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Use of an Iodide-Specific Electrode to Study Lactoperoxidase-Catalyzed Iodination of *l*-Tyrosine

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Abstract □ An *in vitro* method employing an iodide-specific electrode for monitoring lactoperoxidase-catalyzed iodination is described. The method utilized lactoperoxidase, potassium iodide, and a glucose–glucose oxidase system for the generation of hydrogen peroxide and *l*-tyrosine. As iodination of *l*-tyrosine proceeded, the free iodide concentration in solution decreased and was monitored by an iodide-specific electrode. The iodide electrode was reliable when compared to a ^{131}I-method for measuring free iodide changes in solution. Increasing concentrations of resorcinol, a well-known inhibitor of thyroid peroxidase-catalyzed iodination, in the reaction mixture resulted in graded inhibition of the initial rate of lactoperoxidase-catalyzed *l*-tyrosine iodination. This *in vitro* system can be used to assess inhibitory activity of various antithyroid substances.

Keyphrases □ *l*-Tyrosine—iodination, lactoperoxidase catalysis, kinetics, *in vitro*, iodide-specific electrode □ Lactoperoxidase—*l*-tyrosine iodination, catalysis, kinetics, *in vitro* □ Resorcinol—inhibitory activity, kinetics, iodide-specific electrode □ Iodination—*l*-tyrosine, lactoperoxidase catalysis, kinetics, *in vitro*, iodide-specific electrode

Several recent investigations were conducted to study the kinetics of thyroid peroxidase-catalyzed iodination, the reaction responsible for thyroid hormone synthesis (1–4). Since thyroid peroxidase is not unique in its ability to catalyze iodination and thyroxine formation, *in vitro* iodination studies have employed a wide variety of peroxidases (5–10).

Numerous procedures have been utilized to study peroxidase-catalyzed iodination *in vitro* (7, 9, 11–15). The most widely used procedure involves isolation of the iodinated organic compounds and determination of the iodide incorporated into these products. Spectrophotometry is also used to monitor iodination *via* the changing spectral properties of the iodinated products (12, 13). A potentiometric procedure employing various substrates also has been used to follow the kinetics of iodination catalyzed by peroxidase (7, 9, 15).

The objective of the present study was to determine the optimal conditions for the use of an iodide-specific electrode for monitoring lactoperoxidase-catalyzed iodination of *l*-tyrosine. It was determined that lactoperoxidase is an acceptable peroxidase for investigating iodination kinetics (6, 10). Since several of the well-known antithyroid agents used clinically are general peroxidase inhibitors (6, 16–18), the present study also was intended to determine whether the iodide electrode could be used to study the inhibition kinetics of the antithyroid compound resorcinol.

EXPERIMENTAL

Procedure—An iodide-specific electrode[1], a pH expanded-scale millivolt meter[2], and a strip-chart recorder[3] were used to measure the free iodide concentration. As the peroxidase enzyme catalyzed the iodination of an appropriate iodide acceptor at a known initial iodide concentration, the iodide concentration decreased. The reduction in iodide concentration resulted in a potential change, which was related to the iodide-ion activity by the Nernst equation (19). To obtain the actual iodide concentration decrease per unit time (iodination rate), each millivolt change was converted to the corresponding iodide concentration by calibrating the iodide electrode in the concentration range of interest (1×10^{-5}–10×10^{-5} *M*), using appropriate dilutions of a 0.1 *M* sodium iodide standard solution[4].

Lactoperoxidase[5] was quantified by activity units; 1 unit of lactoper-

[1] Model 94-53A, Orion Research Inc., Cambridge, Mass.
[2] Model 12 research meter, Corning Scientific Instruments, Medfield, Mass.
[3] Beckman Instruments, Fullerton, Calif.
[4] Model 94-53-06, Orion Research Inc., Cambridge, Mass.
[5] Lot 430020 B grade, Calbiochem Chemical Works, Los Angeles, Calif.

