Table II—Pharmacology[a]

Compound	LD_{50}, mg./kg. i.p., Mice	LD_{50}, mg./kg. p.o., Rat	Carrageenin-Induced Rat Paw Edema Percent Reduction, 250 mg./kg.
1	>800	>1000	(2) Increase
2	800	>1000	4
3	>800	—	2
6	>800	>1000	(18) Increase

[a] Pharmacological testing was performed by Riker Laboratories, Inc., Northridge, Calif.

***m*-Nitrobenzaldehyde *O*-(*m*-Nitrobenzyl)oxime (Compound 1)**—To 2.4 g. (0.012 mole) of *m*-nitrobenzaldehyde dissolved in 30 ml. hot 95% EtOH was added slowly 1.8 g. (0.012 mole) of *m*-nitrobenzyloxyamine hydrochloride (1) dissolved in 40 ml. 95% EtOH. The solution was stirred for 10 min. and then filtered to give 3.0 g. (85% yield) of a white crystalline solid, m.p. 93–95°. An analytical sample, m.p. 94–96°, was obtained by two recrystallizations from acetone–H_2O.

***m*-Nitrobenzaldehyde *O*-(4-Thiazolylmethyl)oxime (Compound 11)**—To 8.1 g. (0.04 mole) of 4-thiazolylmethoxyamine dihydrochloride (6) dissolved in 600 ml. warm 95% EtOH was added, in portions, 6.0 g. (0.04 mole) of *m*-nitrobenzaldehyde dissolved in 200 ml. warm 95% EtOH. The solution was refluxed for 3 hr. and let stand overnight at room temperature. A small amount (0.5 g.) of nonsulfur-containing solid, m.p. 195.5°, was filtered off and discarded. The filtrate was diluted to 2 l. with H_2O; the resultant crystalline solid was collected and recrystallized from EtOH–H_2O to give 6.4 g. (61%) of cream-colored solid, m.p. 97–99°. One additional recrystallization yielded an analytical sample, m.p. 98–99°.

2-Chloro-4,5-methylenedioxybenzyloxyamine Hydrochloride—To a solution of 16.5 g. (0.05 mole) *N*-(2-chloro-4,5-methylenedioxybenzyloxy)phthalimide (1) in 600 ml. warm anhydrous EtOH was added 2.5 ml. (0.05 mole) hydrazine hydrate (99%), and the solution was refluxed for 4 hr. The reaction mixture was cooled, and the precipitated phthalhydrazide was removed by filtration. Addition of excess ethanolic HCl to the filtrate followed by reduction in volume by evaporation gave 10 g. (84%), m.p. 191–194°. An analytical sample was obtained by dissolving the compound in H_2O, converting to the free base with Na_2CO_3, extracting with ether, drying with Na_2SO_4, and reconverting to the hydrochloride with ethanolic HCl.

Anal.—Calcd. for $C_8H_9Cl_2NO_3$: C, 40.40; H, 3.78; N, 5.88. Found: C, 40.31; H, 4.00; N, 5.73.

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Worldwide Virtual Temperatures for Product Stability Testing

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Abstract □ The market temperatures under which product stability expiration dates for products are to apply should be simulated by laboratory conditions. An actual simulation would involve a pattern of changing monthly temperatures for each location. For those products whose loss rate constant is related to temperature by the Arrhenius relationship, a single virtual temperature can be determined at which the loss rate is equivalent to that of this changing pattern of temperature. This has been calculated for each of 15 cities in the United States and 15 elsewhere. Tables I and II should serve as guides in considering the standard temperature to be incorporated into the laboratory stability test protocol for such products.

Keyphrases □ Product stability testing—simulation of worldwide virtual temperatures □ Expiration dating—guides for determining standard temperatures □ Virtual temperature—application to product stability testing

Drug products stored in pharmacies and warehouses for extended periods of time are exposed to a range of temperatures which, in the United States at least, is narrowed from the ambient range by the widespread use of air conditioning. Storage of some products is governed by restrictions printed on the labels, and these products are not the topic of this paper, although the principle employed may be applied to some of them. According to R. Blythe (1): "Twenty-seven or 68 percent of the reporting [pharmaceutical] companies conduct *field tests* on some of the products. . . in the warmer, more humid areas of the United States. . . Twenty-one

Table I—Temperature Values[a]

