Effect of Temperature on Absorption Rates of Drug Implants

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The absorption rates of drug pellet implants have been shown to be temperature dependent in frogs. For neutral compounds the rate equation tested was $(\bar{R}/\bar{A})r = Kr \cdot Dr \cdot (Ss)r/\delta r$, where $(\bar{R}/\bar{A})r$ is the mean absorption rate per mean area at a given temperature relative to that at 0°C., Kr is a constant, Dr is the relative diffusion coefficient, $(Ss)r$ is the relative solubility of drug, and δr is the relative diffusion layer thickness. To minimize body movement, the frog, *Rana pipiens*, was paralyzed with 10 mg./Kg. of *d*-tubocurarine chloride. After temperature equilibrium was attained in a water or ice bath, a small incision into the dorsal lymph sac was made, and two preweighed pellets of sulfanilamide or acetanilid were introduced. The pellets were removed, dried, and reweighed. A plot of $(\bar{R}/\bar{A})r$ *versus* $Dr \cdot (Ss)r/\delta r$ yielded a straight line with a slope 1.09 for the two compounds.

THE ABSORPTION rates of subcutaneously implanted drug pellets have been shown to be rate limited by the dissolution process at the site of implantation. Although considerable literature has appeared where the pellet implantation technique has been used, apparently no quantitative work has appeared on what influence the animal's body temperature has on the absorption rates of solid implants (1).

The purpose of this study was to determine what the relationship is between the absorption rates of two neutral drugs subcutaneously implanted and the influence of body temperature on the physical and chemical properties of drug and the fluid medium surrounding it.

THEORETICAL

Equations relating absorption rates of implanted drug pellets and drug's physicochemical properties have been derived under conditions of a constant body temperature (2). The following derivations were based, in part, on the assumptions previously made.

The absorption rate of implanted solid drug was assumed to be a diffusion-controlled process where diffusion of drug molecules took place normal to a nearly constant solid surface area. Fick's law in one dimension is

$$da/dt = DA\,dc/dx \qquad \text{(Eq. 1)}$$

where da/dt is the rate of flow of dissolved drug through a plane of area, A, normal to the direction of diffusion, and is (in this case) the absorption rate of the drug implant. The diffusion coefficient is D. The area, A, under these conditions was assumed to be the area of the solid drug pellet at any time. The concentration gradient across the region through which diffusion takes place is dc/dx. If $\bar{R}$, which equals da/dt, is the mean absorption rate, and is the mean pellet area, then rearranging Eq. 1 gives

$$\bar{R}/\bar{A} = D\,dc/dx \qquad \text{(Eq. 2)}$$

where $\bar{R}/\bar{A}$ equals the mean absorption rate per mean pellet area. Under conditions of steady state diffusion from solid, the concentration gradient may be approximately represented

$$\bar{R}/\bar{A} = D\frac{(Cs - C)}{\delta} \qquad \text{(Eq. 3)}$$

where Cs is the concentration of a nearly saturated solution of drug in body fluids in the diffusion layer (3) at a point adjacent to the pellet surface, and C is the drug concentration in the bulk of the body fluids. The boundary or diffusion layer thickness is δ. For most substances which are foreign to the animal, like most drugs, the term C can be assumed to be small compared to Cs and will be neglected. Thus

$$\bar{R}/\bar{A} = DCs/\delta = kDSs/\delta \qquad \text{(Eq. 4)}$$

where Cs equals kSs. For the purposes here Ss is the saturation solubility of a neutral drug in body fluids, and k is a proportionality constant. When the animal's body temperature is varied, the terms D, Ss, and δ all should vary independently with temperature. Equation 4 can be written as

$$(\bar{R}/\bar{A})r = \frac{(\bar{R}/\bar{A})}{(\bar{R}/\bar{A})^\circ} = \frac{(k)}{(k)^\circ}\cdot\frac{(D)}{(D)^\circ}\cdot\frac{(Ss)}{(Ss)^\circ}\cdot\frac{\delta^\circ}{\delta} = Kr \cdot Dr \cdot (Ss)r \cdot (1/\delta_r) \qquad \text{(Eq. 5)}$$

where $(\bar{R}/\bar{A})r$ is the relative absorption rate or the mean absorption rate per mean area, $\bar{R}/\bar{A}$, at an absolute temperature T divided by the mean absorption rate per mean area, $(\bar{R}/\bar{A})^\circ$, at 273.2° A or T°, and the superscript zero associated with other terms in Eq. 5 means that the term also must be evaluated at 273.2° A, Kr is a constant whose value should be unity, Dr is the relative diffusion coefficient, $(Ss)r$ is the relative solubility, and δ_r is the relative diffusion layer thickness.