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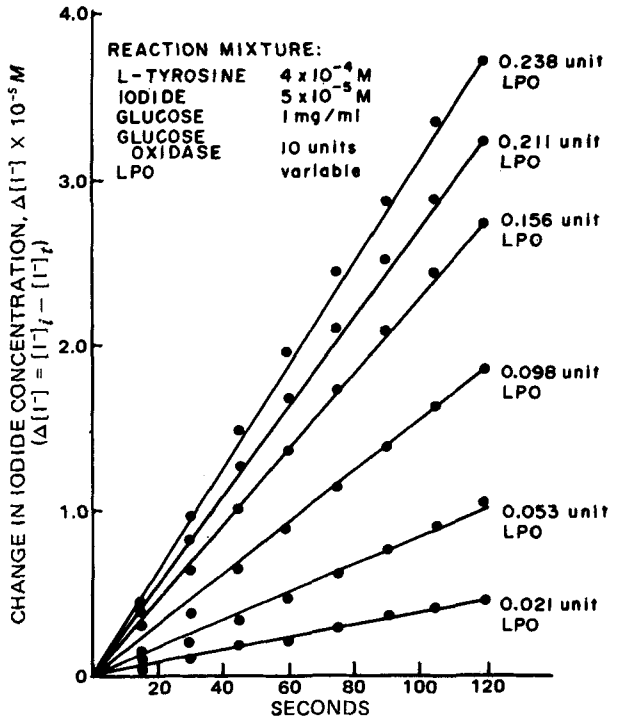

Figure 1—*Effect of varying the lactoperoxidase (LPO) concentration on the change in the initial iodide concentration with time.*

oxidase formed 1 mg of purpurogallin from pyrogallol in 20 sec at pH 6.0 and 20° (20). Hydrogen peroxide was generated in the reaction mixture by a glucose[6]-glucose oxidase[7] system in the presence of molecular oxygen and water. *l*-Tyrosine[8] was the iodide acceptor, while potassium iodide[9] served as the iodide donor.

A mixture of *l*-tyrosine, potassium iodide, and glucose was prepared (0.8:1:1), and 2.8 ml of this mixture (plus inhibitor, if any) was combined in a 50-ml reaction vessel with enough 0.06 *M* phosphate–edetic acid buffer, pH 6.0, to result in a final volume of 20 ml. The reaction vessel was transferred to a constant-temperature bath (37°), and the iodide electrode and single-junction reference electrode[10] were positioned in the reaction mixture. The reaction mixture was allowed to equilibrate for 6 min with constant stirring, and then the initial iodide concentration was measured. The strip-chart recorder was positioned at zero, and the chart speed was set at 2.54 cm/min. Glucose oxidase was added to the reaction mixture, and lactoperoxidase was added exactly 45 sec later. Iodination was allowed to proceed for 2 min.

The initial iodide concentration was calculated from the initial millivolt reading by the Nernst equation. The change in free iodide concentration, $\Delta[I_t]$, was calculated for each 15-sec interval up to 120 sec by calculating the iodide concentration for each cumulative time interval, $[I_t]$, and subtracting $[I_t]$ from $[I_i]$. The change in iodide concentration per unit time was linear over the first 120 sec. The initial iodination rate of *l*-tyrosine, $\Delta[I]$/sec, was determined by linear regression analysis, taking 15 sec as zero time (21).

Optimal Conditions—The effect of varying the lactoperoxidase amount on the iodination rate was investigated by conducting the assay with lactoperoxidase amounts ranging from 0.02 to 0.44 activity unit/reaction mixture. Ten determinations were made for each lactoperoxidase concentration.

