Detection of Myocardial Infarction in Vitro Based on Altered Attenuation of Ultrasound

JAMES W. MIMBS, DONALD E. YUHAS, JAMES G. MILLER, ALAN N. WEISS, AND BURTON E. SOBEL

SUMMARY This study was designed to determine whether attenuation of ultrasound by myocardium is potentially useful in detecting and quantifying infarction. Accordingly, we analyzed 44 regions of myocardium from 11 dogs 4-10 weeks after coronary occlusion. Attenuation of ultrasound in each region was assessed by transmitting a broadband pulse through the tissue in vitro and carefully gating the appropriate pulse into a spectrum analyzer for Fourier analysis (frequency range, 2-9.5 MHz). An ultrasonic index of attenuation was derived from the slope of the best-fit line relating attenuation and frequency obtained from the Fourier transform. Acquisition of ultrasonic data was improved with the use of a specially designed small diameter receiving transducer. Myocardial creatine kinase content was assayed in each region to provide an independent index of regional injury. Results obtained from ultrasonic and biochemical analyses correlated with a correlation coefficient between the two of 0.80 in 24 regions of myocardium from the six dogs studied 4-5 weeks after infarction, and 0.72 in 20 regions from the five dogs studied 9-11 weeks after infarction. These findings indicate that regional infarction is associated with quantitative changes in ultrasonic attenuation.

USE OF ultrasound in medicine, introduced approximately 25 years ago, has been proven to provide a valuable diagnostic tool. Current applications rely on reflection of ultrasound, which occurs at tissue interfaces and is attributable to discontinuities of ultrasonic impedance. Thus, conversion of information derived from reflected ultrasound is the basis of A mode and M mode echocardiography. Primarily because of technical limitations, considerably less attention has been devoted to absorption or attenuation of ultrasonic energy by the tissue. Nevertheless, characterization of attenuation may offer even more definitive information about the physical and biological state of the tissue.

A number of techniques potentially useful in analyzing acoustic properties of tissue have been considered; these include acoustic impediography, acoustic scattering, and spectral analysis.1-7 Lelé and his colleagues have demonstrated that the frequency dependence of the attenuation of ultrasound may be a useful parameter to characterize physical properties of myocardium associated with infarction. The present report describes results of experiments in vitro designed to determine whether the frequency dependence of the attenuation of ultrasound can provide a quantitative measure of the severity of the infarct. The approach used was based on determination of an ultrasonic index which provides a quantitative measure of altered physical (i.e., ultrasonic) properties of myocardium. Results were compared to the creatine kinase content of myocardium, an independent index of tissue damage after ischemia,8 in 44 regions of the hearts from 11 dogs killed from 4-11 weeks after coronary occlusion.

Progress in this investigation initially was impeded by ultrasonic phase-cancellation effects. These phenomena may occur when inhomogeneous wavefronts from ultrasound that has passed through tissue are presented to the face of a conventional ultrasonic receiving transducer. Phase-cancellation effects can be a major source of artifact and may have contributed to problems in interpreting results of some previous studies.9-11 Accordingly, for the present study we improved data acquisition with a small diameter receiving transducer designed to reduce phase-cancellation effects.

Methods

PREPARATIONS OF DOGS

Myocardial infarction was produced in adult, mongrel dogs (15-30 kg). After induction of anesthesia with pentobarbital sodium (25 mg/kg, iv), each dog was intubated, ventilated with room air with a Harvard respirator, and subjected to a left thoracotomy via the fifth interspace. The pericardium was incised, and the left anterior descending coronary artery dissected free immediately distal to the first ventricular branch and ligated. The pericardium was incised, and the left anterior descending coronary artery dissected free immediately distal to the first ventricular branch and ligated. The pericardium was left open, and the chest closed conventionally with intrapleural suction maintained for at least 1 hour with a chest tube. In three dogs we performed a sham procedure including thoracotomy, pericardiotomy, chest closure and drainage but without coronary ligation.

Each dog was anesthetized again, 4-11 weeks after operation, with pentobarbital sodium (25 mg/kg, iv). The heart was rapidly excised and placed in a 0.9% NaCl solution. To prepare the tissue for ultrasonic analysis an
Incision was made at the root of the aorta, continued inferiorly along the left ventricular surface of the interventricular septum to the apex, and extended posteriorly and superiorly, terminating at the base of the heart. This permitted prompt excision of a segment of the anterior and apical wall of the left ventricle. This segment included the area of infarction and a surrounding area of normal myocardium for all animals subjected to coronary ligation.

