Ultrastructural Features of Adriamycin-Induced Skeletal and Cardiac Muscle Toxicity

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In this study, the authors examined the effect of the antitumor agent Adriamycin, a known cardiotoxin, on mouse heart, diaphragm, and gastrocnemius muscle. Using an established model of Adriamycin cardiac toxicity, they found that 4 days after the intraperitoneal injection of 20 mg/kg of Adriamycin, characteristic heart lesions, including vacuolation of the sarcoplasmic reticulum, interstitial edema, and mitochondrial degeneration, were demonstrated in all treated animals. Furthermore, similar, but much more severe, myocyte damage was demonstrated in the diaphragm; muscle toxicity followed a decreasing gradient of injury from the peritoneal to the thoracic surface of the tissue. On the other hand, treatment with Adriamycin resulted in an increase in the size and number of lipid droplets in the red fibers of the gastrocnemius muscle without any other ultrastructural evidence of drug-induced damage to myocytes. An examination of the pharmacokinetics and metabolism of Adriamycin after intraperitoneal treatment revealed that relative drug levels in muscle (diaphragm >> heart >> gastrocnemius) paralleled the degree of ultrastructural damage observed. This study indicates that treatment with Adriamycin can produce significant injury to noncardiac muscle in a fashion that strongly resembles the characteristic pattern of Adriamycin-related damage to the heart, and that the degree of myocyte damage is apparently dependent upon the Adriamycin concentration in the tissue. (Am J Pathol 1985, 118:288-297)

ADRIAMYCIN (doxorubicin) is an antineoplastic antibiotic with a broad spectrum of therapeutic activity in the treatment of hematogenous malignancies as well as solid tumors of the lung, breast, thyroid, and ovary.[1] Unfortunately, this drug produces a peculiar, dose-related form of cardiomyopathy that can seriously compromise its utility in oncologic practice.[2]

Since its introduction into clinical therapeutics more than a decade ago, the morphologic features of Adriamycin cardiac toxicity have been defined both in man[3,4] and in a variety of experimental animal models.[5-7] Independent of species, Adriamycin treatment generally results in a characteristic picture of vacuolar degeneration of the sarcoplasmic reticulum, swelling of cardiac mitochondria with disorganization of the cristae, interstitial edema, and focal myocytolysis.[3] Furthermore, the functional consequences of Adriamycin cardiac toxicity, namely, alterations in the control of both myocardial calcium transport and the mitochondrial electron transport chain are a reflection of the histologic features of this drug-induced cardiomyopathy.[8,9]

It has recently been suggested by several laboratories that the cardiac toxicity of Adriamycin is due to its enzymatic activation to a reactive intermediate in heart mitochondria and sarcoplasmic reticulum.[10,11] It is unknown, however, whether or not metabolic activation of Adriamycin with consequent muscle damage is a specific feature of the myocardial cell. In order to determine the tissue specificity of these potentially toxic reactions, we examined the ability of Adriamycin to injure muscle of the appendicular skeleton and diaphragm. Because the distribution of the flavin-enzyme systems capable of activating Adriamycin is similar in heart and skeletal muscle,[12] we expected that Adriamy-

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cin would prove toxic to all three types of myocytes, even though the drug has not been suggested until very recently to produce skeletal muscle toxicity.[13] Our results indicate that treatment with Adriamycin produces striking myocellular damage to noncardiac muscle; furthermore, the ultrastructural features of this muscle injury strongly resemble the characteristic picture of Adriamycin toxicity in the heart.

