comparable group given adriamycin only. These results indicate that treatment with tocopherol does not impair the responsiveness of the P388 ascites tumor to adriamycin. Further, because it diminishes the dose-limiting toxicity of adriamycin, larger doses of the drug may be administered, leading to a significantly improved therapeutic index. These results provide further evidence in favor of the hypothesis that adriamycin may have at least two mechanisms of tissue damage in the mouse. One, which involves lipid peroxidation, is blocked by tocopherol and appears to play a major role in the development of cardiomyopathy. The other, which may involve binding of adriamycin to DNA, is unaffected by tocopherol and appears to be the major determinant of cytotoxicity for P388 cells. Additional work is needed to determine if any human tumors parallel P388 in these respects. To the extent that a similar differential exists between human cardiac tissue and human tumor, pretreatment with tocopherol or other free radical scavengers might be of therapeutic benefit.

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Experimental Infarct Sizing Using Computer Processing and a Three-Dimensional Model

Abstract. *A method for noninvasive sizing of myocardial infarction, in which data from technetium-99m stannous pyrophosphate scintigrams and a three-dimensional model were used, was tested on experimental, acute anterior infarcts in dogs. The results indicate that the method does size experimental anterior infarcts accurately, but further testing will be necessary to assess the capabilities of the technique for sizing other types of infarcts.*

The major cause of death in patients reaching the hospital with acute myocardial infarction is "pump failure" that occurs as a complication of a large myocardial infarct and is often expressed clinically as cardiogenic shock, medically refractory congestive heart failure, or medically refractory ventricular arrhythmias, or a combination of all (*1*). Hence the ability to size acute myocardial infarcts appears to be important. Quantitative infarct measurements could potentially be used to help assess patient prognosis and to aid in planning subsequent treatment. Myocradial scintigrams, with technetium-99m stannous pyrophosphate (^{99m}Tc-PYP) as an imaging agent, are used to identify and localize acute myocardial infarcts in both experimental animals and in patients (*2*). Since injured myocardial tissue is identified as a bright spot on a ^{99m}Tc-PYP scintigram, information obtained from such scintigrams could probably be used to size infarcts in patients, provided a suitable sizing technique could be developed.

The ^{99m}Tc-PYP myocardial imaging has been used to estimate the size of acute anterior infarcts in experimental animals, but previous estimates have employed "areas of increased activity" from one scintigraphic view as a measure of infarct size (*3*). Area seems to be a relatively poor measure, since it is a two-dimensional quantity and does not take into account the three-dimensional nature of the infarcted tissue. In particular, since infarcts occur in various positions and orientations, no simple two-dimensional measurement will be able to size all types of infarcts successfully. Therefore, we have attempted to develop a relatively simple method for reconstructing infarct size in three dimensions, using the noninvasive ^{99m}Tc-PYP myocardial scintigram.

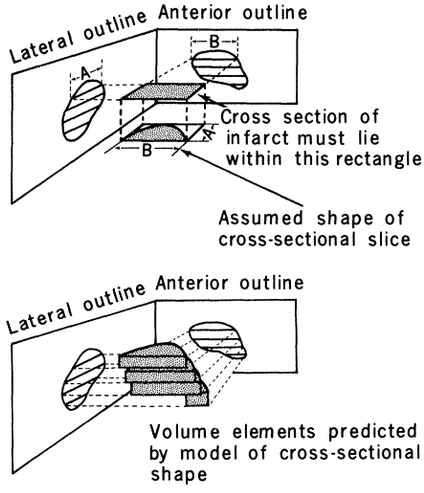

Fig. 1. The model we utilized (C-shaped cross-sectional model) and actual data obtained from two orthogonal projections of infarct outlines. See text for details.