The final *l*-tyrosine concentration (1×10^{-2} *M*, pH 10 stock solution) and the optimal pH for tyrosine iodination under these conditions were determined in the second group of studies. The reaction was conducted at pH 5.0, 6.0, 7.0, and 7.4. Four *l*-tyrosine concentrations, ranging from 2.5×10^{-4} to 1.0×10^{-3} *M*, were used in the assay, with all other components remaining constant. The assay was conducted for all four *l*-tyrosine concentrations at each pH. Six determinations of the iodination rate were made for each *l*-tyrosine concentration at each pH.

The effect of varying the initial iodide concentration was of interest in the third set of studies. The assay was conducted using initial iodide concentrations ranging from 1×10^{-5} to 1×10^{-3} *M* in the reaction mixture; six determinations were made for each initial iodide concentration used.

The fourth study investigated the effect of ionic strength on the iodination rate. Varying amounts of 2 *M* NaCl, 2.5×10^{-3}–2.0×10^{-1} *M* (giving rise to total ionic strengths of 0.097–0.297 *M*), were used to adjust the ionic strength of the reaction mixture.

Iodide Electrode Reliability—Iodine 131, 200 μl, was diluted with 5.0×10^{-5} *M* potassium iodide (1:282)[11]. At 30-sec intervals after initiation of the iodination reaction, 1-ml aliquots were removed and protein was precipitated with trichloroacetic acid. Millivolt readings were taken at the same time. The trichloroacetic acid-treated samples were applied to cation-exchange[12] columns (9 × 50 mm) and washed with deionized, distilled water. Both the eluates and the resin (bound ^{131}I-*l*-tyrosine) were counted in a counter with 4-π geometry (along with ^{131}I-standards). The percent of free iodide was calculated from both the ^{131}I-samples and the millivolt readings obtained from the iodide electrode.

Response of Iodination Reaction to Resorcinol—With a stock concentration of 2×10^{-5} *M* resorcinol[13], the effect of increasing resorcinol concentrations, ranging from 0 to 17.0×10^{-7} *M*, on the initial iodination rate was determined. Six determinations of the initial iodination rate for each resorcinol concentration were conducted.

RESULTS

Procedure—Calibration of the iodide-specific electrode with standard iodide solutions demonstrated that the iodide electrode response was

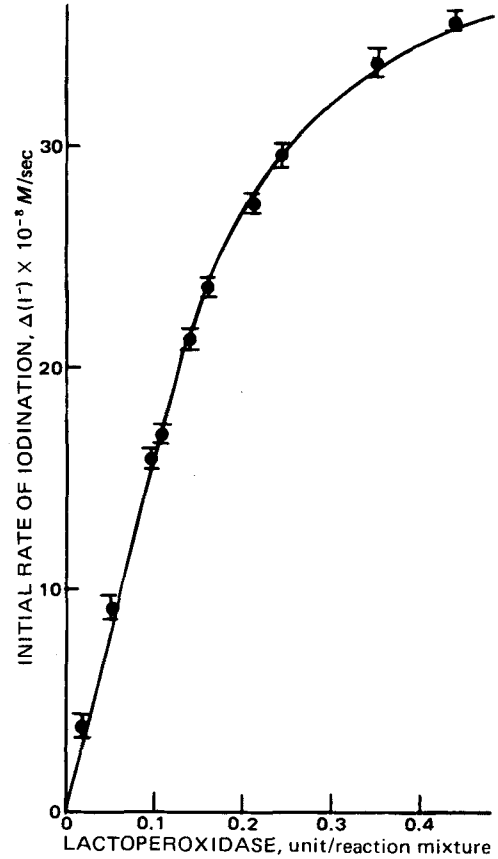

Figure 2—*Effect of varying the lactoperoxidase concentration on the initial iodination rate of* l*-tyrosine.*

[6] Dextrose, Mallinckrodt, St. Louis, Mo.
[7] Lot 501359 A grade, Calbiochem Chemical Works, Los Angeles, Calif.
[8] Lot 64619 A grade, Calbiochem Chemical Works, Los Angeles, Calif.
[9] Fisher Scientific Co., Fair Lawn, N.J.
[10] Model 90-01, Orion Research Inc., Cambridge, Mass.
[11] Na^{131}I (10 μCi/ml), New England Nuclear, Boston, Mass.
[12] Dowex 50X40, Dow Chemical Co., Midland, Mich.
[13] Sigma Chemical Co., St. Louis, Mo.