Relative Diffusion Coefficient, *Dr*.—According to the Einstein-Stokes formula (4), the diffusion coefficient of a large molecule in water may be approximated by

$$D = k'T/6\pi\eta r \qquad \text{(Eq. 6)}$$

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where k' is Boltzmann's constant, T is the absolute temperature, r is the Stokes' radius of the diffusing molecule, and η is the viscosity of the medium. Equation 6 may be rewritten for the two temperatures as

$$Dr = \frac{D}{D^\circ} = \frac{T}{T^\circ}\cdot\frac{\eta^\circ}{\eta} \qquad \text{(Eq. 7)}$$

where it is assumed that the Stokes radius r equals r°, and the other terms have the same meaning as before.

For water it can be shown that

$$\left(\frac{\eta^\circ}{\eta}\right)_{H_2O} = \left(\frac{\eta r^\circ}{\eta r}\right)_{H_2O} \qquad \text{(Eq. 8)}$$

where ηr and ηr° are the relative viscosities of water at temperatures T and T°, respectively. Since the viscosities of frog body fluids containing the drugs used in this study have not been measured as a function of temperature, it is convenient to make use of Hess' rule, which states for a biological medium having a fixed protein concentration that

$$\left(\frac{\eta r^\circ}{\eta r}\right)_{H_2O} \cong \left(\frac{\eta r^\circ}{\eta r}\right)_f \qquad \text{(Eq. 9)}$$

where $(\eta r^\circ/\eta r)_f$ is the ratio of the relative viscosities of body fluids, like blood, serum, or plasma, at temperatures T° and T (5, 6).

Relative Solubility (Ss)r.—If it can be assumed that the ratio of drug solubility in body fluids to that in water is equal to a constant that is independent of temperature, then one can write

$$\left[\frac{(Ss')}{(Ss')^\circ}\right]_{H_2O} = \left[\frac{(Ss)}{(Ss)^\circ}\right]_f \qquad \text{(Eq. 10)}$$

where $[(Ss/(Ss)^\circ]_f$ is the ratio of the solubilities of drug in the fluids at the implantation site at the temperature T and T°.

Relative Diffusion Layer Thickness, δr.—The thickness of the diffusion layer at a given temperature may be estimated from the equation discussed by Jost (7)

$$\delta \cong \sqrt{\eta L/v\rho} \qquad \text{(Eq. 11)}$$

where η is viscosity in poise, L is the linear dimension of surface of solid across which the medium flows in cm., v is the velocity of flow of the medium as a result of stirring in cm. sec.$^{-1}$, and ρ is the density of the medium in Gm. cm.$^{-3}$ Equation 11 may be written as

$$\delta r = \frac{\delta}{\delta^\circ} = \left(\frac{\eta L/v\rho}{\eta^\circ L^\circ/v^\circ\rho^\circ}\right)^{1/2} \qquad \text{(Eq. 12)}$$

where the use of the superscript zero has the same meaning as before. Under conditions of this experiment, it can be assumed with little error that $L = L^\circ$, since only a small weight was absorbed from each pellet, and that $\rho \cong \rho^\circ$, since water density is not a sensitive function of temperature in the 0–30° C. range. The terms v and v° are not necessarily equal, since normal body movement (or stirring velocity) is often temperature dependent (see *Discussion*). In this experiment the influence of body movement was minimized so that $v \cong v^\circ$. The only terms greatly influenced by temperature changes are η and η°. Equation 12 may be simplified to give

$$\delta r \cong (\eta/\eta^\circ)^{1/2} \qquad \text{(Eq. 13)}$$

By making use of the arguments previously proposed, Eqs. 8 or 9 may be substituted into Eq. 13 to give

$$\delta_r = (\eta r/\eta r^\circ)_{H_2O}{}^{1/2} = (\eta r/\eta r^\circ)_f{}^{1/2} \qquad \text{(Eq. 14)}$$

