Relationship between pH and Antibacterial Activity of Glutaraldehyde

Keyphrases □ Glutaraldehyde—relationship between pH and antibacterial activity □ Antibacterial activity—relationship to pH, glutaraldehyde □ Bactericidal activity—aldehyde groups, relationship between pH and antibacterial activity of glutaraldehyde

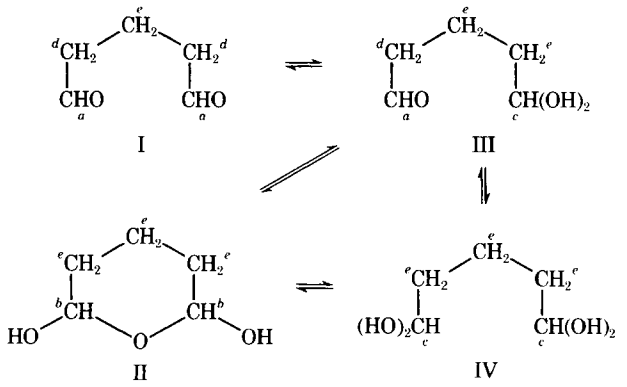
To the Editor:

Considerably enhanced bactericidal activity is achieved by adjusting glutaraldehyde solutions to pH 7.5–8.5 (1–5). In aqueous solution, glutaraldehyde undergoes rapid hydration, the free dialdehyde (I) being in equilibrium with the hydrates II–IV (Scheme I). Using PMR spectroscopy, Hardy *et al.* (6) studied this equilibrium in D_2O solution and reported signals at τ 0.28, 4.7, 5.0, 7.4, and 8.3, corresponding to protons *a*, *b*, *c*, *d*, and *e*, respectively. On the assumption that there was no free dialdehyde (I) present in aqueous solution, these authors described the equilibrium by examination of the ratio of the *b*, *c*, and *d* signal integrals after correction to an equivalent number of protons. Their results indicated that the equilibrium was not significantly modified by concentration.

Rubbo *et al.* (4) concluded, from their study on the bactericidal activities of a series of aldehydes, that the presence of free aldehyde groups was a prerequisite for activity.

Munton and Russell (7) suggested that the enhanced bactericidal activity in alkaline solution probably resulted from a modification of the glutaraldehyde molecule, the bacterial cell, or a combination of both. These workers (8) later suggested that the effect at increased pH was primarily on the outer layers of the cell, since increased polymerization, which is associated with increased free aldehyde concentration, was not observed with mild alkalinization.

No conclusive experimental data have yet been presented to show whether the enhanced bactericidal activity of glutaraldehyde in alkaline solution is a result of: (*a*) change in the free aldehyde–hydrated aldehyde equilibrium, (*b*) modification of the outer layers of the bacterial cell, or (*c*) a combination of both of these effects.



Scheme I

Table I—Effect of pH on Percentage of Free Aldehyde in Aqueous Glutaraldehyde Solutions

Glutaraldehyde Concentration, % w/v	pH	Free Aldehyde in Equilibrium Mixture[a], %
50	2.9	47.00
25	2.9	47.02
10	3.2	47.02
10	6.0	47.03
10	7.9	47.01
5	3.4	46.08

[a] Mean value calculated from corrected τ 8.3/τ 0.25 and τ 8.3/τ 7.3 integral ratios.

The PMR spectrum of aqueous glutaraldehyde was determined[1] (internal standard 4,4-dimethyl-4-silapentane sulfonate, 1%) and exhibited signals at τ 0.28, 0.73, and 0.83 (protons *a*, *d*, and *e*, respectively). Signals due to protons *b* and *c* were not observed due to overlap with the water signal (τ 4.7–5.0).

When assuming the absence of free dialdehyde (I) in aqueous solution (6), examination of the ratios of the integral of the signal at τ 8.3 (protons *c*) with that at τ 7.3 (protons *d*) or τ 0.28 (protons *a*) after correction to an equivalent number of protons (9) gives the ratio of hydrated aldehyde (II and IV) to free aldehyde (III) in the equilibrium mixture. The results (Table I) indicate that the free aldehyde–hydrated aldehyde equilibrium is not significantly modified by adjustment of the glutaraldehyde solution from pH 2.9 to 7.9.

Results of a study undertaken by Rubbo *et al.* (4) suggested that the bactericidal activities of glutaraldehyde and β-methylglutaraldehyde are related to the proportion of free aldehyde in the equilibrium mixture. Glutaraldehyde is less readily hydrated than β-methylglutaraldehyde and exhibits superior bactericidal activity. Thus, it appears that a shift of the free aldehyde–hydrated aldehyde equilibrium in favor of the hydrated aldehyde may result in a reduction in bactericidal activity.