Table I—Effect of Varying Tyrosine Concentrations on the Iodination Rate [a] at Different pH Values [b]

pH	Tyrosine Concentration, M			
	2.5×10^{-4}	4.0×10^{-4}	5.0×10^{-4}	1.0×10^{-3}
5.0	17.35 ± 0.07[c]	17.41 ± 0.10	16.96 ± 0.10	16.68 ± 0.03
6.0	16.00 ± 0.04	16.14 ± 0.09	15.51 ± 0.02	15.05 ± 0.11
7.0	12.23 ± 0.02	11.53 ± 0.13	11.30 ± 0.09	10.28 ± 0.08
7.4	9.05 ± 0.03	8.60 ± 0.04	8.08 ± 0.04	7.12 ± 0.05

[a] Rate is expressed as $\Delta[I] \times 10^{-7}$ M/sec/lactoperoxidase unit. [b] Iodide concentration was 5×10^{-5} M. [c] Rate ± 1 SEM; $n = 6$.

linear with iodide concentration changes in the range defined by the equation, millivolts = 3.846 + 0.624 log [I^-] ($r = 1.00$). Thus, the iodide concentration could be determined for each millivolt change that occurred for any time, t, during the iodination of l-tyrosine.

Optimal Conditions—The result of varying the amount of lactoperoxidase from 0.021 to 0.238 activity unit in the reaction mixture on the iodide concentration change with time was such that as the amount of lactoperoxidase increased, the iodide concentration change increased. The iodide concentration change with time for each lactoperoxidase concentration was linear over the first 120 sec (Fig. 1). Increasing the lactoperoxidase from 0.021 to 0.156 unit in the reaction mixture resulted in a greater iodine concentration change with time than when lactoperoxidase was increased from 0.156 to 0.238 unit. Increasing the lactoperoxidase from 0.021 to 0.160 unit resulted in a greater change in the iodination rate relative to the change associated with increasing the lactoperoxidase from 0.160 to 0.440 unit (Fig. 2).

At pH 6.0, increasing the l-tyrosine concentration from 4.0×10^{-4} to 1.0×10^{-3} M resulted in an iodination rate decrease (Table I). This decrease also occurred at pH 5.0, 7.0, and 7.4. When the l-tyrosine concentration was 2.5×10^{-4} M, the iodination rate was either greater than, or not significantly different from, the rate observed at 4.0×10^{-4} M at all pH values tested. The iodination rate decreased as the pH increased at each l-tyrosine concentration.

Table II shows the effect of varying the iodide concentration on the l-tyrosine iodination rate at pH 6.0. From these data, it appeared that as the iodide concentration increased, the initial iodination rate of l-tyrosine increased as well.

The initial iodination rate was not affected appreciably by increasing the ionic strength from 0.097 to 0.197 M (Table III). When the ionic strength was increased to 0.297 M, however, the iodination rate decreased markedly.

Table IV—Comparison of the Iodide Electrode and the ^{131}I-Methods for Measuring Free Iodide Changes

Seconds	$I^- \times 10^{-5}$ M	Free Iodide, %	
		Iodide Electrode	^{131}I-Method
30	2.99	56.4	58.3
60	1.63	30.8	32.8
90	0.950	17.9	16.9
120	0.785	14.8	8.9

Iodide Electrode Reliability—A comparison of the iodide electrode with the ^{131}I-method for detecting changes in the iodide concentration as l-tyrosine iodination proceeded can be seen in Table IV. The sensitivity of the iodide electrode was comparable to that of the ^{131}I-method but was lower at a low iodide concentration (0.785×10^{-5} M).

