 350–500 Gm. at the beginning of the experiments.

The following parameters were used to estimate alteration in thyroid function in treated animals and controls: (*a*), basal metabolic rate; (*b*), oxygen uptake of slices of liver and kidney tissue; (*c*), 1 and 2-hour I^{131} uptake by thyroid glands.

Basal metabolic rates (BMR) were calculated from oxygen consumption rate determinations carried out in a modified vacuum desiccator in a temperature-controlled room. Soda lime in the desiccator base and in wire gauze wrapped packs in the upper chamber was used for CO_2 absorption. A 10-ml. pipet attached to the desiccator evacuation outlet was used in the measurement of oxygen consumption. The animals were fasted for 12 hours, placed in the desiccator, and left for a 7-minute temperature equilibration period. Oxygen consumption rates were then determined by a stop watch measurement of the time required for a soap bubble to traverse the 10-ml. interval of the pipet. Means of such determinations were used in calculation of BMR values.

Iodine^{131} uptakes were determined by the method of Williams and Doniach (13). Rats were given 10 μc. of I^{131} by intraperitoneal injection and sacrificed by exsanguination under ether anesthesia at 1 or 2 hours following injection. Thyroids were immediately removed, transferred to planchets, and counted by 1 in. NaI (Th.) crystal scintillation detector. Activities were recorded as percentages of those counts given by a standard sample.

Uptake of oxygen by kidney and liver slices was determined by the direct Warburg manometric method. Tissues taken from animals sacrificed by exsanguination were placed in cold Krebs-Ringer phosphate solution with 0.2% glucose buffered to pH 7.4. Slices were immediately prepared by Stadie-Riggs microtome, blotted with filter paper, and weighed. The gas phase was air. Equilibration period was 10 minutes and readings were made every 15 minutes. Rate of oxygen uptake was determined on wet weight basis and expressed as μl. O_2/100 mg. of tissue per 2 hours.

***In Vitro* Administration.**—Drugs were added directly to flasks containing slices of rat thyroid in determination of direct effects on the gland as measured by I^{131} uptake during incubation. Thyroid donors were healthy Sprague-Dawley males, ranging in weight from 350–450 Gm. and differing in age by no more than 3 weeks. The animals were sacrificed by exsanguination under ether anesthesia. Thyroids were removed, sliced in cold Krebs-Ringer phosphate solution, and placed in 25-ml. flasks containing Krebs-Ringer phosphate to which had been added 0.25 μc. of I^{131} and the drug. Each flask contained the sliced pair of thyroids from a single donor. Incubations were carried out for 2 hours at 38° in a Dubnoff incubator. Six such experiments were carried out, one for each of the following: (*a*),

TABLE I.—BASAL METABOLIC RATE, THYROID I^{131} UPTAKE, AND O_2 UPTAKE BY KIDNEY AND LIVER SLICES FOLLOWING CHRONIC, SYSTEMIC DRUG ADMINISTRATION

Treatment	Dose and Route	BMR ± S.E.[a]	Thyroid I^{131} Uptake ± S.E.[a] (% of Standard) 1 hr.	2 hr.	O_2 Uptake in μl./100 mg. Tissue/2 hr. ± S.E.[b] Kidney	Liver
Reserpine	150 mcg./Kg. daily for 28 days, i.m.	36.14[c] ± 2.04 (12)	2.36 ± 0.133 (11)	4.31[c] ± 0.431 (11)	31.89 ± 1.68 (11)	15.13 ± 0.76 (12)
LSD	25 mcg./Kg. daily for 14 days followed by 50 mcg./Kg. daily for 14 days s.c.	59.01 ± 2.33 (16)	2.21 ± 0.117 (11)	3.76[c] ± 0.367 (12)	25.90 ± 1.02 (10)	15.50 ± 0.36 (13)
Iproniazid and 5HTP	Iproniazid, single dose 60 mg./Kg. s.c., followed by 5HTP, 50 mg./Kg. daily for 7 days, followed by 5HTP 25 mg./Kg. daily for 21 days, i.p.	55.51 ± 3.41 (9)	...	3.60[c] ± 0.487 (7)	23.93 ± 3.31 (5)	8.84[c] ± 0.72 (6)
Control		50.20 ± 2.10 (17)	3.34 ± 0.524 (14)	7.86 ± 1.23 (8)	26.47 ± 1.38 (11)	16.66 ± 0.29 (13)

