Table I—Comparison of pKa Values of Medazepam

pKa	Determination by	Reference
4.4	Spectroscopy	2
8.7	Polarography, pK_1	3
4.4	Spectroscopy, pK_1	3
6.17	Spectroscopy	This study
6.19	Solubility	This study

form using the Henderson–Hasselbalch equation.

In the region where the pH approximates the pKa, buffer solutions differing by 0.5 pH unit were used. All pH values of the mixtures were measured potentiometrically with a glass electrode, calibrated with accurate standard buffer solutions. Calculation of all results yielded a pKa of 6.17 ± 0.02. The findings disagreed with previous results (2, 3) (Table I).

Further determinations of the pKa were carried out by the solubility method reported previously (4). The plot of the hydrogen-ion activity against solubility is shown in Fig. 2. The relationship yields an ordinate intercept of $-K$, which can be converted into pKa = $-\log K$. In the equation:

$$[H^+] = \frac{KS}{S_0} - K \qquad \text{(Eq. 2)}$$

K is the acid dissociation constant of the base, S_0 is the low solubility of the free base, and S is the total solubility at a particular pH.

Solubility determinations were carried out at 37° by incubating excess amounts of medazepam with buffer solutions in closed vials in a temperature-controlled water bath for 48 hr. The samples were intermittently shaken, the supernate was filtered at 37° and diluted with buffer solution, and the concentrations of medazepam were determined by UV spectrophotometry at 37°. The pH of the supernate was measured with a glass electrode at 37°.

All determinations were repeated, with a longer equilibrating time, without any deviation in the results. The ordinate intercept of K was 6.5×10^{-7} and yielded a pKa of 6.19. This finding was in good agreement with the value from the spectrophotometric determination.

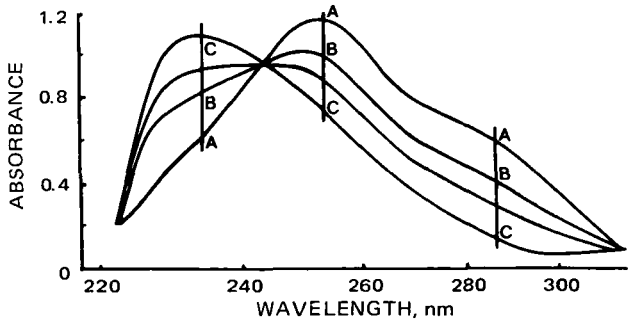

Figure 1—*UV spectra of medazepam in acid (pH 1, points A) and alkaline (pH 12, points C) solutions and at pH 6.0 (points B).*

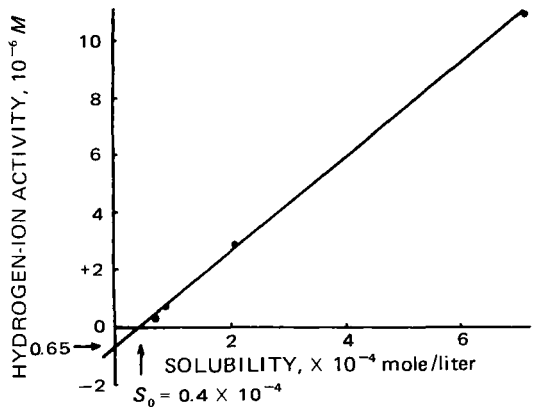

Figure 2—*Determination of* −K *by the method of Green (4) from the relationship between hydrogen-ion activity [H⁺] and solubility,* S, *of medazepam;* S_0 *is the low solubility of the free base.*

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Differences in Ocular Penetration of Pilocarpine in Rabbits of Different Ages

Keyphrases □ Pilocarpine—differences in ocular penetration related to age, rabbits □ Ocular penetration—pilocarpine, related to differences in age, rabbits □ Age, rabbits—effects on ocular penetration of pilocarpine

To the Editor:

In topical ophthalmic drug therapy, generally no distinction is made between age groups. In most cases, from the neonate to the geriatric, the same dosage is administered. The potential hazards of such an approach were recognized by France and France (1), who observed toxic side effects when administering the usual adult dose of phenylephrine to neonates.

It has been our contention for some time that the administration of equal doses of topical ophthalmic drugs to widely varying age groups should result in substantial differences in the amount of drug reaching the aqueous humor. The theoretical aspects of these considerations were discussed recently (2). Such an approach involves both therapeutic and toxicity considerations.

The present communication demonstrates, for the first time, that differences do exist in the penetration of topically applied ophthalmic drugs as a function of age. Such differences may be due to size, weight, tear flow, rate of drainage, and/or structure and function of the various ocular tissues. A more detailed examination of such differences is warranted so that ophthalmic dosage regimens can be placed on a more rational basis.

