Quality Control of Radiolabeled Leukocytes with Monoclonal Antibodies

TO THE EDITOR: A recent letter by Deborah A. Kaminsky in the May 1993 issue of the *Journal* highlights the importance of leukocyte (WBC) purification prior to labeling for autologous leukocyte imaging of infection (*1*). Traditional methods for WBC purification fail to adequately reduce the number of contaminating red blood cells (RBC) and platelets which may lead to false-positive studies or high background levels (*2*).

One method of removing excessive RBC is by hypotonic lysis. This method has been used and results indicate that viable and functional WBC populations are capable of producing reliable images of infected foci when labeled with ^{99m}Tc-HMPAO (*3*).

We have been studying the kinetics of WBC during separation and labeling with ^{99m}Tc-HMPAO by monitoring the expression of activation antigens CD11b and CD18 (*4*) during various stages of purification and labeling.

Results indicate that hypotonic lysis leads to a high degree of neutrophil activation and alters the relative proportions of the leucocytes by eliminating a significant percentage of the lymphocytes (Tables 1 and 2).

TABLE 1
Differential WCC During Separation and Labeling with ^{99m}Tc-HMPAO

Step no.	Differential WCC		
	%Neutrophil	%Lymphocyte	%Monocyte
1 Whole blood	65.9 ± 11.2	22.5 ± 9.4	7.4 ± 3.3
2 After sedimentation	64.9 ± 9.5	22.8 ± 9.8	7.4 ± 3.4
3 After RBC lysis	75.3 ± 1.5	11.0 ± 2.0	8.7 ± 3.2
4 After ^{99m}Tc-HMPAO	74.8 ± 7.2	12.4 ± 5.9	8.5 ± 3.2

Mean and standard deviation of eight patients.
Separation and labeling kinetics of WBCs with ^{99m}Tc-HMPAO.
RBC lysis is achieved by a 20-sec hypotonic lysis followed by re-buffering and washing.

TABLE 2
Neutrophil Activation During Separation and Labeling with ^{99m}Tc-HMPAO

Step no.	Activation index
1 Whole blood	1.00 ± 0.00
2 After sedimentation	1.09 ± 0.15
3 After RBC lysis	2.25 ± 0.94
4 After ^{99m}Tc HMPAO labeling	2.34 ± 1.17

Mean and standard deviation of eight patients.
Activation index = 0.5 (CD11b + CD18) − CD13.
Mean channel fluorescence normalized to whole blood value.
CD13 = Control anti-granulocyte monoclonal antibody.

Although we did not observe any loss in viability with trypan blue, the degree of activation caused by lysis may alter the functional capabilities of WBC, which could lead to variable results when interpreting the nuclear scans (*5*).

Monoclonal antibody activation studies appear to be useful in the quality control of leukocyte separation and labeling procedures.

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Effects of Erythromycin on Alcohol Absorption

TO THE EDITOR: We congratulate Edelbroek et al. (*1*) on a carefully done study relating increased gastric emptying due to erythromycin to an increase in the apparent absorption (systemic exposure) of alcohol, which they attribute in part to reduced metabolism of alcohol by the gastric mucosa. However, we take issue with this proposed mechanism and offer an alternative interpretation of their data.

Edelbroek et al. (*1*) found an inverse correlation between peak blood alcohol concentration (Cmax) and 50% emptying time of liquid during the control period but not the erythromycin period. Inspection of the data strongly suggests that all of the points from both study periods fall along a single regression line; estimation of the values from the graph indicates that the combined r value may be as great as −0.83, implying that up to 69% of the variability in Cmax can be explained simply by differences in gastric emptying. We have recently obtained similar results in a study of the effects of famotidine on the apparent absorption of alcohol (*2,3*). Changes in Cmax induced by famotidine correlated strongly, $r = -0.62$, $p = 0.001$, with changes in the time at which Cmax was achieved (Tmax), with Tmax being an indirect estimate of the liquid emptying rate. Mean plasma ethanol Cmax was increased 23% relative to control ($p = 0.013$) in 24 male subjects when famotidine (40 mg) was given with a standard breakfast 1 hr prior to a small oral ethanol dose (0.15 g/kg).