Several methods for reconstructing approximate cross sections of three-dimensional objects from their projections have been described (*4*). Reconstruction of myocardial infarcts from ^{99m}Tc-PYP images is a formidable task, however, for two reasons. (i) The ^{99m}Tc-PYP uptake is not uniform in infarcted tissue (*2*), and therefore the intensity levels in scintigrams do not accurately indicate the extent of infarction, merely its presence. (ii) Only three or four scintigraphic projections are obtained during routine examination; hence not enough scintigraphic views are available to permit sizing by use of such reconstruction algorithms as those utilized in computerized transaxial tomography (*4*).

Since we do not have enough projections to reconstruct the actual infarct shape, our method uses a three-dimensional "model" of the infarct, and, since intensity levels in the scintigrams can be misleading, we have fit the model according to the infarct boundaries obtained from the scintigram views. Defining infarct boundaries in a ^{99m}Tc-PYP scintigram can also be a difficult problem because the infarct may be partially obscured by the presence of overlapping bones in the images. Computer preprocessing may be useful when isolating an infarct in a scintigraphic image in order to filter the bones from the image and to improve contrast (*5*). However, the preprocessing step must preserve infarct boundaries as much as possible.

The infarct-sizing method we have developed makes use of a slight modification of an elliptical-slice method that has already been described (*6*). Our modifi-

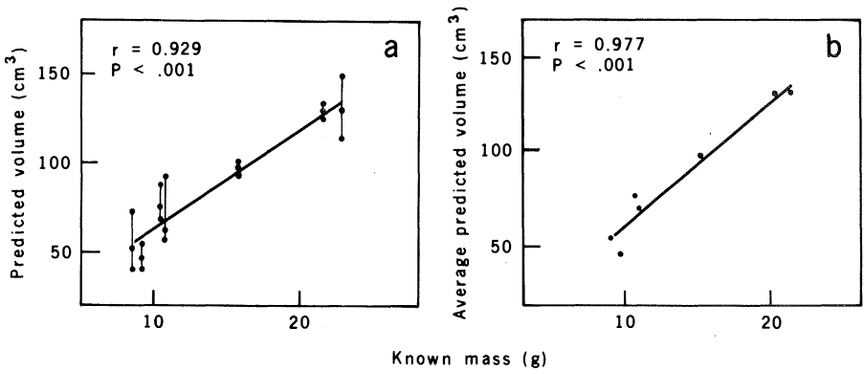

Fig. 2. Correlation between histologically determined infarct mass and infarct volume predicted from the three-dimensional model. (a) Results of three sizing estimates applied to the same data. Overall results obtained when three independently generated sets of infarct outlines were used to predict volumes and a linear regression relationship was constructed for the 21 volume predictions and histological infarct weight. (b) Average scintigraphic sizing value for each animal. The linear regression relationship that was obtained when the mean value of the three volume predictions for each animal is correlated with histological infarct weight. Average predicted volume refers to the mean volume predicted by the infarct model.

cation is to use a semiellipse as the assumed cross-sectional infarct shape (Fig. 1); a semiellipse was chosen because that is approximately the shape of sections that are obtained when one serially cross-sections hearts for histological determinations of infarct size. The initial step in our method is to line up the anterior and lateral projections of the ^{99m}Tc-PYP infarct images that had not been preprocessed. Next, horizontal slices at 0.13 cm are made through the infarct images from top to bottom in both projections. Each pair of corresponding slices from the two projections determines a rectangle within which that level of the infarct is contained (Fig. 1). By using the assumed cross-sectional shape, volume elements are constructed from the slices and the infarct volume is estimated as the sum of the volume elements obtained. Because of the difficulty of precisely isolating infarcts in the images not preprocessed, the outlines and calculations were done three times independently.

In order to test the C-shaped transverse-slice model of myocardium in terms of its ability to provide a meaningful three-dimensional definition of infarct size, we used ^{99m}Tc-PYP myocardial scintigraphic data from experimentally infarcted dogs to predict infarct volumes. Then we correlated the resulting predictions with previously determined histological infarct weights (*3, 7*).