Country	City	Restricted[a] Annual Average Temperature	Virtual Temperatures: Annual Basis	Virtual Temperatures: Six Consecutive Hottest Months	Temperature Range[b]
United States	Atlanta	19.9°	20.4°	23.1°	
	Boston	18.1°	18.2°	19.3°	5.2°
	Chicago	18.5°	18.7°	20.2°	6.9°
	Cleveland	18.4°	18.6°	20.0°	6.3°
	Detroit	18.2°	18.3°	19.5°	5.8°
	Galveston	22.0°	22.7°	26.4°	
	Los Angeles	18.9°	19.0°	20.8°	5.8°
	New Orleans	21.9°	22.7°	26.2°	
	New York	18.6°	18.7°	20.3°	
	Philadelphia	19.1°	19.4°	21.5°	8.0°
	Portland, Oregon	17.4°	17.4°	17.9°	
	St. Louis	19.8°	20.3°	23.0°	10.2°
	San Francisco	17.0°	17.0°	17.0°	0.0°
	Tampa	22.5°	23.1°	26.3°	
	Washington	19.3°	19.7°	21.9°	8.6°
Australia	Sydney	18.8°	18.9°	20.6°	5.2°
Belgium	Brussels	17.1°	17.1°	17.2°	0.8°
Brazil	Porto Allegero	19.9°	20.2°	22.6°	7.6°
	Rio de Janeiro	23.0°	23.1°	24.8°	5.8°
	Salvador, Bahia	25.4°	25.4°	26.4°	3.3°
Cuba	Havana	25.1°	25.2°	27.0°	5.6°
England	London	17.0°	17.0°	17.0°	0.2°
Germany	Munich	17.0°	17.0°	17.0°	0.4°
India	Bombay	27.1°	27.1°	28.0°	5.8°
Japan	Tokyo	19.2°	19.5°	21.6°	8.8°
Pakistan	Karachi	26.0°	26.5°	29.4°	10.0°
E. Pakistan	Dacca	25.8°	26.1°	28.5°	9.7°
W. Pakistan	Lahore	24.8°	26.1°	30.7°	16.6°
Puerto Rico	San Juan	25.6°	25.6°	26.5°	3.1°
South Africa	Johannesburg	18.2°	18.2°	19.3°	2.9°

[a] 17° has replaced any lower monthly values in these computations including that for restricted annual average temperature. [b] Hottest month to coldest month (with heat, if necessary).

of the companies indicated in a Pharmaceutical Manufacturers' Association survey that they used special tests for products shipped to foreign markets." The current prevalence of air conditioning probably has led to a reduction of the amount of field testing within the United States. He also indicates that three companies use "...cyclic testing in which temperature and humidity are alternately increased and decreased in a given pattern...."

Currently, much interest is being shown in drug dating, as indicated by the 1969 conference on dating of pharmaceuticals (2, 3). Also, some laboratories store the product at elevated temperatures and/or at a "room" temperature. This widespread interest in dating makes it desirable to consider carefully the determination of a standard temperature used to establish the expiration date on a product. The purpose of this note is to present the method of calculating the virtual temperature (defined later), which will guide the choice of a standard temperature for dating purposes. Also, the virtual temperatures for 30 cities are given, and an approximate formula is given which simplifies the calculation for other places.

DISCUSSION

It is well known that changes in the actual field storage temperatures cause the reaction rate constant of some products to change according to the Arrhenius relationship (4). The mathematical development given here applies only to such products. For example, if the storage temperature were 20° for 1 year and 40° for the next year, the final concentration of the active ingredient of such a product may be 85% of the initial value; the average temperature is 30°, but 2 years of storage at 30° would not result in 85% potency (assay error assumed to be negligible). The better way to determine a single equivalent temperature would be to calculate rate constants (of the proper order) for the 1st and 2nd years and average them. This average rate constant could be substituted into the Arrhenius equation, which could then be solved for the corresponding temperature, here named the virtual temperature. Two years' storage of the product of the example at the virtual temperature (perhaps 31°, dependent upon the magitude of the heat of activation) would result in 85% potency.

The pharmacist with the tasks of exploratory work on stability of new formulations, of estimating overages for accepted formulations, and of providing data for dating purposes needs a reference storage temperature for incorporation into his laboratory stability test protocols for both "room" temperature studies and elevated temperature studies. Conceptually, the *average* of the 12 monthly reaction rate constants based on frequent observations at a given geographical location could be calculated. If this average reaction rate constant is substituted into the Arrhenius equation, it then can be solved for the single corresponding temperature, named the annual virtual temperature. The potency data for the product held in the *laboratory* at this virtual temperature for 12 months would yield, upon calculation, a reaction rate constant equal to the average mentioned previously, with due allowance for observation variation. (The annual virtual temperature value would differ to some degree from the annual temperature and usually would equal none of the monthly temperatures; the virtual temperature may be said to exist in effect but not in fact.)