Substitution of Eqs. 7–10, 12, and 14 into 5 gives for an aqueous *in vitro* system that is slowly stirred

$$(\bar{R}/\bar{A})_r = Kr(T/T^\circ)\,(\eta r^\circ/\eta r)_{H_2O}{}^{1.5}[(Ss')/(Ss')^\circ]_{H_2O} \qquad \text{(Eq. 15)}$$

and for an *in vivo* system

$$(\bar{R}/\bar{A})_r = Kr(T/T^\circ)(\eta r^\circ/\eta r)_f{}^{1.5}[(Ss/(Ss)^\circ]_f \qquad \text{(Eq. 16)}$$

Thus, if $(\bar{R}/\bar{A})_r$ is plotted against all the righthand terms in Eqs. 15 or 16 (except Kr), a linear plot should result having a slope of Kr.

MATERIALS AND METHODS

Implants.—Thin, cylindrical disks of acetanilid and sulfanilamide having weights in the range of 51.0 to 83.0 mg. and of 63.1 to 100.7 mg., respectively, were compressed in a similar manner to that described previously (2), except that the drug powders were first moistened with distilled water and punched wet. The pellets were then air dried for 48 or more hours before weighing. Pellets formed by this technique were hard and more suitable for implantation than those prepared by the dry method.

Drug grade materials were used without further purification. The melting points of acetanilid and sulfanilamide were 114° C. and 163° C. (corrected), respectively. No binders, excipients, dilutents, or lubricants were used.

TABLE I.—ACETANILID ABSORPTION DATA AND PROPERTIES OF DRUG AND MEDIUM AT VARIOUS TEMPERATURES

t, °C.	No. of Animals	$\bar{R}/\bar{A}$[a] Gm./hr./cm.^{2} × 10^4	$(\bar{R}/\bar{A})_r$	Solubility Gm./100 ml. H_2O	$(Ss)_r$	$\frac{Dr}{\delta r}$[b]	$\frac{(Ss)_r Dr}{\delta r}$
0	8	3.57 (±0.033)	1.00	0.361	1.00	1.00	1.00
9	6	7.59 (±0.68)	2.13	0.432	1.20	1.59	1.91
15	6	13.69 (±0.15)	3.83	0.497	1.38	2.08	2.87
20	7	16.60 (±0.27)	4.65	0.563	1.56	2.55	3.98
25	6	22.50 (±0.31)	6.30	0.641	1.78	3.10	5.52
29	6	27.36 (±1.00)	7.66	0.712	1.97	3.59	7.07

[a] Mean absorption rate per mean area, followed by the 95% confidence interval in parentheses. [b] Values in this column can be calculated by either of two methods: Substitution into the empirical equation which holds for the temperature range 0 to 33° C. $[Dr/\delta r = 1.00 + 5.5713 \times 10^{-2} t + 1.0572 \times 10^{-3} t^2 + 3.65 \times 10^{-6} t^3]$ where t is in degrees C. $Dr/\delta r$ at 15° C. = 2.08. Substitution into Eqs. 7, 8, and 14. At 15° C. the ratio equals $Dr/\delta r = T/T^\circ(\eta r^\circ/\eta r)_{H_2O}{}^{1.5} = (288.2°/273.2°)^{1.5}(1.000/0.6363)^{1.5} = 2.08$.

TABLE II.—SULFANILAMIDE ABSORPTION DATA AND PROPERTIES OF DRUG AND MEDIUM AT VARIOUS TEMPERATURES

t, °C.	No. of Animals	$\overline{R}/\overline{A}$[a] Gm./hr./cm.^{2} × 10^4	$(\overline{R}/\overline{A})_r$	Solubility Gm./100 ml. H_2O	$(Ss)_r$	D_r/δ_r	$(Ss)_r D_r/\delta_r$
0	6	0.0907 (±0.026)	1.00	0.158	1.00	1.00	1.00
10	7	0.3096 (±0.037)	3.41	0.302	1.91	1.66	3.18
15	6	0.6149 (±0.193)	6.78	0.400	2.53	2.08	5.27
20	11	0.8201 (±0.085)	9.04	0.530	3.35	2.55	8.54
25	6	1.4033 (±0.13)	15.47	0.728	4.61	3.10	14.29
29	6	2.1155 (±0.429)	23.32	0.931	5.89	3.59	21.15

[a] Mean absorption rate per mean area, followed by the 95% confidence interval in parentheses.

