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Dr. Smith responds:

To the Editor:

My purpose in investigating the effect of hemolysis on the accurate assessment of catecholamines and DOPA concentrations by the "Cat-A-Kit" radioenzymatic method was to provide a basis for rejecting hemolyzed specimens, and thereby reduce the ever-growing backlog of specimens awaiting analysis in my freezer. I was surprised and disappointed to find that I could not discard hemolyzed samples as being "unsuitable for analysis." I chose freezing as the means of inducing hemolysis because this best approximates the conditions encountered in the clinical setting, i.e., erythrocyte disruption from physical trauma (shaking of the blood-collection tube, use of a small-bore collection device, excessive vacuum, or excessively hard or prolonged centrifugation). Hemolysis induced by hypotonic solutions does not represent this situation, nor is it physiological.

There are several differences between the Cat-A-Kit method (1) and that of Brown and Jenner (2) used by Causon et al., including the specific activity of the labeled compound, the enzyme preparation, the extraction solvents used, and, perhaps most important, the concentration of nonisotopic O-methylated metanephrines added as carrier proteins. Saar et al. (3) demonstrated that the extraction efficiency of 3H-labeled methylated derivatives depends on the carrier concentration. The method of Brown and Jenner involves only 0.5 μg of carrier per assay tube or only 3% of the lowest concentration investigated by Saar et al. Because the low concentration of added carrier reduces the extraction efficiency of 3H-labeled metanephrines, and because the extraction of both 3H- and 3H-labeled metanephrines should be equivalent, then the ratio of 3H:3H-labeled metanephrines to [14C]metanephrines recovered (the basis for the estimation by Causon et al. of endogenous catecholamine concentrations from standard) will change with that reduced efficiency. The Cat-A-Kit method involves about 40 μg of each derivative (metanephrine and normetanephrine) per incubation volume, which is nearly the amount of carrier substance (45 μg) described by Saar et al. as "optimal for sensitivity." Possibly the addition of hemolysate to the incubation mixture may only further diminish the poor recovery and variability observed by Causon et al. Increasing the concentration of carrier may solve this problem, and I would be interested in seeing the results of a similar study after this change.

In my original paper I did not mean to imply that all radioenzymic methods were unaffected by specimen hemolysis. Surely, this must be determined for each method. However, I maintain that when sample hemolysis is believed to be caused by collection or processing techniques but is not associated with patient's stress, the specimen should be considered acceptable for measurement of free or sulfate-conjugated catecholamines or DOPA with the Cat-A-Kit.

References


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Instrumental Bias in Blood Gas Analysis of Tonometered Whole Blood

To the Editor:

Our laboratory became increasingly concerned with the problem of instrumental bias (1) when we became involved in a statewide program for proficiency testing. As a "reference" laboratory in the program, we reviewed quarterly data submitted by satellite laboratories. The program included blind analysis of a commercially prepared, aqueous quality-control product (General Diagnostics, Morris Plains, NJ 07950) provided in three vials, each of which had a different value for pH, PO2 and PCO2. Each laboratory used its own blood gas instrument for these measurements. Early in the program, the members of the reference committee noted a wide range of reported PO2 values in the test results from the participating laboratories and decided to determine whether the inconsistencies were due to the control product or to instrumental bias. Four laboratories from the initial program agreed to participate in a study of the variations that had appeared.

To eliminate any bias due to the aqueous product, we elected to use tonometered whole blood and five blood gas instruments that were automated to various degrees. These instruments represented all of the manufacturers whose machines had been used in the California proficiency testing program and thus were typical of the equipment with which the wide range of PO2 values had been obtained.

Under ideal conditions, all five instruments would have been studied in one laboratory (2). Because this was not practical, five technicians in the four laboratories, who had demonstrated their expertise in the original program, were chosen. The instruments and the laboratories were as follows: (a) a three-month-old semiautomated Corning 168 (Corning Medical, Medfield, MA 02052) at the Presbyterian of the Pacific Hospital, San Francisco; (b) a seven-year-old manual Radiometer BMSSMKII (London Co., Cleveland, OH 44145), with external cuvettes for measuring PO2 and PCO2 at Los Angeles County Medical Center; (c) a three-year-old IL 813 (Instrumentation Laboratory, Inc., Lexington, MA 02137) at Loma Linda University Medical Center; (d) a fully automated three-month-old Radiometer ABL-3 (London Co.) at University of California Medical Center at San Diego; and (e) a three-year-old IL 813 at University of California Medical Center at San Diego.

Following a 1977 protocol by Leary et al. (3) on the use of equilibrated blood for blood gas quality control, each laboratory obtained venous blood samples in EDTA-containing tubes.

This blood was screened and pooled to provide the necessary 60 mL each day for seven days.

All laboratories used an Instrumentation Laboratory tonometer (Model 237) to equilibrate their samples. Each operator was provided with specific instructions for tonometry (even though all of the laboratories, except Loma Linda, routinely used IL 237 tonometers for daily quality control). Tonometer bath temperature was regulated at 37°C before sample preparation and was closely monitored during the process. To preclude any contamination, the tonometer vessels, vessel chambers, and gas lines were sterilized before use. Before sample preparation, the entire system was purged of room air by allowing the equilibrating gas to run through the tonometer for 5 min at a flow rate of 500 mL/min. At the same flow rate, 8 mL of whole blood was added to the tonometer vessel and