The excised segment of myocardium was mounted on a sample holder which consisted of a rectangular, metallic, hollow rim with pins along its periphery. This device provided stabilization of the tissue sample and avoided interference with ultrasonic analysis. The mounted sample was placed into a saline (0.9% NaCl) bath maintained at 20-23°C, a temperature selected to provide reproducible results when analyses were repeated within 1 hour. The saline bath chamber was constructed so that the mounted tissue sample could be analyzed ultrasonically at four discrete sites, with the transmitted ultrasonic beam occupying a circular surface area of about 1 cm diameter. Each region analyzed was separated from the adjacent site by 0.7 cm. Tissue was placed into the bath so that the endocardial surface faced the transmitting transducer. Ultrasonic measurements were initiated within 3 minutes after the dog was killed, and were completed within 30 minutes.

### ASSAY OF MYOCARDIAL CREATINE KINASE ACTIVITY

When ultrasonic studies had been completed, the sample holder and tissue were removed from the saline bath. A wire grid, with openings spaced to correspond to the four sites analyzed ultrasonically, was placed over the tissue and stored at -20°C. Each region analyzed was separated from the adjacent site by 0.7 cm. Tissue was placed into the bath so that the endocardial surface faced the transmitting transducer. Ultrasonic measurements were initiated within 3 minutes after the dog was killed, and were completed within 30 minutes.

### ULTRASONIC ANALYSIS

#### Instrumentation and Methods

The instrumentation employed for ultrasonic analysis is depicted in Figure 1. The transmitting transducer (Panametrics V309, nominal center frequency = 5 MHz, 1.3 cm diameter, unfocused) and driver (Panametrics 5050 PR) yielded an ultrasonic pulse with frequency components of sufficient amplitude to permit operation over a range of ~2-9.5 MHz. This transmitter was placed 5 cm from the endocardial surface of the tissue sample. In a preliminary series of experiments, a receiving transducer identical to the transmitting transducer was placed 5 cm from the epicardial surface. For the results reported here, a receiving transducer 0.2 cm in diameter was placed 1 cm from the epicardial surface. Broadband characteristics were achieved by designing this transducer to operate over the frequency range of about 2-10 MHz on the low frequency tail of the response of a nominally 30-MHz center frequency quartz plate. Under control of a timing unit, broadband ultrasonic pulses were gated into a slowly sweeping analogue spectrum analyzer (Hewlett-Packard 8553/8552A). Output pulses from the spectrum analyzer, comprising the Fourier transform of the received ultrasonic pulses, were converted by a sample-and-hold unit into a slowly varying (DC) signal which was then transmitted to a signal averager (Hewlett-Packard 5480B).

The procedure used for the quantitative measurement of attenuation following transmission of ultrasound through tissue was somewhat similar to the substitution technique described by Schwan and Carstensen.14 Before tissue was placed in the path of the ultrasonic beam, the response characteristics of the system were measured and stored in the signal averager. The recorded voltage as a function of frequency ($\nu$) is

$$ V_A(\nu) = \log[R(\nu)\exp(-\alpha_zz_0)[I(\nu)]], $$

where $R(\nu)$ is the transfer function for that part of the system not involving the ultrasonic field, $\alpha_z$ is the attenuation coefficient of the saline carrier medium, and $z_0$ is the distance between the transmitter and the receiver. The frequency dependent term $I(\nu)$ describes the production, propagation, and detection of the ultrasonic field. This term is dependent upon the geometrical properties of the transmitting and receiving transducers, and upon the properties of the intervening media.