Temperature Bath.—One to two 2000-ml. beakers, each containing layer of about 1 cm. of tap water, were immersed to a depth of about two-thirds their height into a constant temperature bath regulated to ±0.5°. A cardboard cover with a small hole was placed over each beaker opening to minimize the formation of air convection currents. A thermometer was inserted through the cover, and the bulb was rested in the water layer. A short piece of laboratory rubber tubing about 30 cm. long was placed on the bottom of the beaker with the coil touching the edge.

Animals.—Frogs of each sex (*Rana pipiens*) having a weight range of about 30 to 70 Gm. were injected in the ventral lymph sac with a solution of *d*-tubocurarine chloride (Upjohn). The dose of this drug needed to cause complete paralysis has been previously reported (8–11), although the dosage reported by King (9) seems to be low by a factor of 10. The dose used in this work was 10 mg./Kg., which usually gave a paralysis of sufficient duration to complete the experimental run. If any voluntary movement was observed during the course of an experiment, the animal received another smaller maintenance dose.

For temperatures above 0° C., three to four paralyzed frogs were partially immersed in the water layer of each beaker with their pharyngeal regions resting on the rubber tubing. The frogs were kept there for 15 minutes or longer until temperature equilibrium between them and the bath was attained. For the 0° C. point, all the paralyzed frogs were placed in an insulated chest containing chopped ice (12). Most of their bodies were covered with a thin layer of ice.

After temperature equilibrium was attained, the frogs were removed briefly one at a time from the temperature controlled environment, and an incision of about 0.75 cm. was made through the dorsal skin. Two preweighed pellets were inserted into the dorsal lymph sac. The frogs were then rapidly returned to their previous environment for the duration of the experimental run. At that time, the pellets were removed from the animals, placed on filter paper, and gently moved about to remove traces of biological fluid from their surfaces. The pellets were air dried for 48 hours or more. The time of implantation was adjusted so that pellets would lose from 5 to 10 mg. during the experiment. Data obtained from frogs which did not have an observable heart beat after the experiment were discarded. The mean absorption rates per mean areas of the pellets were calculated by a graphic method (13), except that a "ghost" correction was not made, since the duration of implantation was short.

Solubilities and pK Values.—Numerous reports on the water solubility of acetanilid as a function of temperature have appeared (16–19). The data given by Logan (18, 19) were used in this study because they seemed most reliable. The solubilities were recalculated to give solubility of drug in grams per 100 ml. of water. For purposes of interpolation the data were fitted to a third degree polynomial in temperature to give [solubility in H_2O (Gm./100 ml.) = $0.3611 + 6.3034 \times 10^{-3} t + 1.7064 \times 10^{-4} t^2 + 9.9729 \times 10^{-7} t^3$] where t is in degrees centigrade and includes the range from 0–30° (see Table I, column 5). Literature values for sulfanilamide water solubilities at various temperatures were converted to solubility in grams per 100 ml. water (20–22). A plot of log solubility *versus* $1/T$ was made over the temperature range 0–38°. A smooth curve was fitted by eye, and only points falling near or on this line were used in estimating the water solubility. Sulfanilamide solubilities were fitted to [solubility in H_2O (Gm./100 ml.) = $0.158 + 1.4101 \times 10^{-2} t - 1.8289 \times 10^{-4} t^2 + 2.1225 \times 10^{-6} t^3$] where t is in degrees centigrade (see Table II, column 5). Salt effects on the solubilities of the two drugs were considered small in biological fluids and were ignored.

The influence of drug pK on its solubility can be ignored at physiological pH's. The pK's for acetanilid (23–25) and sulfanilamide (20, 26–28) indicate that the compounds behave as neutral species under conditions of this experiment. Drug solubility should, therefore, be independent of diffusion layer pH (2).

RESULTS

Tables I and II summarize experimental and literature values needed to solve Eq. 16 for acetanilid

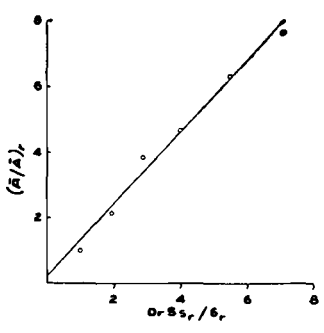

Fig. 1.—A plot of $(\overline{R}/\overline{A})_r$ *vs.* $Dr\ (Ss)r/\delta r$ for acetanilid. The line represents a plot of the regression equation, $Y = 0.203 + 1.09\ X$. The standard error of the estimate, S.E., is 0.271.