Identical doses of 25 μl of 1×10^{-2} *M* tritiated pilocarpine solution were instilled into the eyes of 20- and

Table I— Concentration of Pilocarpine in the Aqueous Humor of 20- and 60-Day-Old Rabbits following Instillation of 25 μl of 1×10^{-2} *M* Pilocarpine

Minutes	Concentration[a], μg/ml	
	20-Day-Old Rabbits	60-Day-Old Rabbits
5	0.84 (0.03, 10)	0.55 (0.06, 8)
10	1.67 (0.05, 8)	1.03 (0.14, 11)
15	1.88 (0.17, 8)	1.11 (0.11, 8)
20	2.06 (0.10, 8)	0.98 (0.12, 10)
30	1.51 (0.18, 8)	0.93 (0.12, 9)
45	1.17 (0.12, 7)	0.42 (0.04, 8)
60	0.88 (0.12, 8)	0.44 (0.04, 7)
90	0.38 (0.03, 8)	0.19 (0.02, 7)
120	0.17 (0.01, 9)	0.11 (0.01, 8)

[a] Mean concentrations. The first number in parentheses refers to standard error, and the second number is the number of eyes sampled at that time point.

60-day-old male, New Zealand albino rabbits[1]. At various times postinstillation, rabbits were sacrificed and the aqueous humor was aspirated from the anterior chamber. Liquid scintillation counting allowed conversion of counts to micrograms of pilocarpine per milliliter of aqueous humor. All experimental procedures have been well established (3–5).

The aqueous humor concentration *versus* time profiles in the two categories of test animals are reported in Table I. At every time point, the concentration of pilocarpine was significantly higher in the 20-day-old rabbits as compared to the 60-day-old rabbits. The calculated areas under the curves were different by approximately a factor of two. The implication is that it should be possible to reduce substantially the dose administered to the 20-day-old rabbits while simultaneously maintaining aqueous humor concentrations equivalent to the 60-day-old rabbits. We make no comment, at this time, regarding the required concentration to produce a pharmacological effect.

Many studies currently appearing in the ophthalmic literature do not concern themselves to any extent with the age or size of animals used. These studies, although using relatively large differences in age, certainly point out the need for standardization of test animals. Also apparent are the inherent difficulties in comparing studies between laboratories without adequate knowledge concerning age and size of test subjects.

More importantly, further investigation into the development of ophthalmic pediatric dosage regimens is warranted. When one considers differences in the aqueous humor volume in the eye, as well as differences in the surface area available for absorption and the existence of immature membranes, it becomes apparent that some dosage adjustments may be in order, at least during the rapid growth phase of the eye (*i.e.*, birth to 3 years).

In addition, it is known that tear production and instilled volume drainage account for a large loss of drug from any topically applied dose (6, 7) and can affect the ocular bioavailability of drugs (8). No one to date has quantitatively considered these effects as applied to the bioavailability of topically administered drugs in children, nor have the potential toxic effects due to drainage been quantitated.

Also worthy of mention is the fact that numerous functional changes take place in the eyes of geriatrics. Decreased tear flow and volume are not uncommon with age and could cause differences in instilled drug concentration. One also might suspect changes to take place in the drainage of instilled solutions and, potentially, in the integrity of ocular membranes. All such changes have the potential to cause differences similar to those noted here and may warrant dosage adjustments.

Finally, one major problem in topical ophthalmic drug therapy of both infants and geriatrics is compliance with the medication dosage regimen. It is hoped that, by quantitating and maximizing dosage regimens, therapy can be simplified and the degree of compliance increased.

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[1] At the 20th postnatal day, the rabbit's globe is about two-thirds of adult size; at 60 days, the rabbit's globe attains 90% of adult size. The human eye is about two-thirds of adult size at birth and about 90% of adult size at 3 years of age.

Culture of Isotopically Substituted Plants of Pharmacological Importance: Conservation and Recycling of Stable Isotope Substrates

Keyphrases □ Isotopes, stable—^{2}H- and ^{13}C-substituted tobacco plants cultured, sealed growth chambers, isotope substrates conserved and recycled □ Plant culture—^{2}H- and ^{13}C-substituted tobacco plants cultured, sealed growth chambers, isotope substrates conserved and recycled □ Tobacco plants—*Nicotiana tabacum*, ^{2}H- and ^{13}C-substituted, cultured in sealed growth chambers, isotope substrates conserved and recycled

To the Editor:

We have successfully cultured algae, bacteria, protozoa, molds, yeasts, and fungi in fully deuterated form (1). Deuterated metabolites have been isolated from