[a] Numbers in parentheses indicate number of animals. [b] Numbers in parentheses indicate number of flasks. [c] Differs significantly from control at 95% probability.

reserpine, 0.5 mcg./ml.; (*b*), LSD, 1 mcg./L.; (*c*), serotonin, 0.5 mcg./ml.; (*d*), methimazole, 5 mcg./ml.; (*e*), thyrotropin, 0.6 m u./ml.; and (*f*), control (no drug). Concentrations were chosen which would approximate those presented to tissues on systemic administration within usual therapeutic or experimental dosage range. Eleven flasks were incubated in each experiment, ten containing thyroid slices and a standard containing no tissue. Iodine^{131} uptake for each treatment was determined by comparing activities of 0.1-ml. samples from thyroid-containing flasks with the activity of a 0.1-ml. sample taken from the standard flask. These activity differences were expressed as percentages of standard sample counts. Activities were determined by use of a scintillation detector as previously described.

RESULTS

Behavioral effects and other observable changes were recorded during the 28-day periods of systemic drug administration. Reserpine-treated animals began to present signs of decreased activity after 2 or 3 days of drug administration. Ptosis was observed in all reserpinized animals beginning during the first week and continuing through the course of the experiment. Animals receiving LSD did not demonstrate signs of extreme behavior alteration; however, there were occasional signs of erratic activity such as backing movements and purposeless activity usually occurring within 20–30 minutes after injection. Animals receiving 5HTP following iproniazid presented evidence of drug toxicity in lethargy, anorexia, and weight loss. Four animals in this group died during the first week of treatment. this lethality necessitated a reduction in dose. Signs of toxicity persisted, however, and at sacrifice, kidneys of all animals in this group were found to be reduced in size and altered in color and consistency. Microscopic examination of stained sections showed tubular damage. Because of loss of animals during the dosing period, I^{131} uptakes were limited to 2-hour determinations for this group.

Table I presents data for BMR, I^{131} uptake, and tissue slice oxygen uptake for the four groups of systemically treated rats. Table II presents data from measurement of direct effect of the several treatments on thyroid tissue slices.

TABLE II.—I^{131} UPTAKE BY THYROID SLICES ON INCUBATION WITH CERTAIN DRUGS

Drug	Concentration	Flasks, No.	Uptake %, ± S.E.
Reserpine	0.5 mcg./ml.	10	47.4[a] ± 2.1
Serotonin	0.5 mcg./ml.	10	9.4[a] ± 1.7
LSD	1 mcg./L.	10	12.0[a] ± 2.0
Methimazole	5 mcg./ml.	10	0[a]
Thyrotropin	0.6 m u./ml.	10	35.0 ± 3.3
Control	...	10	30.4 ± 6.6

[a] Differs significantly from control at 95% probability.

Two of the parameters, BMR and 2-hour I^{131} uptake, gave evidence of a decreased thyroid activity in response to chronic reserpine administration. In the direct *in vitro* tests, however, reserpine increased I^{131} uptake by thyroid slices. This increased uptake was found to be significant at 95% probability when compared with uptakes of both the control and 0.6 m u./ml. of thyrotropin.

Increasing serotonin level by chronic administration of 5HTP following an initial iproniazid injection (14, 15) failed to alter BMR and oxygen consumption of isolated kidney slices but did reduce significantly the uptake of I^{131} at 2 hours and reduced the consumption of oxygen by liver slices. Serotonin added directly to incubating thyroid slices also significantly reduced I^{131} uptake.