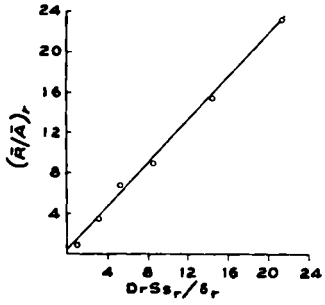

Fig. 2.—A plot of $(\bar{R}/\bar{A})r$ *vs.* $Dr(Ss)/\delta r$ for sulfanilamide. The line represents a plot of the regression equation, $Y = 0.130 + 1.09\ X$. The standard error of the estimate, S.E., is 0.450.

and sulfanilamide absorption. Figures 1 and 2 show plots of $(\bar{R}/\bar{A})r$ *versus* $Dr(Ss)r\ (1/\delta r)$ for the two compounds. Examination of Figs. 1 and 2 indicates that the slope, Kr, for the two compounds is approximately unity. For acetanilid and sulfanilamide, the equations for the straight line by the least squares method were $Y = 0.203 + 1.09\ X$ with a standard error of the estimate, S.E., of 0.271, and $Y = 0.130 + 1.09\ X$, with a standard error of the estimate of 0.450, respectively. Thus, there is a reasonable agreement between the theoretically predicted slope of unity and the experimental slopes.

DISCUSSION

Absorption Kinetics.—The linear relationship shown in Figs. 1 and 2 gives additional support to our view that the rate-limiting step in absorption after implant of the neutral drugs used was the dissolution process at the absorption site. The two drugs were largely dissimilar with respect to their chemical, physical, and pharmacological properties; yet, when suitable approximations were made for the temperature dependency of some of the terms in Eq. 16, the relative absorption rate could be described in terms of the physicochemical properties of drug and the fluids at the absorption site.

Variables in Absorption Equations.—In Eq. 1 the diffusion coefficient term, D, should be considered as being an apparent one. When neutral drugs are dissolved into biologic media at the dissolution site, drug-protein interactions would be expected to occur. If M refers to a drug molecule and P refers to one of many different protein molecules present which could potentially interact with the drug, then a large number of drug-protein species could be postulated having formulas of the form, $M_x \cdot P_y$, where x and y could have values of one or greater. Each species could be a diffusable one, or be a carrier for the drug, and would have a diffusion coefficient of its own. Under steady state conditions at any temperature, the apparent diffusion coefficient for the overall process equals the diffusion coefficient for a particular drug containing species times the fraction of the total diffusing drug represented by this species summed over all possible drug containing species. If it can be assumed that the extent of protein binding for each species is relatively independent of temperature, then the mathematical treatment shown in Eqs. 3 and 5 should be approximately correct. In this connection, Klotz and Urquhart (32) have shown with certain organic anions that the heat of protein binding is quite small.

In Eqs. 6 to 9 it was assumed that the relative diffusion coefficient of drug in water and biological fluids as a function of temperature could be estimated satisfactorily by the Einstein-Stokes equation (4), assuming that the Stokes law radius of the diffusing species in solution was temperature independent. A consideration of Longsworth's data (15) for dextrose, a neutral molecule having a molecular weight of the same order of magnitude as acetanilid and sulfanilamide, allows one to make an *in vitro* test of this assumption. Table III shows the experimental diffusion coefficients for dextrose at the four temperatures 1, 13, 25, and 37°, the observed relative diffusion coefficient, which equals the diffusion coefficient at any temperature divided by the value at 1° C., and the calculated relative diffusion coefficient using the relationship

$$Dr \text{ calcd.} = \frac{T}{T^{1°}} \cdot \left(\frac{\eta r^{1°}}{\eta r}\right)_{H_2O} \quad \text{(Eq. 17)}$$

When Eq. 8 is substituted into Eq. 7, an expression similar to Eq. 17 is found, except that the reference temperature for Eq. 17 is 1° C. instead of 0° C. The percentage error made by Eq. 17 did not exceed 2.7% over the 36° C. temperature range. Thus, the temperature dependency of the Dr term used in Eqs. 15 and 16 could be satisfactorily accounted for by the application of the Einstein-Stokes and the Einstein-Stokes-Hess equations.