Response of Iodination Reaction to Resorcinol—The iodide concentration change with time practically was linear over the first 120 sec for control (no inhibitor present) and in the presence of increasing resorcinol concentrations (Fig. 3). Therefore, the initial reaction rate could be calculated by applying linear regression analysis over the first 120 sec. By calculating the initial iodination rate in the presence of each resorcinol concentration, a log dose–inhibition curve was constructed (Fig. 4), in which the dose represented the resorcinol concentration and the response was the percent of the control rate (the initial iodination rate in the presence of resorcinol divided by the initial iodination rate in the absence of resorcinol).

DISCUSSION

The initial iodination rate of l-tyrosine changed in a biphasic manner with increasing lactoperoxidase concentrations (Fig. 2). The rate was dependent on the amount of lactoperoxidase present up to 0.16 activity unit. Between 0.16 and 0.44 unit, there was a slope change so that smaller increments in the rate occurred with large changes in lactoperoxidase concentration. Between 0.16 and 0.44 unit of lactoperoxidase, the generation of hydrogen peroxide and/or one substrate possibly became rate limiting as well. Use of 0.02–0.16 unit resulted in rates dependent on the lactoperoxidase amount present in the reaction mixture. Thus, all rates calculated in these studies were expressed in terms of the total number of lactoperoxidase units present so that the rate per lactoperoxidase unit was used for comparison, standardizing the rates obtained for all experiments.

Table II—Effect of Varying Iodide Concentrations on the Iodination [a] Rate [b]

Experiment	Iodide Concentration, M					
	10^{-5}	3.0×10^{-5}	5.0×10^{-5}	7.5×10^{-5}	10^{-4}	10^{-3}
1	6.24	14.10	16.25	17.24	19.13	53.88
2	6.23	14.10	16.43	18.29	19.11	45.39
3	6.28	14.09	16.12	17.56	18.90	57.17
4	6.27	14.11	16.12	17.72	19.10	47.77
5	6.27	14.22	16.20	17.60	18.95	59.75
6	6.25	13.85	16.12	18.28	18.88	52.88
$\bar{X}$[c]	6.26	14.08	16.20	17.78	19.01	52.79
S_x[d]	0.02	0.12	0.12	0.42	0.12	5.47
$S_{\bar{x}}$[e]	0.01	0.05	0.05	0.17	0.05	2.23

[a] Tyrosine concentration was 4×10^{-4} M. [b] Rate is expressed as $\Delta[I] \times 10^{-7}$ M/sec/lactoperoxidase unit. [c] Mean. [d] One standard deviation. [e] One standard error of the mean.

Table III—Effect of Ionic Strength on the Iodination [a] Rate [b]

Experiment	Total Ionic Strength, M					
	0.097	0.100	0.107	0.147	0.197	0.297
1	16.17	16.19	16.15	16.29	16.10	14.15
2	16.02	16.53	16.94	16.94	15.94	14.65
3	16.10	15.81	16.28	15.97	15.88	14.38
4	16.29	15.98	16.70	16.31	16.07	14.30
5	16.15	16.21	16.28	16.01	15.83	14.99
6	16.21	16.00	16.17	16.45	15.91	14.15
$\bar{X}$[c]	16.16	16.12	16.42	16.33	15.96	14.44
S_x[d]	0.09	0.25	0.33	0.35	0.11	0.33
$S_{\bar{x}}$[e]	0.04	0.10	0.13	0.14	0.05	0.13

[a] Tyrosine concentration was 4×10^{-4} M, and iodide concentration was 5×10^{-5} M. [b] Rate is expressed as $\Delta[I] \times 10^{-7}$ M/sec/lactoperoxidase unit. [c] Mean. [d] One standard deviation. [e] One standard error of the mean.

