LETTERS TO THE EDITOR

Re: Spatial and Temporal Quantitation of Plane Thallium Myocardial Images

We read the recent pair of papers from Drs. Watson and Berger (*1*, *2*) with great interest, and agree that a quantitative approach to thallium image analysis becomes increasingly important as imaging techniques improve.

There are several questions of some importance regarding the derivation of normal criteria. The first is the make-up of the normal group: 16 subjects (Group A) were angiographically normal, but were not further defined (chest pain, mitral prolapse?). The other nine (Group B), who also may have had clinical syndromes, were considered normal partly on the basis of a "normal" stress thallium image. The normal limits for Group B's stress thallium images were derived from Group A. Then Groups A and B were used to derive normal limits for the rest of the study. It seems rather circular to define a normal thallium image on the basis of a group defined as normal in part by virtue of having a normal thallium image. It similarly seems tenuous to use the stress thallium image as one basis for defining the limits of normal washout. If it is true (as we believe it is) that washout analysis offers information different from, and in some ways superior to, that derived from single-image analysis, perhaps washout criteria should be used to define normal single-image distribution limits. Were there subjects with "normal" stress images but abnormal washout involving only one or two segments? How were they interpreted?

The second question deals with the use of an "upslope" (as opposed to "downslope") washout curve as the limit of normal. Although this criterion is convenient and clinically satisfactory, it does not appear to be entirely supported by the data presented in Figs. 7 and 8. If we use 2 s.d. from the mean of the normal group, a washout coefficient of approximately −0.05 seems to be the limit of normal. Whether this is practically different from a coefficient of 0 is unclear, but is not discussed in the papers, which arbitrarily chose 0 as the upper limit of normal washout rate. We emphasize that we do not differ with the choice of 0 as the cutoff if it is clinically the most useful, but feel that it should be clear that the choice was somewhat arbitrary, and not necessarily physiologic or based on the data.

The questions raised in no way negate the value of this excellent series of studies. Rather we hope to provoke discussion about the definition of normal and the difficult nature of the phenomena being studied.

JOEL SKLAR
PETER STEELE
DENNIS KIRCH
Veterans Administration Med. Ctr.
Denver, Colorado

REFERENCES

1. WATSON DD, CAMPBELL NP, READ EK, et al: Spatial and temporal quantitation of plane thallium myocardial images. *J Nucl Med* 22:577-584, 1981
2. BERGER BC, WATSON DD, TAYLOR GJ, et al: Quantitative thallium-201 exercise scintigraphy for detection of coronary artery disease. *J Nucl Med* 22:585-593, 1981

Reply

The letter from Drs. Sklar, Steele, and Kirch addressed several important questions which, we quite agree, are complex and deserve more careful discussion than was allowed in the published paper. In fact, these points were elaborated in the original manuscript, but the discussions were deleted in deference to the reviewers.

Their first question concerns our small group of 25 normal subjects reported in the first paper (*1*). The 16 subjects in Group A were derived from a much larger group of patients, all of whom had normal cardiac anatomy and resting left-ventricular function. Only those patients with normal electrocardiographic responses to exercise, normal physical examination, and chest pain (without ischemia) were included. Thus, these 16 patients represent a highly selected group. Since our criteria for normality consisted of angiographic, ventriculographic, and clinical data, it is quite likely that these patients represent true cardiac normals. The nine patients in Group B did not undergo catheterization because the likelihood of coronary artery disease could be reduced to $\leq 1\%$ following serial Baysian analysis of age, sex, symptoms, and resting and exercise electrocardiography. Although the normality of these Group B patients was further substantiated by uniform initial thallium uptake, these data were excluded from the computation of average initial uptake. Consequently, the method was not circular, although it may have appeared so on first examination.

The normal patients from the prospective study reported in the second paper (*2*) included all consecutive patients who had angiographically nonsignificant coronary artery stenoses. Necessarily, many of these patients did have "nonsignificant" coronary artery abnormalities, other heart disease, or typical symptoms that brought them to cardiac catheterization, and we did not wish to use this group of patients as reference normals. Therefore, data from the 25 "normal-normals" were obtained to satisfy our curiosity to examine a group of subjects which should have *completely* normal thallium studies, and was helpful in establishing and understanding the criteria for scan interpretation.

However, the normal limits that we use cannot be entirely derived from a group of normal patients. These limits must be chosen and evaluated in terms of how well they *separate* normal and abnormal patients within the unselected group of patients referred for clinical evaluation. This was the subject and the reason for the second paper (*2*). The washout criteria are a good example. The absolute value of thallium washout rates in the delayed images is necessarily dependent upon the level of exercise achieved at the time of injection, and also depends directly on the residual blood levels of recirculating thallium in the postexercise period—a factor that is not related to coronary blood flow and may depend upon such tenuous variables as the patient's state of exercise between the initial and delayed images. The limits of normal washout must be broad enough to include this normal physiologic variability encountered in the clinical population. Adopting the criterion of upslope compared with downslope proved to be an adequate discriminant in this setting, and could also be more simply and reliably used compared with a slope coefficient defined mathematically from a least-squares curve analysis. In our paper, it was stated "The use of upslope against downslope provides a discriminant that requires no mathematical computations, and encom-

passes the normal physiologic variability in net washout rate." Drs. Sklar et al. in their letter indicated that while this criterion is convenient and clinically satisfactory, it does not represent exactly ±2 s.d. from the mean of the slopes obtained from the normal group. This is an entirely correct interpretation of our paper.

