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The authors of the paper in question respond (Mrs. McCluskey is deceased):

To the Editor:

Dr. Levine has correctly drawn attention to potential theoretical weaknesses in the technique developed in our laboratories. We believe, however, that his criticisms somewhat misrepresent our findings and may not be completely warranted. The following points might be made:

(a) There appears to be some confusion in the basis for Dr. Levine's critique. He equates Roth's (1) method, as used by us, with that of Krasner et al. (2, 3). Though both are fluorescent techniques, with similar (but not identical) spectral characteristics, there are substantive differences. Krasner et al. did experiments under approximately physiological conditions while our experiments were in the presence of high concentrations of phosphoric acid. Under the latter conditions, it is almost certain that the properties of the binding site have altered significantly. Thus, we do not see how it is possible for Levine to extrapolate from results reported by Krasner et al. concerning both the fluorescence yield and the results of competition experiments (with particular respect to sulfisoxazole) in order to repudiate our results.

(b) Our method also differs slightly from that of Roth in that we used human serum albumin rather than bovine serum albumin and there are distinctive differences in the mode of binding of bilirubin to these two protein molecules (4). Further, a titration of a constant concentration of bilirubin with variable albumin by our technique gives a curve very similar to the curve in Figure 2A of Beaven et al. (5), viz. a sharp inflexion at 1:1, followed by a much more gradual rise in fluorescence intensity.

(c) We would emphasize that Levine's criticisms of our work are based on results reported from experiments designed to determine the reserve binding capacity of albumin. We have felt it more important to try and assess the excess bilirubin beyond that tightly bound to albumin, as this is the problem in the jaundiced neonate.

(d) We do not believe that any meaningful statement can be made about the properties of photoxidation products of bilirubin (which could remain bound to albumin) in the absence of knowledge of their identity.

(e) The correlation between "albumin-titratable bilirubin" and the bilirubin/albumin ratio was only considered as supportive evidence that a clinically significant value was being observed. The more important correlation was considered to be with the saturation index of Odell et al. (6). This appears to measure a similar biological function by an independent method and has proved of predictive value (7).

(f) Finally, we consider it unfortunate that Dr. Levine feels that our results should be dismissed on theoretical grounds, without advancing supportive experimental evidence for his views. We have advanced the method as a possible clinical test, and follow-up studies of the patients involved is the only way of establishing its usefulness.

References


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Misuse of Calibration
Sera in Automated Laboratory Testing Instruments

To the Editor:

A common practice in the operation of automated biochemical testing instruments is to test a single sample of "calibration serum" after every 10th or 20th patients' sample. Results for each such control are used immediately to "adjust" (recalibrate) the instrument for the constituent involved. Unless the constituent can be tested with perfect precision (zero variance of the results), this practice is certain to increase the testing error, just the opposite of what is intended.

The truth of this statement is illustrated with a hypothetical example. For instance, consider glucose assay. (In the following discussion, all values are in mg/100 ml.)

Assume that an instrument was initially calibrated perfectly, and then samples from a serum pool with a "true concentration" of 100 were tested many times without adjusting the instrument. A distribution of the results like that shown at the top of Figure 1 might be obtained. Their average would be 100, but the results would vary. This variation is the "indeterminate error" (random variation) of the biochemical method, instrument, and variation of the aliquots. The vertical lines indicate the ±2 SD (95%) range of the results. Each test is independent of all others, i.e., there is no "drift" or correlation of any kind among results.

In the above example, if the instrument were calibrated accurately, and left alone, it would produce the most accurate and precise results possible. But consider what happens if small, immediate adjustments are made with results of tests of single calibration samples interspersed among the patients' samples.

When the first calibration sample came along, suppose a reading of 97 was observed, and the analyst adjusted the scale to obtain 100 (first adjustment). This has the effect of adding 3 to all following results, as is illustrated. When the second calibration