Materials and Methods

Drug Treatment

For these experiments, CDF_1 male mice weighing 18–20 g were obtained from Simonsen Laboratories, Gilroy, California. The mice had been raised on Wayne Lab-blox mouse pellets with water available *ad libitum*; they were caged on hardwood bedding and were housed in a constant temperature (22 C) environment with alternating 12-hour wake and sleep cycles. Adriamycin was obtained from Adria Laboratories, Inc., Columbus, Ohio; Adriamycin was reconstituted in 0.85% sterile sodium chloride on the day of administration and was protected from light until used. In these studies, experimental animals were housed 5 to a cage. The two experimental groups consisted of 5 mice treated with Adriamycin and 5 saline-treated controls. Following an established protocol for our previously published morphologic and biochemical model of anthracycline cardiac toxicity,[6,14] Adriamycin was administered at a dose of 20 mg/kg body weight by intraperitoneal injection in a constant volume of saline. This drug dose was chosen because our previous studies had indicated that 1) it resulted in a reproducible degree of cardiac injury 96 hours after drug administration which had all of the characteristic features of adriamycin cardiomyopathy,[6] 2) on the day of sacrifice there was essentially no animal mortality from noncardiac drug-induced toxicity, 3) when appropriately converted to an equivalent dose in man on the basis of body surface area it was remarkably similar to drug dosage regimens routinely used in the clinic,[1,15] and 4) there was no renal damage and only very mild hepatic toxicity produced by this dose of Adriamycin in the CDF_1 mouse.[6] Control animals were treated simultaneously with identical volumes of 0.85% sterile sodium chloride. Adriamycin and saline treatments occurred at 8 AM (Pacific Standard Time).

Tissue Preparation and Electron Microscopy

Four days after drug treatment, mice were sacrificed by cervical dislocation. The adbominal and thoracic cavities were quickly exposed and flushed on all surfaces, including the cardiac interior, with buffered aldehyde fixative (2% formaldehyde, 2% glutaraldehyde, 0.1 M cacodylate buffer, 0.02% $CaCl_2$, pH 7.4). Simultaneously, the leg was removed and skinned, and the gastrocnemius muscle was flooded with fixative. Strips of the diaphragm were also carefully removed, tagged for identification of the thoracic surface for future orientation during sectioning, and immersed in chilled aldehyde fixative. Samples of the left ventricle and midportion of the gastrocnemius were excised, minced, and also immersed in chilled aldehyde fixative. After 2–3 hours, the tissue samples were rinsed in buffer and postfixed in 1% O_sO_4, 0.1 M cacodylate buffer, 0.02% $CaCl_2$, pH 7.4, for 2–3 hours. Following osmication and buffer rinses, the tissue was dehydrated with graded ethanol, transferred to propylene oxide, and infiltrated and embedded in Epon. The tissue was sectioned, stained with lead citrate and uranyl acetate, and examined by electron microscopy.

Pharmacologic Studies

To examine the relative distribution of Adriamycin in cardiac and skeletal muscle after intraperitoneal drug administration, we treated 6 experimental animals per time point with 20 mg/kg of Adriamycin intraperitoneally and 3 with an equal volume of physiologic saline. Two and 24 hours after Adriamycin administration, control and drug-treated animals were sacrificed; and diaphragmatic, cardiac, and gastrocnemius muscle were processed, as previously described,[6] prior to tissue homogenization. Levels of Adriamycin, Adriamycinol, and the collected aglycones of these species in muscle were detected by the method of Bachur and colleagues.[16] In brief, the tissues were pooled after washing so that each sample consisted of organs from two mice; the samples were then extracted into chloroform/methanol (2:1, vol/vol) by homogenization for 2 minutes with a Brinkman model PCU-2-110 Polytron (Brinkmann Instruments, Inc., Westbury, NY) on ice. The homogenate was then filtered and evaporated to dryness under a stream of nitrogen. The dried extract was redissolved in chloroform/methanol (2:1) and chromatographed on scored Silica Gel 60 thin-layer plates (250 μ) in the two-phase system described by Bachur et al.[16] The relative fluorescent intensity of Adriamycin and its metabolites was determined from a linear calibration curve with the use of a Perkin-Elmer model 650-10S spectrofluorimeter with activation and emission wavelengths of 470 and 585 nm, respectively. An Adriamycin standard as well as chemically prepared Adriamycinol and aglycone standards were chromatographed on each plate. Experiments in which daunorubicin was added as an internal standard prior to

homogenization to tissues from animals both treated and untreated with Adriamycin revealed an average recovery of 75% for the anthracycline antibiotics in these studies. In all determinations, background organ fluorescence, as determined in control animals, was converted to equivalent drug levels and subtracted from the experimental results. Data were analyzed with the two-tailed Student t test for independent means ($P > 0.05$ was considered insignificant).

