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Silver et al. respond:

To the Editor:

We welcome Mr. Irish’s interest in our paper (1) and are grateful for this opportunity to clarify and expand on our brief reference to accelerated degradation testing. The papers referred to by Irish review the use of the Arrhenius equation to relate the degradation rate constant (k) to absolute temperature (T). In simplest form,

\[ \ln(k) = \ln(A) - \frac{E}{RT} \]  

where A is a constant, R is the gas constant, and E is the activation energy of the degradation process. Measurement of the degradation rate at several temperatures allows determination of values of A and E for the material under test so that the absolute degradation rate at low temperature may be predicted. Such a result is of great importance in defining the storage stability of biological standards, and this justifies the careful experimental methodology described by Kirkwood (2).

In the development of immunodiagnostic reagents, a simple preliminary test of likely storage stability may be helpful. It is a widely held rule of thumb (especially among those involved in the production of commercial assay kits) that “stability for a week at 37 °C is equivalent to stability for at least a year at 4 °C.” The basis of this simple rule may be understood as follows. Relative stability (degradation rates) at two different temperatures, To (upper) and TL (lower), may clearly be inferred from equation 1, because

\[ \ln(\frac{k_T}{k_L}) = \frac{E}{RT_L} - \frac{E}{RT_U} \]  

Moreover, only the value of E is required for application of equation 2. Because the degradation process is assumed to be a first-order process (2), the time (t) required for degradation to proceed until a fraction (F) of original potency remains is given by

\[ t = -\frac{\ln(F)}{k} \]  

and hence equation 2 may be written

\[ \ln(\frac{t_I}{t_U}) = \frac{E}{RT_L} - \frac{E}{RT_U} \]  

which now relates the different times taken to reach the same extent of deterioration at the two temperatures. If a reagent formulation is stable (according to appropriately defined performance criteria) for the time tU at temperature TL then it may be predicted to be stable for time tI at temperature TI.

From equation 4 it is readily shown that those using the simple accelerated stability rule are assuming, either implicitly or explicitly, that E is about 20 kcal/mol or greater. Such a range has presumably become accepted, because experience shows that it often yields correct predictions of the actual long-term stability at 4 °C of well-formulated products. Protein denaturation and enzyme inactivation—likely causes of deterioration in diagnostic reagents—may typically show activation energies in this region (3). An E value of 20 kcal/mol would also be appropriate for degradation proceeding by solvolysis (4) and such a value is common in practice in similar preliminary accelerated stability testing in the pharmaceutical industry (5).

We now have some premixed antiserum reagents (1) that has been stored at 4 °C for two years and this shows no decrease in binding in the standard curve as compared with newly-prepared premixed reagent. Quality-control samples read 8.3, 33, 62, and 125 mg/L on using two-year-old reagent as compared with 8.5, 32, 61, and 135 mg/L with new reagent. We therefore conclude that in this case the simple accelerated-stability rule gave a valid prediction.

The studies required for the full application of the accelerated degradation test to characterize completely the stability properties of a given material (2) are too complex for routine use in most laboratories. The simple rule as outlined above is widely used and we believe it can be of value provided that its limitations are recognized.

References


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Effect of Time of Exposure of Serum to Gel-Barrier Tubes on Results for Progesterone and Some Other Endocrine Tests

To the Editor:

In a recent Letter, Smith (1) points out that gel-barrier tubes, if used as sample-collection devices for progesterone assays, can cause results to be decreased as compared with samples collected in plain tubes. In his study the blood was exposed to the gel for only 1–2 h, whereas in practice the blood could be centrifuged in a gel-containing tube and stored in the same tube for one or two weeks. To investigate whether the value measured for progesterone would decrease as a function of time exposed to the gel, we did the following experiment. We collected

Fig. 1.

Time (Days)

Progesterone (mol/L)

Plain Tube

Tube with Gel Barrier

20

40

60

0 1 2 3 4 5 6

CLINICAL CHEMISTRY, Vol. 33, No. 1, 1987 203