Acute anterior myocardial infarcts were produced in seven anesthetized (pentobarbital, 30 mg/kg) dogs by occluding their proximal left anterior descending coronary arteries. The ^{99m}Tc-PYP myocardial scintigrams were obtained approximately 30 hours later by using a Pho/Gamma III (Searle Radiographics) camera with a high-resolution collimator. Anterior and lateral views were obtained from each dog, and the images were stored in digital form on magnetic tape for subsequent processing on a DECsystem-10 computer. The ^{99m}Tc-PYP myocardial images were displayed on a Tektronix 4012 graphics terminal with eight levels of intensity. Although the levels of gray were adjusted to maximize contrast, no processing was done to filter bones from the images. The infarcted regions were manually outlined on hard-copy images taken from the graphics terminal, and infarct volumes were calculated as previously described.

Histological infarct size was measured as follows (*3*). The hearts were removed, weighed, and divided into five or six transverse slices, which were immersed in phosphate-buffered 10 percent formalin. The left ventricular portion (free wall and septum) of each transverse slice was dissected free and divided into several tissue blocks, and each block was weighed. Histologic sections were prepared from each block and were stained with hematoxylin and eosin (H and E) or with the periodic acid–Schiff (PAS) technique. Each PAS-stained section was placed in a photographic enlarger, and a negative photographic print of the section was prepared at a standard magnification. Areas of infarcted myocardium were traced on the photographic prints after microscopic examination of both the H and E and the PAS sections so as to correlate the changes in the PAS sections with classic features of necrosis in the H and E sections. For each section the total area and the area of infarcted myocardium were measured by planimetry, and the percent of infracted myocardium was calculated. The mass of infarcted myocardium in each block was calculated by multiplying the average percentage of infarcted myocardium by the weight of the block. The reproducibility of the histological infarct-sizing method was assessed by measuring the mass of necrotic tissue in 12 tissue blocks on two separate occasions. The mean relative variation between the two analyses was 5.6 percent (coefficient of variation, 0.056).

Correlation between calculated infarct volumes, based on the C-shaped model, and the morphologically determined infarct weights is shown in Fig. 2. The overall correlation provided by the three sets of calculations is statistically significant ($r = 0.929, P < .001$), as is the correlation obtained by averaging the three separate volume predictions obtained for each dog infarct. The variability in predicted infarct volumes appears to be due primarily to difficulty in precisely reproducing the infarct outlines; this indicates a need for further improvement in image quality and probably can be helped by computer processing of the images.

Previous work from our laboratory has shown that experimental anterior infarcts can be relatively accurately sized by use of a simple planimetric measurement of infarct area with or without computer processing of the image (*3*). However, the correlation coefficients obtained in our earlier investigations relating scintigraphic and histological infarct size have never been as good as the present ones, and our earlier methods were unable to accurately size inferior and nontransmural infarcts since only two-dimensional estimates were used.

The results suggest that computer-aided sizing of myocardial infarcts from ^{99m}Tc-PYP scintigrams, with the use of a C-shaped cross-sectional model, may be used to measure the size of experimental myocardial infarcts in dogs with occlusion of the proximal left anterior descending coronary artery. The method described has the advantage of being relatively simple, quick to calculate, and minimal in equipment requirements. Possible improvement in results could be achieved if two more orthogonal scintigraphic views (for example, 45° left anterior oblique and 45° right anterior oblique) were routinely available and if computer preprocessing of the images was performed. Since infarct volume predicted by this method depends strongly on the manually outlined infarct borders, the accuracy of the method depends on having clear images and carefully drawn outlines.

Further work should be done to improve the quality of the ^{99m}Tc-PYP im-

ages and to broaden the scope of the model. In particular, the ability of this reconstruction technique to size experimental infarcts needs to be tested in animals with acute inferior and nontransmural (subendocardial) myocardial infarcts. The present data, however, suggest that a three-dimensional model may be used for accurate estimation of experimental infarct size in dogs with acute anterior infarcts. This method has potential application for the quantitation of infarct size in humans.