After this initial measurement, a tissue specimen was placed in the path of the ultrasonic beam. For a specimen of thickness $\Delta z$, the voltage stored in the signal averager is

$$ V_B(\nu) = \log[R(\nu)\exp(-\alpha_zz_0)\exp[(\alpha_0 - \alpha_\Delta\Delta z)P^*[I(\nu)]], $$

where $\alpha_\Delta$ is the attenuation coefficient of the tissue, $z_0$ is the distance between the transmitter and the receiver, and $P$ is the power of the transmitted ultrasonic pulse.
where $\alpha$ is the tissue attenuation coefficient, and $T^2$ represents the product of the amplitude transmission coefficients for the saline-tissue and tissue-saline interfaces. The term $\exp[(\alpha - \alpha_s)\Delta z]$ describes the attenuation due to the presence of a thickness $\Delta z$ of tissue replacing an equivalent thickness of saline. The expression $I(\nu)$ is replaced by $I(\nu)$ and reflects the change in the ultrasonic field presented to the receiving transducer that is caused by the presence of the intervening tissue.

The voltages $V_A(\nu)$ and $V_B(\nu)$ are subtracted digitally by the signal averager. The resulting signal loss as a function of frequency is

$$V_A(\nu) - V_B(\nu) = (\alpha - \alpha_s) \Delta z \log e - \log T^2 + \log \frac{I(\nu)}{I(\nu)} \tag{3}$$

For a tissue specimen in a saline bath, two approximations may be made to simplify Equation 3: (1) the attenuation coefficient of saline is negligible compared to that of tissue ($\alpha_s \ll \alpha$) and (2) negligible loss in signal occurs due to reflection at the saline-specimen interfaces ($T^2 \approx 1$). With these approximations, Equation 3 may be restated

$$V_A(\nu) - V_B(\nu) \approx \alpha_s \Delta z \log e + \log \frac{I(\nu)}{I(\nu)} \tag{4}$$

Signal Loss $\approx$ (I) Attenuation Coefficient (II) Phase-Cancellation Contribution

Equation 4 segregates the two major sources of signal loss. Term I describes signal loss attributable to absorption and scattering of ultrasound within the specimen. Term II describes signal loss associated with geometrical distortion of ultrasonic wavefronts by the specimen and their detection at the face of a spatially extended piezoelectric receiving transducer (phase cancellation). These wavefront distortions may result from transmission of ultrasound through tissue with variations in surface characteristics or internal structure, or both. In the absence of phase-cancellation effects, $I(\nu) = I(\nu)$ and term II becomes zero. Thus, Equation 4 may be solved to give the ultrasonic attenuation coefficient,

$$\alpha_s = \frac{V_A(\nu) - V_B(\nu)}{\Delta z \log e} \tag{5}$$

In the present study Equation 5 was used to determine the ultrasonic attenuation of tissue over the range of frequencies 2–9.5 MHz.

Figure 2 depicts results of analysis of the frequency dependence of attenuation with castor oil, a standard used in ultrasonic studies, serving as a specimen. Figure 2 depicts a plot of $V_A(\nu)$ with no specimen present (upper curve) and a plot of $V_B(\nu)$ with a castor oil 'specimen' of $\Delta z$ = 0.4 cm (lower curve). The result of subtraction of the two curves in Figure 2 is displayed as the attenuation-frequency plot in the bottom panel with data points calculated from Equation 5. Thus, these results are based on the assumption that phase-cancellation effects (term II of Equation 4) are insignificant. This is a valid assumption when the sample is homogeneous, as is the case with castor oil. Figure 2 agrees well with results of others, in that the function exhibits a frequency $\nu$ dependence of the form $\nu^{\nu/2}$. 15

Analysis of Data from Myocardium

For each region of myocardium, the loss in ultrasonic signal strength ($V_A - V_B$) was determined over the frequency range of 2–9.5 MHz, and an attenuation-frequency plot similar to that of Figure 2 was generated for each analysis. Figure 3 depicts two examples of attenuation-frequency plots from a single region of normal myocardium. Plot A was obtained with the small diameter (0.2 cm) receiving transducer used in the present study. Previous studies have demonstrated that the ultrasonic attenuation coefficient for soft tissue varies nearly linearly with frequency. 16 This relationship is evident in plot A, Figure 3. Accordingly, we used the calculated slope of the best-fit straight line conforming to the data as an index of the frequency dependence of ultrasonic attenuation. This analysis assumes no significant signal loss from phase-cancellation effects (compare Eq. 4, above).