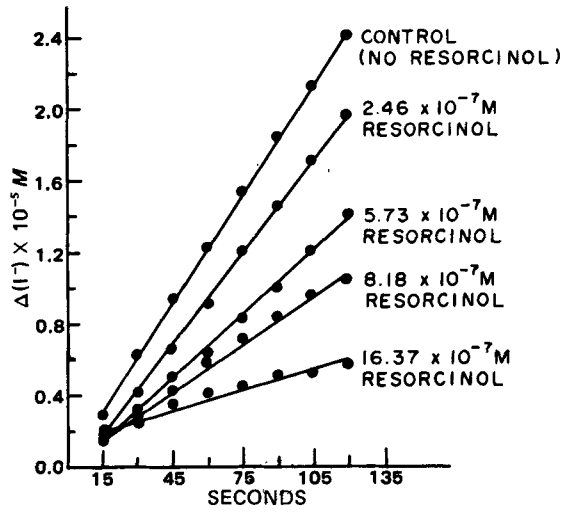

Figure 3—*Effect of increasing resorcinol concentration on the iodide concentration change with time.*

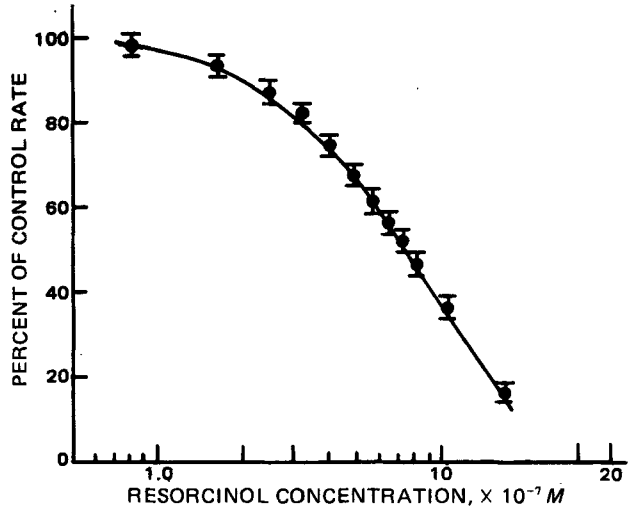

Figure 4—*Effect of graded resorcinol doses on the* l-*tyrosine iodination* (n = 6; *bars represent* ±SEM).

Although the iodination rates observed at pH 5.0, when the *l*-tyrosine concentration of the reaction mixture was varied, were greater than those observed at pH 6.0, the latter pH was chosen for this system since it was closer to physiological pH (Table I). A large reduction in the iodination rate was noted with all *l*-tyrosine concentrations at pH ≥7.0. The iodination rates obtained with *l*-tyrosine concentrations of 2.5×10^{-4} and 4.0×10^{-4} *M* at pH 6.0 were very similar; but as the *l*-tyrosine concentration was increased above 4.0×10^{-4} *M*, the rate decreased. This decrease was expected since adding enough of the alkaline stock *l*-tyrosine solution to the reaction mixture to produce 5.0×10^{-4} and 1.0×10^{-3} *M* resulted in a pH change of 0.2 and 0.5 unit, respectively. From these data, it was concluded that, at pH 6.0, an *l*-tyrosine concentration of 4.0×10^{-4} *M* should be used in subsequent studies since a satisfactory rate was achieved with only a minute pH change (0.05 unit).

When the iodide concentration was increased, the *l*-tyrosine iodination rate increased (Table II). A disproportionate rate increase was observed at very high iodide concentrations, and it was concluded that molecular iodine formation was occurring as well. This hypothesis was supported by previous observations that molecular iodine formation was favored at very high iodide concentrations (6, 22, 23). The iodide electrode, however, would not be able to distinguish between the two reactions since it was sensitive only to the total free iodide change. When the iodide concentration was increased from 3.0×10^{-5} to 1.0×10^{-4} *M*, the iodination rate increased. Any one of these concentrations appeared to be suitable for the assay. Therefore, the concentration used was 5.0×10^{-5} *M*.