In Eq. 10 it was assumed that the ratio of a neutral drug's solubility at two temperatures in water equalled the ratio of the drug's solubility at the same two temperatures in biological fluids. In the case of sulfanilamide this statement seems to hold. The van't Hoff equation is

$$\log Ss = \frac{-\Delta H}{2.303\ RT} + \text{constant} \quad \text{(Eq. 18)}$$

where Ss is the drug solubility in biological fluids, ΔH is the heat of solution in Kcal. per mole, T is the absolute temperature, and R is the gas constant. An analogous expression could be written for the solubility, Ss', of drug in water. If Eq. 10 is valid, then the heats of solution of drug in biological fluids and water should be nearly identical. Clark, *et al.* (29), found the solubilities of sulfanilamide in human serum to be 0.981 and 1.970 Gm. per 100 Gm. solvent at 25 and 37° C., respectively. The calculated value for ΔH from their data was 10.7 Kcal./mole. This value agrees favorably with the heat of solution of 10.9 Kcal. per mole reported by Kienle and Sayward (30) for the drug in water over the same temperature range. Clark, *et al.* (29), also gave water solubility values for sulfanilamide at these two temperatures, but their value at 25° C. seems high, while the value at 37° C. is in close agreement with that given by Kienle and Sayward (30).

TABLE III.—DIFFUSION COEFFICIENTS (15) FOR DEXTROSE

t, °C.	1	13	25	37
$D \times 10^6$ cm.^{2} sec.$^{-1}$	3.137	4.736	6.728	9.088
Dr observed	1.000	1.510	2.145	2.897
Dr calcd.	1.000	1.502[a]	2.107	2.820

[a] Dr calculated (Eq. 17) = [(286.2)/(274.2)·(1.0000)/(0.6947)] = 1.502.

Of the four independent variables in Eq. 11 for the thickness of the diffusion layer, only two are highly sensitive to changes in temperature. They are the viscosity of the medium, η, which was corrected for by a modification of Hess' rule, and the velocity of flow of the biological medium, v, as a result of body movement or stirring. In biological systems body movement is often temperature dependent. Crozier and Stier (31) have shown that the pharyngeal respiratory rhythm of frogs (*Rana pipiens*) was temperature dependent and followed the Arrhenius equation

$$k = A \exp[-E/RT] \qquad \text{(Eq. 19)}$$

where k is the reaction rate, A is a constant, E is the activation energy, and R and T have the same meanings as before. Crozier and Stier (31) found that the experimental activation energy for the rate of frog pharyngeal movement was 8.8 Kcal. In this experiment, it was important that animal body movement should be controlled so that the influence of temperature on drug absorption rate could be readily estimated. The method chosen here was that of paralyzing the frog with large doses of *d*-tubocurarine chloride, so that the velocity of stirring or movement of body fluids was biologically nearly zero. Body movement never can be reduced to zero, since heart function must be maintained. The isolated frog (*Rana temporaria*) heart rate has been shown to be temperature dependent (14). Little could be done to control this variable in body movement, except to note that *d*-tubocurarine in the frog was reported to cause slowing of heart rate in addition to its other effects (11).

The fact that the slopes of the lines in Figs. 1 and 2 are slightly greater than unity indicates that the term Kr might not be completely temperature independent. If experimental errors are ignored, there are at least three reasons why the slope should be greater than unity. First, the value for Dr as calculated from Eq. 17 is always slightly smaller than Dr observed, excepting the 1° C. value. Second, the density of water (and presumably body fluids saturated with drug) decreases at temperatures below and above 4° C. In Eq. 12 at temperatures a little greater than 8° C., ρ° for water is slightly greater than ρ. And third, heart rate, a source of body movement even in curarized animals, is probably greater at higher temperatures.

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ERRATUM

In the paper titled "Production of Pyridoxine and Niacin by *Chlorella vulgaris* and *C. pyrenoidosa*" (1), Table III at page 152 should be clarified to read:

Table III.—Value of *C. vulgaris* Relative to *C. pyrenoidosa* as a Source of Vitamin B_6 and Niacin at Different Harvest Times

Vitamin	mmcg. per mg. Dry Wt. 2 wks.	mmcg. per mg. Dry Wt. 3 wks.	mmcg. per ml. Culture 2 wks.	mmcg. per ml. Culture 3 wks.
	1	*2*	*3*	*4*
B_6	0.66	0.90	0.57	0.75
Niacin	1.06	1.18	0.93	1.07

(1) Pratt, R., and Johnson, E., This Journal, **53**, 151 (1964).