Table I—Temperature-Dependent Solubilities [a] (Millimolar) of Lidocaine, Mepivacaine, and Bupivacaine in 0.5 *M* Phosphate Buffer, pH 7.4 [b], and 1–4 m*M* NaOH [c]

Temperature [d]	Lidocaine		Mepivacaine		Bupivacaine	
	0.5 *M* Phosphate Buffer	1–4 m*M* NaOH	0.5 *M* Phosphate Buffer	1–4 m*M* NaOH	0.5 *M* Phosphate Buffer	1–4 m*M* NaOH
14.5°	30.3 ± 1.4	—	17.1	—	1.35	—
14.9°	—	18.5 ± 0.5	—	13.6 ± 0.3	—	0.375 ± 0.003
25.0°	22.9 ± 0.1	16.3 ± 0.1	13.5	10.2 ± 0.4	0.850 ± 0.020	0.318 ± 0.002
34.5°	—	14.6 ± 0.1	—	9.91 ± 0.20	—	0.313 ± 0.004
37.0°	16.5	—	9.90	—	0.575 ± 0.008	—

[a] Either the result of a single determination or average (two or three determinations) ± *SE*. [b] Composition at the time of preparation was 0.427 *M* Na_2HPO_4 and 0.0710 *M* NaH_2PO_4, pH 7.40 ± 0.01 at 25° and 7.38 ± 0.01 at 37°. [c] The pH varied from 10.43 (1 m*M* at 34.5°) to 11.86 (4 m*M* at 14.9°). [d] Temperature control was ±0.1°.

in the phosphate buffer (Table I). Since the pKa of these bases varied at 23° from 7.78 for mepivacaine to 8.09 for bupivacaine (5), at pH 7.4 these local anesthetics existed as a mixture of protonated and unprotonated forms. Thus, the solubilities of the unprotonated base species were determined in 1–4 m*M* NaOH. The solubilities in these high pH media were independent of hydroxyl-ion concentration and increased with decreasing temperature (Table I), although this trend was not as great as in the phosphate buffer.

The difference in temperature dependency is now being studied in our laboratories and is probably due to a measurable decrease in the pKa values of the bases with increasing temperature whereas the phosphate buffer pH remains relatively constant (Table I, footnote *b*). Our preliminary determination of lidocaine pKa values at different temperatures by the solubility method indicated that they vary from 8.2 at 15° to 7.7 at 35°. With increasing temperature, therefore, the gap between the medium pH and the pKa diminishes. Thus, the proportion of the soluble protonated base, BH^+, decreases with increasing temperature, which is reflected greatly in the total solubility, S_t, of the base at pH around 7.4; *i.e.*, S_t is given by (6):

$$S_t = [B] + [BH^+] = S_0\left(1 + \frac{[H^+]}{K_a}\right) \qquad \text{(Eq. 1)}$$

where $[B] = S_0$ is the solubility of the unprotonated species and K_a is the acid dissociation constant of BH^+. Since S_0 decreases and K_a increases as the temperature is increased (Eq. 1) at pH 7.4, both of these effects contribute to lower solubility at higher temperatures.

Since the unusual temperature-dependent solubility is likely to extend over a fairly wide pH range, including physiological pH, the following clinical problem could arise. In parenteral dosage forms of these drugs as local anesthetics, solution is effected by means of their hydrochloride salts. Therefore, the pH of these preparations can be as low as 3.0–4.5 (7). The results of the present study suggest possible precipitation of the base at the injection site from the following two points: (*a*) an increase in pH to the tissue pH after injection, as was suggested earlier for lidocaine (8); and (*b*) a lowering of free base solubility at body temperature relative to ambient temperature.

For most organic compounds, solubilities are usually assumed to increase with temperature. These local anesthetics, however, behave differently. Even if the decrease in solubility with increasing temperature at pH values of around 7.4 is mainly attributable to a decrease in the fraction of protonated species because of a shift in pKa with increasing temperature, the unprotonated species also show atypical temperature-dependent solubilities. Only a few organic medicinal compounds are known to exhibit such behavior. These include anhydrous ampicillin (9), dactinomycin (10), aminopyrine and propyphenazone (11), and colchicine (12).