Several questions concerning uptake and washout were raised in the letter. First, since the absolute washout rate depends upon several variables aside from myocardial thallium uptake, the washout rate cannot be used to imply or to substitute for the measurement of initial thallium distribution. The initial thallium distribution, redistribution, and segmental washout rates are probably best viewed as three separate entities (even though they are not completely independent). A myocardial segment can have reduced uptake and normal washout, which would be observed as a persistent defect. A myocardial segment may have reduced initial uptake with delayed washout, compared with normal myocardial segments, and this would produce classical redistribution (i.e., delayed normalization of the defect). In this case, the abnormal segment washes out more slowly than the normal segment, but does not necessarily have an absolute washout that is outside normal limits. In more severe defects, redistribution may result from increasing uptake of the abnormal segment. Increasing uptake in all myocardial segments in the absence of significant initial defects can occasionally be observed in cases of diffuse symmetric multiple-vessel disease, in which case no normal myocardial segment is available for comparison. In these cases, an *apparent* "reverse redistribution" can occasionally be observed when we compare two abnormal myocardial segments both of which have similarly reduced initial uptake but dissimilar washout rates. We have not quantitatively substantiated the case of *true* "reverse redistribution" resulting from a segment that has completely normal initial uptake but abnormal washout rate, which would produce a reverse defect in the delayed images. This would require a myocardial segment with normal blood flow and normal extraction coefficient, but with abnormal cellular washout rate, and would be illogical in the context of coronary artery disease. Reverse redistribution occasionally appears on scintiphoto images, but we have found on quantitative evaluation that it is nearly always the result either of comparing two abnormal myocardial segments under the incorrect assumption that one of the segments is "normal" or, in some cases, a photographic distortion resulting from the use of nonlinear gray-scale reproduction.

We wish to thank Drs. Sklar, Steele, and Kirch for their comments and for providing this forum for discussion.

DENNY D. WATSON
ROBERT S. GIBSON
CHARLES D. TEATES
GEORGE A. BELLER
University of Virginia Hospital
Charlottesville, Virginia

REFERENCES

1. WATSON DD, CAMPBELL NP, READ EK, et al: Spatial and temporal quantitation of plane thallium myocardial images. *J Nucl Med* 22:577-584, 1981
2. BERGER BC, WATSON DD, TAYLOR GJ, et al: Quantitative thallium-201 exercise scintigraphy for detection of coronary artery disease. *J Nucl Med* 22:585-593, 1981

Re: Indium-111 Tropolone Versus Oxine

As a research biochemist having developed an aqueous ethanol-free In-111 oxinate preparation that proved to be an efficient cell-labeling agent especially for leukocytes and platelets (*1*), I would like to comment on the article by Dewanjee et al. titled: "Indium-111 Tropolone, A New High-Affinity Platelet Label: Preparation and Evaluation of Labeling Parameters (*2*)."

The statements concerning the solubility of oxine and the need for ethyl alcohol as a solvent are erroneous, and the statements about the ability of indium-111 tropolone to label platelets in a plasma environment are misleading and may raise false hopes in experiments.

The second line of the Summary contains the following statements: "Unlike oxine, which must be dissolved in ethyl alcohol, tropolone is soluble in isotonic saline." However, oxine used in the concentration levels current in cell-labeling procedures is soluble in saline without the help of ethyl alcohol or a solubilizer (*3*).

In the sixth line of the Summary I read that indium-111 tropolone would be able to yield 60-70% labeling efficiency with platelets in an ACD plasma medium. From Fig. 2, however, it is clear that only in cases of extremely low plasma concentrations, below 50 μl/ml, can labeling efficiencies between 40 and 50% be obtained. When the incubation mixture contains 250 μl/ml (25%) plasma, the labeling efficiency is only about 20%. For indium-111 oxinate and incubation mixtures containing more than 50% plasma, labeling efficiencies over 20 and up to 50% are obtained (*4*). Consequently there is no advantage in using tropolone instead of oxine. Is it realistic to speak of "plasma medium" if it contains only 50 μl plasma per ml incubation mixture?

In the Discussion there is an erroneous statement that HEPES or Tris buffer should be necessary as a solvent if acetylacetone is to be used. HEPES and Tris function as buffers. They don't function as solubilizers and they don't interfere with platelet function.

Let me conclude with a suggestion. Why not use the correct chemical names for indium chelates, such as indium-111 oxinate, indium-111 tropolonate, indium-111 acetylacetonate?

W.TH. GOEDEMANS
Byk-Mallinckrodt CIl B.V.
1755 ZG Petten, The Netherlands

REFERENCES

1. GOEDEMANS WTH: Simplified cell labelling with indium-111 acetylacetonate and indium-111 oxinate. *Br J Rad* 54:636-637, 1981
2. DEWANJEE MK, RAO SA, DIDISHEIM P: Indium-111 tropolone, a new high-affinity platelet label: preparation and evaluation of labeling parameters. *J Nucl Med* 22:981-987, 1981
3. GOEDEMANS WTH: GB patent application 2066664. Published July 15, 1981
4. SCHEFFEL U, TSAN MF, MCINTYRE PA: Labeling of human platelets with [^{111}In] 8-hydroxyquinoline. *J Nucl Med* 20: 524-531, 1979

Reply

I tend to disagree with Dr. Goedemans regarding the solubility of oxine and In-111 oxine in water. It is likely that a trace amount of oxine and In-111 oxine might be in solution, but the major fraction of In-111 oxine is in insoluble form without alcohol. The exact physical form of these neutral In-111 complexes in water is not known. A major fraction of the complex is retained in the filter paper (0.22 μm Millipore or Nuckopore filter), and most of these complexes tend to be sticky. The exact physical form is irrelevant as long as we obtain constant labeling efficiency maintaining cell viability.

In an ideal cell-labeling system, we would like to add minimum amounts and kinds of chemicals including buffer or organic solvent. In an In-111 tropolone preparation we use In-111 chloride, 20-25