Plot B in Figure 3 is an example of nonmonotonic dependence of attenuation on frequency when the same tissue site as that analyzed in plot A was evaluated with a 1.3-cm diameter receiving transducer identical to the transmitting transducer. The absence of a monotonic attenuation-frequency dependence implies substantial artifact, i.e., nonphysiological contributions to the observed signal loss. When 20 analyses of normal and necrotic myocardium were performed with the 1.3-cm diameter receiving transducer, results yielded monotonic attenuation-frequency plots in only 25% (Fig. 4). However, utilization of the 0.2-cm diameter receiving transducer to
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Figure 3 Two examples of attenuation-frequency plots obtained from a single region of myocardium. Plot A was obtained with a 0.2-cm diameter receiving transducer and depicts monotonic variation of the ultrasonic attenuation coefficient with frequency. Plot B was obtained with a 1.3-cm diameter receiving transducer and depicts nonmonotonic variation of the ultrasonic attenuation coefficient with frequency. The variability of the attenuation-frequency relation in plot B is an example of attenuation artifact due to phase-cancellation effects.

minimize phase-cancellation effects provided monotonic data in 38 of the 44 ultrasonic analyses (86%) (Fig. 4).

Because phase-cancellation effects can distort results and mask the anticipated monotonic and nearly linear frequency dependence of attenuation, a data acceptance criterion was established for the present study based on the goodness of the straight line fit to the signal loss data. With the use of the straight line fit and the measured signal loss data, a root mean square deviation (RMSD) was calculated as:

$$\text{RMSD} = \left( \frac{1}{N_2 - N_1} \int_{N_1}^{N_2} \left[ y_m(v) - y_c(v) \right]^2 dv \right)^{1/2}$$

where $v$ is the ultrasonic frequency with $(N_2 - N_1)$ defining the frequency interval of the measurement, $y_m(v)$ is the measured signal loss at frequency $v$, and $y_c(v)$ is the calculated signal loss, determined by a least squares straight line fit to the data. The units of RMSD are the same as those of $y(v)$, in this case decibels (dB). An objective criterion was used to segregate monotonic data ($\text{RMSD} \leq 1.3 \text{ dB}$) from nonmonotonic data ($\text{RMSD} > 1.3 \text{ dB}$) that presumably reflected unavoidable phase-cancellation induced artifact. As shown in Figure 4, 86% of the data in the experiments performed with the 0.2-cm diameter receiver met the acceptance criterion.

Results

MYOCARDIAL CK CONTENT AND MEASURED INDEX OF ATTENUATION

Table 1 contains data obtained from the ultrasonic and enzymatic analyses of each region of myocardium studied (44 sites from 11 dogs studied 4–11 weeks after coronary occlusion). Myocardial CK content was determined for each dog as an estimate of the extent of myocardial necrosis, because regional CK depletion after coronary occlusion correlates with infarction estimated by gross, microscopic, and ultrastructural criteria, and with the severity of ischemia measured with radioactively labeled microspheres.6, 15, 17 The negative value for CK depletion, noted in some regions, results from variability of the assay and regional CK content such that CK observed in a specific region is less than average CK from the control biopsy sites. Myocardial infarction, produced by occlusion of the left anterior descending coronary artery, resulted in a wide range of values for CK depletion among the sites.

The calculated index of ultrasonic attenuation for each myocardial site is shown in Table 1. The value of the index in each case was determined as the slope of the best-fit straight line to a plot of the measured coefficient of attenuation vs. frequency. In addition to the calculated slope, the root mean square deviation (RMSD) was determined for each site. The value of RMSD reflects the conformity of the attenuation-frequency data to the acceptance criterion.

ANALYSIS OF ULTRASONIC DATA

Figure 5 illustrates the relationship between the ultrasonic index of attenuation and the enzymatic estimate of myocardial injury in sites from hearts of all dogs sacrificed 4–5 weeks after infarction. Data from 17 of 20 (85%) sites investigated in these five dogs met the acceptance criterion ($\text{RMSD} \leq 1.3$) in addition to data from a sham operated dog killed 5 weeks after operation. Regional myocardial CK depletion and the corresponding regional ultrasonic index of attenuation correlated with an $r$ of 0.80. In seven of eight sites with CK depletion $> 40\%$ the index of attenuation was markedly elevated (Fig. 5). A similar relationship was evident with data from dogs killed 9–11 weeks after infarction. Seventeen of 20 sites (85%) met
the acceptance criterion, and the correlation coefficient between enzymatic and ultrasonic indices was 0.72.