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Naomi I. Nakano

Hokkaido Institute of Pharmaceutical Sciences
Otaru 047-02, Japan

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Carbocyclic Analog of Cytarabine

Keyphrases □ Cytarabine, analogs—synthesis, carbocyclic analog, antileukemic activity □ Antineoplastic agents—cytarabine, carbocyclic analog, synthesis □ Antineoplastic activity—cytarabine, carbocyclic analog

To the Editor:

Cytarabine (1-β-D-arabinofuranosylcytosine, Ara-C) inhibits a variety of experimental neoplasma (1, 2), produces remissions in some patients with acute myelocytic or acute lymphocytic leukemia (3–5), and is considered the most effective single-drug treatment for acute myelogenous leukemia (6). It is also effective in suppressing the replication of certain DNA viruses (2) and is a useful agent for the clinical treatment of herpes virus infections (7). Recently, we reported (8) the synthesis and activity of the carbocyclic analog (carbodine, I) of cytidine against a murine leukemia (L-1210). Brockman *et al.* (9) showed that carbodine inhibits both DNA and RNA synthesis in

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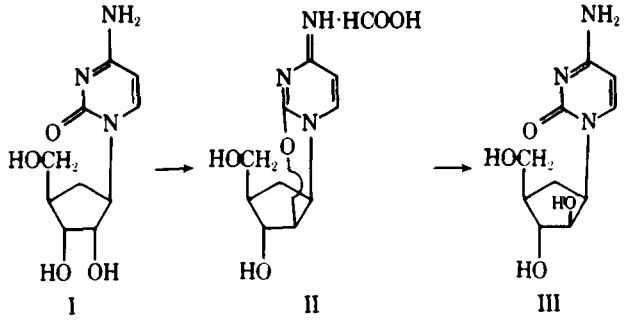

Scheme I

L-1210 cells and that it is phosphorylated to the mono-, di-, and triphosphate stages. Collectively, these facts provided the rationale for the synthesis of the carbocyclic analog (III) of cytarabine.

Carbodine served as the precursor of III (Scheme I). Treatment of I with dimethylformamide–thionyl chloride reagent in excess dimethylformamide, a method reported (10) for the conversion of cytidine to cytarabine, produced the carbocyclic analog (II) of 2,2′-anhydrocytidine (cyclocytidine). A solution of I (500 mg) in the reagent solution (0.75 ml of thionyl chloride in 5 ml of dimethylformamide stirred for 0.5 hr at room temperature prior to the addition of I) was stirred for 3 hr at room temperature, diluted with water (2.5:1), sparged with nitrogen to remove sulfur dioxide, and applied to a column of a cation-exchange resin[1] in the pyridinium salt form. Elution of the column with 0.1 *M* pyridinium formate, combination of fractions containing II[2], and concentration of the solution *in vacuo* furnished a viscous syrupy residue that contained II, as well as pyridinium formate.

A solution of this residue in 1 *N* aqueous ammonia was heated at 80° for 15 min to hydrolyze II to III. The reaction solution was concentrated *in vacuo*, and an aqueous solution of the residue was chromatographed on a strong cation-exchange resin[1] in the pyridinium salt form, as already described. Fractions containing III[2] were concentrated *in vacuo*, and an aqueous solution of the residue was applied to a column of a strongly acidic cation-exchange resin[3]. The column was washed thoroughly with water and then eluted with 2 *N* aqueous ammonia.

The portion of the eluate containing III[2] was concentrated to dryness *in vacuo*, and the white, solid residue was recrystallized from ethanol–water; 60% yield from I; mp 227–230° dec.; mass spectrum (1 m*A*, direct-inlet probe, 240°): *m/e* 242 (M + 1), 241 (M), 224 (M − OH), 223 (M − H_2O), 210 (M − CH_2OH), and 138 (cytosinyl group + C_2H_4); UV: λ_{max} 283 (ϵ 14,500) and 214 (10,100) at pH 1, 274 (10,000) and 225 (sh) at pH 7 or pH 13; PMR (dimethylsulfoxide-d_6, δ in parts per million downfield from tetramethylsilane): 1.5–2.05 (m, CH_2 and CH at positions 5 and 4 of the cyclopentane ring), 3.25–3.7 (m, CH_2OH and CH at position 3 of the cyclopentane ring), 3.7–3.9 (m, CH at position 2 of the cyclopentane ring), 4.46–4.85 (m, OH of CH_2OH), 4.85–5.14 (m, CH at position 1 and OH at positions 2 and 3 of the cyclopentane ring), 5.65 (center of doublet, CH at position 5 of the pyrimidine ring), 6.94 (broad singlet, NH_2), and 7.57 (center of doublet, CH at position 6 of the pyrimidine ring).