The effect of the total ionic strength of the reaction mixture on the iodination system was investigated since ion activity is a function of ionic strength (19). Little change in the initial rate was demonstrated when the ionic strength was increased to 0.197 *M* (Table III). The ionic strength of the reaction mixture used for iodide electrode calibration was 0.097 *M*. The greatest contributor to this ionic strength was the phosphate buffer. Table V summarizes the reagents and their final concentrations to be used in subsequent studies in the *in vitro* model of thyroid peroxidase-catalyzed iodination.

Table V—Assay for Lactoperoxidase-Catalyzed Tyrosine Iodination at pH 6.0, 37°

Reagent	Stock Concentration	Reaction Mixture (20 ml) Volume, ml	Concentration
l-Tyrosine	10^{-2} *M*, pH 10	0.8	4×10^{-4} *M*
Potassium iodide	10^{-3} *M*	1.0	5×10^{-5} *M*
Glucose	20 mg/ml	1.0	1 mg/ml
Glucose oxidase	0.20 mg/ml (170 units/mg)	0.3	10 units total
Lactoperoxidase	0.20 mg/ml	0.05[a]	0.10–0.16 unit total
pH 6.0 buffer	0.06 *M* phosphate, 10^{-3} *M* edetic acid	To 20	No change

[a] Of a 1:4 dilution of stock.

The reliability of the iodide electrode for monitoring the free iodide concentration change as *l*-tyrosine iodination proceeded was comparable to that of a ^{131}I-method for monitoring iodination (Table IV). The iodide electrode accurately measured the free iodide change in the reaction, except that its reliability dropped slightly compared to the ^{131}I-method after a significant amount of *l*-tyrosine iodination had taken place. Since only the first 120 sec was of concern in these studies, this drop was not considered to be a serious problem.

The lactoperoxidase-catalyzed iodination of *l*-tyrosine at pH 6.0 appeared to be a good model for assessment of the inhibitory activity of resorcinol, a well-known inhibitor of thyroid peroxidase-catalyzed iodination *in vivo* and *in vitro* (6). Over a resorcinol concentration range of 0.8×10^{-7}–17.0×10^{-7} *M*, a sigmoidal log dose–inhibition curve was obtained (Fig. 4). The results were similar to those reported for a method for monitoring iodination without an iodide-specific electrode (6), in which the resorcinol concentration corresponding to 50% inhibition of the thyroid peroxidase-catalyzed iodination of bovine serum albumin was 7.10×10^{-7} *M*. In this study, the resorcinol concentration that produced 50% inhibition of lactoperoxidase-catalyzed iodination of *l*-tyrosine was 7.64×10^{-7} *M*.

It was concluded from these data that the iodide-specific electrode could be used to monitor the lactoperoxidase-catalyzed iodination of *l*-tyrosine. In addition, this potentiometric method is ideal for studying the kinetics of inhibition of lactoperoxidase-catalyzed iodination by known or suspected inhibitors of the thyroid peroxidase enzyme.

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Sustained Drug Delivery Systems II: Factors Affecting Release Rates from Poly(ϵ-caprolactone) and Related Biodegradable Polyesters

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Abstract □ The release rates of several steroids from films and capsules of homopolymers and copolymers of ϵ-caprolactone, DL-lactic acid, and glycolic acid were measured *in vitro* and *in vivo* for up to 200 days. Relatively constant release rates from capsules (reservoir devices) were observed only under certain conditions. Factors that influence the drug release kinetics were evaluated. Release from poly(ϵ-caprolactone) and poly(ϵ-caprolactone-co-DL-lactic acid) was diffusion controlled. Release from poly(DL-lactic acid-co-glycolic acid) was associated with polymer degradation. Release from poly(DL-lactic acid) was very slow when diffusion controlled.