Recently, it has been suggested that there is no need to attribute changes in alcohol absorption induced by H_2-receptor antagonists to alterations in the activity of gastric alcohol dehydro-

genase (ADH) (*4*). The findings can be explained adequately by differences in hepatic first-pass metabolism related to the rate of delivery of alcohol to the liver: with more rapid delivery, more alcohol bypasses hepatic alcohol dehydrogenase. This explanation is supported by the finding that famotidine, which is not believed to have an appreciable effect on gastric ADH, can increase apparent alcohol absorption, and that a similar effect can be demonstrated in animals that lack gastric ADH (*5*).

We would, therefore, disagree with the final suggestion of the authors: that the effect of erythromycin on alcohol absorption might be of more concern in individuals with low gastric alcohol dehydrogenase activity, including those taking cimetidine. First, the effect of erythromycin in the subjects studied by Edelbroek et al. (*1*) was to make alcohol *fully* bioavailable. The volume of distribution of ethanol has been shown to be equivalent to total body water (~0.64 liter/kg) (*6*), so that the theoretical Cmax in this study resulting from complete distribution of the dose of 0.5 g/kg in 0.64 liter/kg would be 78 mg/dl, almost identical to the peak alcohol level observed −77 mg/dl. How could alcohol be made any more than fully bioavailable in selected populations? That would require *de novo* synthesis of alcohol! Second, the incremental increase in alcohol bioavailability with erythromycin is likely to be diminished, rather than enhanced, in those with low first-pass metabolism (an effect previously attributed to diminished activity of gastric ADH), such as females, fasting males and patients taking H2-receptor antagonists. In these subjects, alcohol is already more "fully" bioavailable, so that there is less alcohol remaining, out of the amount ingested, to become available for enhanced absorption due to accelerated gastric emptying. Third, the effect of H2-receptor antagonists is only demonstrable with very low alcohol loads (0.15 g/kg, compared with the 0.5 g/kg used by the authors), where even complete bioavailability would not raise Cmax alarmingly. In contrast, the motility effects of erythromycin, as shown nicely in this study, have an effect even with substantial alcohol loads.

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REPLY: Drs. Palmer and Burnham are correct in that, if our data are pooled (which would be statistically incorrect), about 70% of the variance in peak blood alcohol concentrations is accounted for by the rate of gastric emptying. In view of recent observations, including those made by the authors, the relative importance of hepatic and gastric alcohol dehydrogenase in first-pass metabolism of alcohol is contentious and dependent on the alcohol load (*1,2*).

We therefore agree that the impact of either reduced levels of gastric alcohol dehydrogenase, or decreased exposure to gastric alcohol dehydrogenase as a result of more rapid gastric emptying after erythromycin is uncertain.

Our study confirms that first pass metabolism of alcohol (either gastric or hepatic) is significant, in that the area under the curve (AUC) for alcohol was substantially greater after erythromycin.

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Application of the Effective Dose Equivalent to Nuclear Medicine Patients

TO THE EDITOR: The international nuclear medicine community has cause to be extremely grateful to the MIRD Committee for its seminal work on internal dosimetry and for the magnificent service it has provided over the years in tabulating invaluable basic data. We read the recent article on the application of effective dose equivalent (*1*) with great interest, but find it necessary to express serious concern. The opinions expressed therein on behalf of the MIRD Committee could unfortunately be described as ill-founded and unhelpful. They threaten to set back progress made in comparison of potential hazard from different medical procedures by ten years or more.

The effective dose equivalent (now known as effective dose) is indeed a weighted sum of doses to individual organs where the weighting factors are based upon estimates of relative risk of stochastic effects from irradiation of the different tissues. The concept was introduced by the ICRP as a means of relating inhomogeneous irradiation of the human body to a comparable whole-body radiation and its purpose was indeed initially to facilitate the protection of workers occupationally exposed to radiation. Its use has since been widely recommended for comparison of doses to patients from medical diagnostic procedures (*2–5*) and it has been found to be very useful for this purpose (*6,7*). It is accepted that its use is not appropriate for therapeutic procedures, where deterministic (nonstochastic) processes predominate.

Dr. Poston and the MIRD Committee now pronounce that use of this quantity for individual patients undergoing diagnostic nuclear medicine procedures is "inappropriate." They cite four reasons and we shall deal with each in turn:

1. It is stated that the effective dose equivalent was intended for radiation protection purposes and that the risks were to be compared with mortality in safe industries. This is not an argument against using the effective dose equivalent as a single figure indicator of hazard. The fact that a quantity is