Compound III moved slightly faster than I on a thin-layer plate of silica gel with 2-propanol–1 *M* ammonium acetate (7:3) as developing solvent.

Anal.—Calc. for $C_{10}H_{15}N_3O_4$: C, 49.79; H, 6.27; N, 17.42. Found (after drying at 100° for 4 hr): C, 49.57; H, 6.30; N, 17.45.

The carbocyclic analog (II) of cyclocytidine was isolated from another reaction of I with dimethylformamide–thionyl chloride. The mixture of II and pyridinium formate, obtained by chromatographing the reaction product on the pyridinium cation-exchange resin[1], as already described, was stored in a high vacuum for an extended period to volatilize pyridine and formic acid. The residue was triturated several times with ether and then with cold ethanol. Ethanol evaporation, addition of a second portion of cold ethanol, and drying of the crystalline precipitate at 56° afforded II formate as a hydrate; TLC, one spot [40 or 80 μg on silica gel, 2-propanol–1 *N* ammonium acetate (7:3)[4], UV detection]; mass spectrum: *m/e* 223 (M), 192 (M − CH_2OH), and 46 (HCOOH); UV: λ_{max} 269 (ϵ 9500) and 232 (9000) at pH 1.

Anal.—Calc. for $C_{10}H_{13}N_3O_3 \cdot HCOOH \cdot \frac{3}{4}H_2O$: C, 46.39; H, 5.84; N, 14.76. Found: C, 46.29; H, 5.54; N, 15.28.

Kikugawa and Ichino (10) reported the preparation of cytarabine from cytidine through the intermediate formation of cyclocytidine with dimethylformamide–thionyl chloride reagent. The overall yield of cytarabine was about 27%. When the first procedure outlined for the preparation of III was applied to the preparation of cytarabine from cytidine, the yield of product obtained from the second ion-exchange column[1] was 41%.

Analysis of the product by high-pressure liquid chromatography showed it to be a mixture of cytarabine and cytidine (55:45) and indicated an overall yield of about 23% of cytarabine. In comparison, the yield of III free of I was 68% at the same stage.

In tests performed by standard protocols (11), the carbocyclic analog of cytarabine was active against L-1210 leukemia in mice (Table I).

Table I—Tests of Compound III against L-1210 Leukemia [a]

Dose, mg/kg/day	Weight Change[b], T − C	Average Lifespan, T/C	Increase in Lifespan, %
150	−2.2	16.6/8.1	104
100	−0.8	13.8/8.7	58
67	−1.5	11.4/8.1	40

[a] Mice were implanted intraperitoneally with 10^5 L-1210 cells on Day 0. Treatment was begun on Day 1 and continued through Day 9 (*q.d.* 1–9). T = treated mice; C = control mice. [b] The weight change is the difference on Day 5 between the average change in weight of treated mice and the average change in weight of control mice.

[1] Styrene-sulfonic acid-type resin, analytical grade (AG-50W-X4, Bio-Rad Laboratories, Richmond, Calif.).

[2] Fractions of the eluate containing II or III were located by monitoring the effluent with a UV spectrophotometric monitor.

[3] Amberlite CG-120, H^+ form.

[4] In this TLC system, II moved more slowly than I and III.