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Transport Interaction of Cystine and Dibasic Amino Acids in Renal Brush Border Vesicles

Abstract. *The uptake of cystine by vesicles prepared from rat kidney brush borders occurs by two distinct transport systems. The higher affinity system is inhibited by the dibasic amino acids lysine, arginine, and ornithine. The lower affinity system, unaffected by dibasic amino acids, appears to correspond to that observed by studying uptake of cystine by kidney slices.*

Human cystinuria, an inherited disorder characterized by hyperexcretion in the urine of cystine and the dibasic amino acids, lysine, arginine, and ornithine, has focused attention on the nature of the renal tubule reabsorptive mechanism for these substances. Dent and Rose (*1*) postulated that these four amino acids have a common transport process in the renal tubule cell, which is defective in this disease, a conclusion strengthened by the fact that lysine infusion in both man and dogs produced an increase in cystine excretion (*2*). Experiments with both rat and human kidney cortex slices in which the cellular uptake of radioactive cystine, lysine, and arginine was studied revealed that the dibasic amino acids shared a common transport system, but that cystine uptake was by an independent mechanism; lysine and arginine did not inhibit cystine uptake by the renal cortical slice (*3, 4*). Indeed, this dichotomy was strengthened by the finding that dibasic amino acid uptake by renal cortex slices from human cystinuric patients was defective, but that of cystine was not (*4*). Further support for the separate nature of the renal transport process for cystine and dibasic amino acids came from the description of patients with hyperdibasic aminoaciduria without cystinuria (*5*) and with cystinuria without dibasic aminoaciduria (*6*) as well as from dogs with cystinuria without significant dibasic aminoaciduria (*7*).

However, Greth *et al.* (*8*) have demonstrated in vivo that cystine can enter the renal tubule cell via the basal lateral membrane in rats under conditions where luminal transport does not occur. It thus appeared that in the rat kidney cortical slice significant uptake of cystine might occur through the basal lateral membranes, obscuring transport at the luminal brush border membrane and leaving the interaction of cystine and dibasic amino acids at the luminal membrane as a distinct but undetectable possibility. Silbernagl and Deetjen (*9*) have reported that, in micropuncture studies of rat proximal tubules, arginine inhibits the tubule reabsorption of cystine, thus supporting an interaction of the amino acids in a brush border transport process.

Although the "black box" experimental techniques used previously have provided valuable insights into renal tubule transport mechanisms, the data regarding the nature of events at the luminal brush border membrane have been inferential. The ability to isolate rat renal tubule brush border membranes (*10*) and to study the entry characteristics of amino acids into vesicles prepared from these membranes (*11, 12*) provides the opportunity to examine the interaction of cystine with dibasic amino acids at the membrane locus of transport. The results of such studies form the basis of our report.

Rat brush border membranes were isolated by the method of Booth and Kenny (*13*). Vesicles were prepared by homogenization and centrifugation in hypotonic THM buffer [1 m*M* tris plus Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) plus 100 m*M* mannitol, *p*H 7.4] as previously reported (*14*) and uptake of cystine in the presence of a sodium gradient at *p*H 7.4 determined by methods described by McNamara *et al.* (*12*) in a THM (20 m*M* tris plus 20 m*M* Hepes plus 60 m*M* mannitol). ^{14}C-labeled cystine (291 mc/mmole) was purchased from New England Nuclear Corporation and found to be chromatographically pure. The vesicle preparation showed an alkaline phosphatase enrichment of 12-fold compared to the starting material. Michaelis-Menten constants (K_m) were calculated assuming a two-component system by linear and nonlinear regression analysis as reported (*12*).

Figure 1 shows a Lineweaver-Burk plot of the concentration dependence of the initial rate (0.5 minute) of vesicle uptake of cystine from 0.018 to 0.89 m*M*. The data reveal a two-limbed curve with observed transport parameters of K_{m_1} of 0.031 m*M*, V_{max_1} of 0.322 nmole per milligram of protein per 0.5 minute, and K_{m_2} of 0.481, V_{max_2} of 1.80. By regression analysis of the data, the calculated