Rats given LSD for 28 days showed a reduced 2-hour thyroid I^{131} uptake, but BMR values and oxygen uptakes by kidney and liver slices were not significantly different from controls. The direct effect of LSD on thyroid slices was a decrease in uptake of I^{131}.

DISCUSSION

Some evidence of a reduced rat thyroid activity in response to chronic reserpine administration was provided by reduction in both BMR and 2-hour I^{131} uptake. However, failure of reserpine to reduce I^{131} uptake of incubating thyroid slices when added directly to the medium suggested that the effects of reserpine on the thyroid were not direct. Since reserpine releases serotonin from binding sites and is believed to effect many of its actions through this medium, the direct effect of serotonin on thyroid slice I^{131} uptake was measured. Results of this

experiment indicated a significant capacity on the part of serotonin to reduce thyroid activity. This suggests that the thyroid effects of chronically administered reserpine are accomplished through the intermediation of serotonin. If this were true it might be expected that LSD through serotonin antagonism (16, 17) would increase thyroid activity, and that an increase in serotonin blood level induced by monoamine oxidase inhibition and 5HTP would reduce thyroid activity. However, the data obtained in testing these assumptions were not conclusive or consistent. There are possible explanations for failure of these attempts at confirmation. That both LSD and serotonin decreased I^{131} uptake by thyroid on *in vitro* administration may be only further evidence for the lack of specific antagonism between the two. In evaluating the role of free serotonin maintained in excess as presumably was accomplished by use of the drug combination, the possibility that toxicity may have altered parameters must be considered.

The evidence for a thyroid inhibiting effect for LSD is consistent with results obtained by others (9, 10). The significance of this in relation to the reserpine mechanism is not apparent. The finding that reserpine *in vitro* significantly increased I^{131} uptake by thyroid slices was not in agreement with Mayer, *et al.* (1), who used calf thyroid and a considerably higher concentration of reserpine. This phase of the work will receive further attention as also will the lethality of the combined effects of iproniazid and 5HTP.

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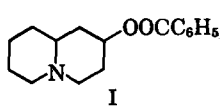
Esters of Bicyclic Aminoalcohols IV

Local Anesthetic Esters Derived from 2-Hydroxyquinolizidine

By CHANDULAL N. PATEL and TAITO O. SOINE

Several pairs of substituted benzoate esters derived from 2-hydroxyquinolizidine and 3-(2-methylpiperidino)propanol were synthesized in order to enable comparison of each pair for relative duration of local anesthetic activity in the rabbit cornea. The tests indicate, in general, the 2-hydroxyquinolizidine esters provided a greater duration of activity than the corresponding esters of 3-(2-methylpiperidino)-propanol. Some of the implications of this increase in duration of action with respect to the structures are discussed.

RHODES AND SOINE (1), in 1956, reported the synthesis of a series of 2-hydroxyquinolizidine esters specifically designed as anticholinergics. The selection of 2-hydroxyquinolizidine as the aminoalcohol was based on its formal relationship to tropine with respect to the relative positions of the amine and alcohol functions. No mention was made of local anesthetic activity although the relationship of 2-hydroxyquinolizidine to ecgonine would be analogous. In 1960, Counsell and Soine (2) described the preparation of selected esters of 1-, 2-, and 3-hydroxyquinolizidines and (among the activities reported) noted local anesthetic activity associated with most of their esters. In connection with the comparative local anesthetic activities, two observations were of particular significance with respect to the present report: *a*, the activity of 2-hydroxyquinolizidine benzoate (I) was 3 and 5 times greater, respectively, than the activities of the isomeric 1- and 3-hydroxyquinolizidine benzoates, and *b*, the activity of I was 1.72 times greater than that of piperocaine (II) which was used as the standard for compari-



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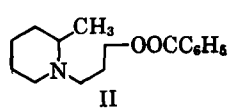

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