Keyphrases □ Polyesters, biodegradable—poly(ϵ-caprolactone), poly(DL-lactic acid), copolymers, steroid release, films, capsules □ Drug delivery systems—kinetics, biodegradation, diffusion, films, capsules □ Poly(ϵ-caprolactone)—homopolymers, copolymers, release rates, films, capsules □ Sustained-release systems—biodegradable polyesters, poly(ϵ-caprolactone), films, capsules

Polymer systems for sustained subdermal drug delivery may be based on principles of drug diffusion and/or polymer degradation. The diffusion coefficients of steroids in poly(ϵ-caprolactone) and poly(ϵ-caprolactone-co-DL-lactic acid) are comparable to values reported for poly(dimethylsiloxane) and, *a priori*, these polymers may be used in diffusion-controlled delivery systems that biodegrade after drug exhaustion (1, 2). In contrast, diffusion in poly(DL-lactic acid) is much slower (2); consequently, diffusion, leaching, and biodegradation may contribute to the drug delivery rates reported in studies of this polymer (3–5).

This paper describes the release rates of several steroids from films and capsules of homopolymers and copolymers of ϵ-caprolactone, DL-lactic acid, and glycolic acid *in vitro* and *in vivo* and factors that determine the observed kinetics.

EXPERIMENTAL

Synthesis—Poly(ϵ-caprolactone), poly(DL-lactic acid), their copolymers, and poly(DL-lactic acid-co-glycolic acid) were prepared by bulk polymerization of the purified monomers at 130° *in vacuo* in the presence of stannous octoate (50–500 ppm). The polymers were purified by precipitation from methylene chloride with methanol, followed by rapid and thorough washing with water in a blender[1]. Copolymer composition was determined by NMR spectroscopy.

Films were prepared by casting a common solution of the steroid and polymer in methylene chloride onto a glass plate and spreading with an adjustable applicator[2]. When a thickness greater than 100 μm was required, thinner films were stacked and compression molded at 100–130° to ensure even drug distribution. Sandwiched films, *i.e.*, a drug–polymer layer completely encased by drug-free polymer, were prepared by casting a polymer–drug solution on a drug-free polymer film. The resulting double-cast film was cut to leave smaller squares, over which a drug-free film was cast. The triple film then was cut between the squares.

Polymer tubing was prepared by melt extrusion or, when limited material was available, by rolling polymer film around a short polytef tube and annealing *in vacuo* while mechanically rotating the tube about its axis. Capsules were prepared by heat sealing the tubing with warm pliers. Steroids were micronized[3] (<5 μm) and dispersed in a vehicle using a tissue grinder (10 min) prior to capsule filling.

Release Rate Measurement—Films or capsules were immersed totally in distilled water (80–400 ml) at 37°. Mixing was accomplished using a rotating shaker[4] maintained at 135 rpm. Increasing the shaker speed to 195 rpm did not change the release rate. The aqueous reservoir was changed daily (capsules) or more frequently (films); where release rates were very fast, a flow system was used to keep the aqueous drug concentration low. In most cases, the frequency of solution change was such that the drug concentration did not exceed 10% of its aqueous solubility and typically was <1%. Drugs were either tritium or carbon 14 labeled, and release rates were determined by radioassay.

In vivo release rates were determined by radioassay of the feces and/or urine after subdermal implantation of the films or capsules in female New Zealand White rabbits or Charles River rats *via* incisions about the dorsal midline.

THEORETICAL

The diffusion-controlled release of a drug from a monolithic film or slab of unit area into an infinite aqueous sink may be described by Eqs. 1 and 2. Equation 1 applies when the drug is dissolved completely in the polymer (6); Eq. 2 applies when the drug solubility is exceeded and the

[1] Waring.
[2] Boston-Bradley.
[3] Wig-L-Bug, Crescent Dental Manufacturing.
[4] Eberbach.

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