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Y. Fulmer Shealy ˣ
C. Allen O'Dell
Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, AL 35205

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Absorption of Insulin Delivered to Rabbit Trachea Using Aerosol Dosage Form

Keyphrases □ Insulin—aerosol dosage forms, tracheal administration, absorption, rabbits □ Dosage forms, aerosol—insulin, tracheal administration, absorption, rabbits □ Proteins—aerosol dosage forms, insulin, tracheal administration, absorption, rabbits

To the Editor:

Insulin, an endogenous hormone used in treatment of diabetes mellitus, has been administered parenterally rather than orally because it readily decomposes in the GI tract. However, some effective approaches have been developed for oral (1–3), nasal (4), and rectal and aerosol (5, 6) dosage forms. The aerosol dosage form has been receiving much attention because it can be used by diabetic patients with little difficulty.

Wigley *et al.* (5) reported that insulin delivered to patients and volunteers by aerosol inhalation using a nebulizer was absorbed through the respiratory tract epithelium and was an effective therapeutic form for diabetes mellitus (5). However, little has been mentioned about the pharmaceutical factor that governs insulin absorption from aerosol dosage forms.

The present study was undertaken to investigate the bioavailability of powdered aerosol insulin when administered intratracheally to rabbits.

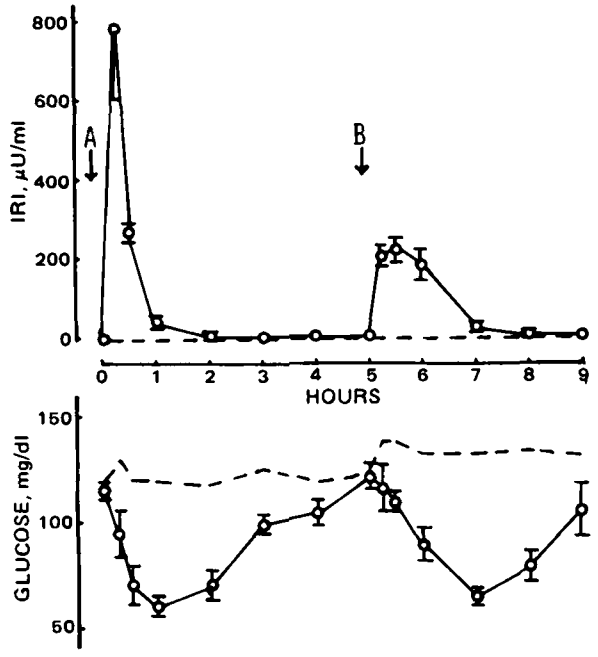

Figure 1—*Plasma concentration profiles of immunoreactive insulin (IRI) and glucose after intravenous (A, 1 U/kg) or intratracheal (B, 2.5 U/kg) insulin administration. Each point represents the mean ± SEM of three experiments. Broken lines denote immunoreactive insulin and glucose levels in sham-operated rabbits.*

White male rabbits, 2.8–3.1 kg, were anesthetized with pentobarbital sodium and maintained under anesthesia for the entire experiment. After the animal was secured in a supine position, insulin solution (1.0 U/kg) or physiological saline (as the control) was injected into the ear vein.

Blood samples were collected from the femoral vein at timed intervals to monitor immunoreactive insulin and glucose levels in plasma. Four hours after intravenous insulin injection, the rabbit trachea was exposed and polyethylene tubing was inserted through a tracheal incision. Insulin aerosol was sprayed into the trachea 1 hr after surgical treatment, and the appearance of immunoreactive insulin and the blood glucose level then were determined at timed intervals.

The insulin aerosol formulation was composed of: monocomponent porcine insulin[1], 5 mg; lactose, 75 mg; acetylglycerin monostearate, 50 mg; dichlorodifluoromethane, 3.6 g; and dichlorotetrafluoroethane, 2.4 g. A metered aerosol valve was designed to release 0.058 mg (1.5 U) of insulin/delivery. The plasma glucose level was measured by the method of Hyvärinen and Nikkilä (7), and immunoreactive insulin in plasma was determined by the method of Desbuquois and Aurbach (8).

Figure 1 shows the time courses of immunoreactive insulin and glucose concentrations in plasma after intravenous injection and after intratracheal aerosol insulin administration. After intravenous administration, there was a sharp increase in plasma immunoreactive insulin and a decrease in plasma glucose. The decline in plasma glucose continued for about 3 hr, although the plasma immuno-

[1] Crystals, lot S 837301 (biological potency 26.6 I.U./mg), Novo Industry Co., Copenhagen, Denmark.

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