Obviously that method of calculating the virtual temperature is not feasible since it would require stability studies at each location or in the laboratory with temperatures changing as at the location. Since the loss rate constant (of the pertinent order) in any month is, according to the Arrhenius relationship:

$$k_i = Ae^{-\Delta H/RT_i} \qquad i = 1, \ldots, 12 \qquad \text{(Eq. 1)}$$

with A = a constant for a given reaction; ΔH = heat of activation, calories mole^{-1} (a constant for a given reaction); R = universal gas constant, 1.987 cal. mole^{-1} degree^{-1}; and T_i = the absolute tempera-

Table II—Extreme Virtual Temperature Values from Table I

Extreme	Annual Virtual Temperature	Hot Season Virtual Temperature
Smallest in U. S. cities shown	17.0°, San Francisco	17.0°, San Francisco
Largest in U. S. cities shown	23.1°, Tampa, Florida	26.4°, Tampa, Florida
Smallest in Table I	17.0°, San Francisco, London, Munich	17.0°, San Francisco, London, Munich
Largest in Table I	27.1°, Bombay	30.7°, Lahore, West Pakistan

ture in the ith month; the average of the 12 values of k_i is proportional to the average of the 12 values of $e^{-\Delta H/RT_i}$. Monthly temperatures, rather than weekly or daily temperatures, are used because the typical storage situation prevents the product from changing temperatures rapidly; also they are more readily available. The T_i values can be obtained easily for most locations and, with a suitable value of ΔH, used to calculate the average of the 12 $e^{-\Delta H/RT_i}$; this numerical value is defined to be equal to $e^{-\Delta H/R(VT)}$, where VT stands for the virtual temperature. The solution of this for VT in degrees absolute is given in Eq. 2:

$$(VT°\text{K.}) = \left[\frac{(\Delta H/R)}{-\ln\left(\frac{e^{x_1} + e^{x_2} + \cdots e^{x_{12}}}{12}\right)}\right] \qquad \text{(Eq. 2)}$$

where $x_1 = -\Delta H/RT_1$ for the 1st month, *etc.*, and ln stands for log base e.

A heat of activation of 14 kcal. mole^{-1} represents products near the middle of the range of 10–20 kcal. mole^{-1} chosen by Kennon (5) and is appropriate for products with reaction rate constants that approximately double as the temperature increases by 10° within the normal range.

Equation 2 was applied to the monthly temperatures secured from United States Weather Bureau records (6) for 20 or more years for 15 continental United States cities and 15 other cities chosen to represent extremes (7). Temperatures below 17° (65°F.) were replaced by 17° in Eq. 1, because it was assumed the storage area would be heated; the annual temperature calculated in this fashion is called the restricted annual temperature. The annual virtual temperature values are shown in Table I as well as virtual temperature values for the six consecutive hottest months. Table II presents the extreme values of Table I.

A rule-of-thumb (obtained from a plot of the data) that gives essentially the same result as Eq. 2 uses the temperature *range* (with 17° substitution for any period if appropriate) to estimate the annual VT°C. (*a*) If the temperature range is 4.5° or less, the approximate annual VT°C. is estimated by the restricted *annual* temperature. (*b*) If the range is over 4.5°, approximate (VT°C.) = (restricted annual temperature) + 0.1 (range − 4.5°).

Equation 2 was also used with ΔH = 20 kcal. mole^{-1}, the average value quoted by Kennon (5), and ΔH = 30. The former corresponds approximately to an increase in the rate constant by a factor of 3 per 10° increase, and the latter by a factor of 5.5. The virtual temperatures obtained from these equations were practically unchanged for some cities and became larger for the rest: by less than 0.3° for 20 kcal. mole^{-1} and by less than 0.5° for 30 kcal. mole^{-1} for 29 cities. The only exception was Lahore which had increases of 0.4 and 1.0°, respectively. The slightness of these changes is understandable when it is realized that if the geometric mean k were appropriate rather than the arithmetic mean k, the corresponding "virtual" temperature would equal the harmonic mean of the 12 monthly temperatures regardless of the value of ΔH. The near equality of the arithmetic mean k and the geometric mean k for most of these cities is reflected in the slightness of the dependence of the virtual temperatures reported here upon ΔH.

The largest annual virtual temperature in these 15 United States cities is 23.1° for Tampa for the 30-kcal. mole^{-1} case. The difference between annual and virtual temperature in these 15 United States cities is largest for Tampa also: 0.8° for 14 kcal. mole^{-1} and 1.3° for 30 kcal. mole^{-1}. A 1° temperature difference translated into a difference in rate constants for 14, 20, and 30 kcal. mole^{-1} products is 8, 12, and 19%, respectively, which corresponds to sizable differences in overage.

This information provides a basis for choosing a standard stability temperature for the United States. No allowance has been made for air conditioning in pharmacies and warehouses, so these values may be considered as upper limits for virtual temperature. The oft-quoted room temperature of 25° for dating purposes (5, 8–10) is safe for the United States and is probably high for such products. An annual virtual temperature of 24° would be consonant with the data presented here. If a survey of air conditioning were undertaken, it probably would support an annual virtual temperature of 23°.

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