Figure 6 depicts the relationship between CK content and ultrasonic attenuation-frequency dependence in a different way. Figure 6A illustrates the attenuation-frequency dependence in sites from dogs killed 4-5 weeks after infarction. For this figure a composite of all attenuation-frequency plots from sites with CK depletion >40% (indicative of significant injury) was compared to a composite from sites with CK depletion <20%. Each of the two composite plots was constructed by averaging values from all sites for the attenuation index at each specified frequency.

A comparison of the two plots in Figure 6A reveals a clear separation of the attenuation data for plots from sites with CK depletion >40% compared to those with CK depletion <20%. Although the delineation is not distinct in the range of 2.0-6.0 MHz, the separation becomes significant at the 95% confidence level for all points in the frequency range of 6.5-9.5 MHz. Figure 6B illustrates a similar comparison of attenuation-frequency dependence among sites from dogs killed 9-11 weeks after infarction.

**Figure 5** The correlation between the ultrasonic index of attenuation, expressed as the slope of the best-fit line (least squares method), and an enzymatic estimate of myocardial injury (expressed as percent CK depletion) in all regions with monotonic data from the dogs studied 4-6 weeks after infarction or sham operation.

**Figure 6** (A) the composite attenuation-frequency plot for all data from animals sacrificed 4-5 weeks after myocardial infarction or sham operation. (B) the attenuation-frequency plot for all data from animals sacrificed 9-11 weeks after operation. As noted in the text, CK depletion of <20% was considered to be indicative of normal samples or those with trivial amounts of infarct within them (○). CK depletion of ≥40% (A) or ≥20% (B) was used to define samples considered to exhibit infarction (×). Results expressed are means ± SE of the number of different samples (n) studied at each frequency.
In Figure 6B, the sites with CK depletion ≥ 20% are contrasted to depletion sites with CK < 20% because of the relatively small number of sites studied.

**Discussion**

The results of this study indicate that an index based on the frequency dependence of ultrasonic attenuation correlates with an independent index of myocardial injury, depletion of creatine kinase activity. The study was undertaken because we anticipated that one or more of the well recognized morphological changes accompanying infarction, such as loss of myofibrillar protein or deposition of collagen, would alter the ultrasonic properties of the heart. Reports from other laboratories suggest that ultrasonic impedance and attenuation properties of tissue may be determined primarily by structural components containing collagen. The present study demonstrated that altered attenuation correlates with the severity of tissue injury (reflected by CK depletion), but it does not directly assess the possibility that the cause of increased attenuation might be an increase in connective tissue associated with scarring.

Measurement of the frequency dependence of attenuation was initially described by Gericke in studies designed to detect flaws in metals. Lele and his colleagues adapted these techniques for the ultrasonic characterization of tissue and correlated results with histological criteria in studies of acute myocardial infarction. Preliminary studies of acute myocardial infarction in our laboratory, however, revealed no statistically significant differences in ultrasonic attenuation between regions of infarct and normal myocardium for tissue studied at 24 hours after ligation of the left anterior descending coronary artery (unpublished observations). This apparent disparity between our results and those of Lele remains unresolved, but differences in methods used make comparisons somewhat difficult; these include: transducer configuration, differences in the character of infarct produced by occlusion of the circumflex rather than anterior descending coronary artery, and variation in the time of ultrasonic analysis after coronary occlusion.

Preliminary studies in the present investigation provided further stimulus for development of a receiving transducer that is relatively insensitive to phase-cancellation effects. Phase-cancellation effects recently have been identified as a source of problems in the measurement of attenuation by inhomogeneous media such as tissue. A detailed evaluation of the consequences of phase-cancellation effects for measurements of the frequency dependence of attenuation of myocardial tissue is presented elsewhere. As noted in Figure 3 and in the section on data analysis, results from a preliminary series of experiments employing a 1.3-cm diameter receiving transducer exhibited marked deviation from the theoretical monotonic curve in 75% of the cases that precluded adequate quantitative analysis (Fig. 3B). In addition, minimal changes in the orientation of this transducer with respect to the tissue during each experiment induced marked changes in the attenuation-frequency plots. These observations suggested the presence of severe phase-cancellation effects.