Since no significant change in the free aldehyde–hydrated aldehyde equilibrium over the pH 2.9–7.9 range was observed (Table I), clearly the enhanced bactericidal activity of glutaraldehyde in alkaline solution is not a result of modification of the existing equilibrium. Therefore, it would appear that the alkali exerts its effect solely on the bacterial surface.

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Effect of Plasma Protein Binding on Elimination of Warfarin

Keyphrases □ Warfarin—effect of plasma protein binding on elimination, pharmacokinetics □ Protein binding, plasma—effect on elimination of warfarin, pharmacokinetics □ Elimination, warfarin—effect of plasma protein binding, pharmacokinetics

To the Editor:

Studies in humans and rats have shown pronounced intersubject variation in the elimination of the coumarin anticoagulants dicumarol and warfarin (1–4). Detailed investigations in rats revealed that this intersubject variation may be associated with large differences in the distribution of these drugs in the body (3, 4). Similar changes in distribution and elimination were produced in perfused isolated rat liver preparations by varying the concentration of plasma proteins in the perfusion solution (5). These findings raised the possibility that some intersubject variation in the elimination of coumarin anticoagulant drugs may be related to differences in the binding of these very extensively protein bound drugs to plasma proteins. It is well recognized that differences in the activity of coumarin drug-metabolizing enzyme(s), particularly due to enzyme induction or inhibition, can also account for some intersubject variation in the elimination of these drugs (6, 7). The purposes of this communication are: *(a)* to present a theoretical pharmacokinetic formulation of the relationship between plasma protein binding and the kinetics of elimination of drugs such as dicumarol and warfarin, and *(b)* to describe, preliminary to a more detailed report, experimental data in support of the pharmacokinetic theory.

Let it be assumed that: *(a)* the kinetics of elimination are apparent first order, *(b)* elimination occurs from the central compartment, *(c)* the driving force of the rate-limiting steps of the elimination processes is the concentration of free (nonprotein bound) drug, *(d)* elimination is rate limited by these processes rather than by organ perfusion rate, *etc.*, and *(e)* the extent of plasma protein binding is essentially constant over the concentration range of therapeutic or experimental interest. The last assumption is realistic for drug concentrations at which only a small fraction of the binding sites on plasma proteins is occupied (8).

The sites of drug elimination (in the liver and elsewhere in the central compartment of the body) may be viewed as being surrounded by an aqueous solution of free drug. This aqueous solution represents a physiological space consisting of plasma water and other water exclusive of dissolved macromolecules capable of binding the drug. The volume of this space is designated as V_w. The rate of elimination, $-dA/dt$, of the drug is then proportional to the amount of drug[1] in V_w:

$$-dA/dt = k'fC_pV_w \qquad \text{(Eq. 1)}$$

where k' is a first-order elimination rate constant (which may be the sum of several rate constants for different elimination processes), which relates the amount of drug[1] in V_w to the rate of elimination[2]; C_p is the concentration of total (free and protein bound drug) in the plasma; and f is the fraction of free drug in the plasma. Designating the product of k' and V_w as k'' (which may be viewed as an intrinsic clearance constant) and converting $-dA/dt$ to a concentration term yield:

$$-(dC_p/dt)V_{area} = k''fC_p \qquad \text{(Eq. 2)}$$

where V_{area} is the volume of distribution of the drug in the body as previously defined (9). Unlike V_w, which is considered to be a physiological constant, V_{area} is a parameter that is affected by changes in protein binding. Rearrangement of Eq. 2 yields:

$$-(dC_p/dt)V_{area}C_p^{-1} = k''f \qquad \text{(Eq. 3)}$$

or:

$$\text{total plasma clearance} = k''f \qquad \text{(Eq. 4)}$$

Thus, a plot of the total plasma clearance of a drug *versus* the fraction of nonprotein bound drug in the plasma should be linear and go through the origin.

The elimination rate constant and the apparent volume of distribution of warfarin were determined in a selected group of 13 adult male Sprague–Dawley rats as previously described (4), except that the drug was administered intravenously. Serum protein binding[3] was determined by equilibrium dialysis using ^{14}C-warfarin. The fraction of free drug differed widely between animals (from 0.002 to 0.015), but it

[1] This is free drug only, by definition.
[2] The constant k' may also incorporate a partition coefficient to account for possible differences in the concentration of free drug in plasma water and other parts of V_w (such as intracellular water), perhaps due to differences in pH.
[3] Serum rather than plasma was used to prevent possible interference by anticoagulants in the protein binding determination.