To determine whether phase-cancellation effects were responsible for artifact in these preliminary experiments, we designed and used a small diameter receiving transducer (0.2 cm instead of 1.3 cm). As noted in Table 1, monotonic dependence of attenuation on frequency was observed in 86% (38 of 44) of sites analyzed. This improved percentage of monotonic data (compared to 25% in the preliminary series of experiments) supports the concept that artifact due to phase-cancellation had been reduced substantially.

Although the ultrasonic and biochemical indices of injury correlated in sites from dogs killed 4–5 weeks after infarction ($r = 0.80$) and from dogs killed 9–11 weeks after infarction ($r = 0.72$), several factors probably contributed to an imperfect correlation. The method for analysis of myocardial CK employed in this study entailed a standard deviation of 12% with small tissue biopsies (~0.5 g) (data not shown). In addition, a single site subjected to ultrasonic analysis often exhibited fibrosis and necrosis detectable grossly in one portion and apparently normal tissue in a contiguous region. Such inhomogeneity within a site would lead to variation in the ultrasonic attenuation-frequency data depending upon which region within the site was included within the geometric field of view of the small diameter receiving transducer.

Other sources of potential error may be related to the dependence of the measured attenuation index not only on the signal loss voltage but also on tissue thickness (compare Eq. 5, above). In the present study thickness was determined by hand but more precise measurements, such as those based on transit time of an acoustic pulse, may be useful.

The occurrence in the present study of nonmonotonic data (14% of analyses) despite the refinement of transducer design suggests that while phase-cancellation effects were reduced, they were not eliminated entirely. Thus, phase-cancellation effects may also have affected the quality of the monotonic data to some extent as well as invalidating the nonmonotonic data. We are currently developing a large diameter receiving transducer based upon the acoustoelectric effect. This new approach to transducer design appears to eliminate phase-cancellation effects.

Results of the present study demonstrate that ultrasound can be used to characterize physical properties of tissue quantitatively in vitro. With the use of transmitted ultrasound to investigate myocardium after coronary occlusion, we have observed a correlation between the change in an ultrasonic index based on attenuation and an independent index of myocardial injury, creatine kinase depletion. Phase-cancellation effects were recognized and reduced by the use of a small diameter receiving transducer. Although the present results were obtained with transmitted ultrasound, the ultrasonic indices used are based on intrinsic properties of tissues. Thus, they may be amenable to measurement in vivo in studies using reflected ultrasound as well.

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Trophic Influence of the Sympathetic Nervous System on the Rat Portal Vein

OCTAVIO APRIGLIANO AND KENT HERMSMEYER

SUMMARY Adrenergic denervation of the rat portal vein was produced in vivo by the sympatholytic agent 6-hydroxydopamine (6-OHDA). Treatment of rats with 6-OHDA decreased the responses of the portal veins to nerve stimulation, reduced $^3H$-norepinephrine (NE) uptake, and decreased catecholamine fluorescence, indicating that partial adrenergic denervation was achieved. The main findings of this study indicate that the in vivo denervation produced: (1) a (time-dependent) increase in sensitivity of the veins to NE, which was not of prejunctional origin, (2) an increase in sensitivity to BaCl$_2$, and (3) a partial depolarization of the myovascular cells. The results suggest that the in vivo denervation of the portal veins by 6-OHDA produces a postjunctional alteration, which may be due to the removal of a trophic influence of the sympathetic nervous system. It is proposed that the partial depolarization and associated ionic changes may be components of the mechanism. These results provide the first direct evidence that membrane excitability changes are involved in trophic nerve-muscle interactions in blood vessels.

MOTOR innervation can control and modulate in many ways the homeostasis of effector cells (for reviews see Thesleff, Guth, Gutmann, and Fleming et al.†). These interrelationships between nerve and effector cells have been generally classified as trophic phenomena. The trophic influences of nerve on muscle may be defined as long term interactions affecting or regulating the muscle cells in addition to the more immediate phenomenon of synaptic transmission. The common approach to the study of neurotrophic influences is to interrupt the normal nerve-muscle contact by surgical or functional denervation and to observe the changes in some muscle cell function during and after denervation and the reversal of these changes on reestablishment of normal innervation. Thus,
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J W Mimbs, D E Yuhas, J G Miller, A N Weiss and B E Sobel

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