Results

Cardiac Muscle

Adriamycin has been demonstrated previously to produce cardiac toxicity in the mouse when administered by either the intravenous or the intraperitoneal route.[5] In this study, our observations of Adriamycin cardiomyopathy after intraperitoneal drug treatment are consistent with those of prior investigations by several laboratories.[5] We found that myocardial damage was focal; heavily damaged cells were often adjacent to those that appeared normal (Figure 1). There was a variable degree of damage to heart mitochondria; however, mitochondrial swelling, disruption of the cristae, and the presence of paracrystalline bodies were demonstrated in some fields. The most consistent feature of Adriamycin-induced cardiac injury was vacuolar degeneration of portions of the sarcoplasmic reticulum; the presence of myelin figures and an array of dense bodies also characterized the Adriamycin-damaged myocytes (Figure 2). Finally, myofibrillar disorganization and interstitial edema, as well as occasional frank myocytolysis, were also observed. A blinded quantitative evaluation of this myopathic injury was independently performed by the three investigators. Grading of our cardiac samples according to the 0–3 scale established by Billingham et al[4] revealed a mean pathology grade of 2.14 ± 0.44 (mean ± SD; n = 5). The pathology grade of cardiac tissue from saline-treated control mice was not significantly different from 0.

Diaphragm

The diaphragm in the mouse is composed of fibers that are categorized as white, red, and intermediate.[17,18] Red fibers are distinguished by numerous large, rounded mitochondria that are distributed throughout the sarcoplasm and in clusters beneath the sarcolemma by a thickened and electron-dense Z-line, and by an abundance of triglyceride droplets. White fibers have small, elongated mitochondria, fewer in comparison with red fibers, and these are most abundant adjacent to the Z-lines. Intermediate fibers share features of red and white fibers. It should be noted that the diaphragm in humans is also of the mixed fiber type, with, however, a preponderance of white or intermediate types.[17] Because it can be somewhat difficult to distinguish the abdominal from the thoracic surface of the diaphragm, our specimens were tagged in a consistent manner at fixation in order so that orientation could be maintained throughout all of the tissue preparation procedures. Control samples revealed intact abdominal and thoracic surfaces lined by a thin mesothelium, and a uniform distribution of muscle fibers throughout the diaphragm (Figures 3 and 4). Red, white, and intermediate fibers did not appear to have any special distribution pattern within the diaphragm.

The administration of Adriamycin intraperitoneally resulted in a dramatic gradient of damage across the diaphragm in all treated animals (Figure 5). Large, clear spaces, probably representing interstitial edema, consistently marked the abdominal side of the diaphragm and extended approximately halfway across the muscle. The mesothelium on the abdominal surface of the diaphragm either was absent or severely fragmented (Figures 5 and 6). Whereas tissue damage was acute nearer the abdominal side of the diaphragm, the thoracic side was unaffected morphologically. The only change evident on the thoracic side was an apparent loss of cytoplasmic lipid droplets from the red fibers.

Changes observed ultrastructurally in diaphragm myocytes after Adriamycin treatment were similar in kind to those in the heart but were markedly more severe. The focal nature of the drug's action was nonetheless still evident, because apparently normal cells were observed immediately juxtaposed to severely damaged cells (Figures 6 and 7). There was no evidence to suggest that adriamycin had in any way preferentially selected between red, white, or intermediate myocytes. Normal appearing myocytes were of all three types, and light to moderate damage was evident in all three muscle fibers. The type of muscle fiber in the most severely injured cells could not be determined because of extensive organelle degradation. The effects of Adriamycin varied from slight to severe between neighboring cells; at times variation in damage was observed even with respect to the organelles within a single cell. This is consistent with the features of Adriamycin toxicity in the heart and probably reflects the fact that the structural manifestations of Adriamycin cytotoxicity have varying time courses.

Nuclear changes included framentation of the nucleolus, an overall granular appearance of the chromatin, and marked patches of heterochromatin (Figure 7). Changes within the sarcoplasmic reticulum ranged between moderate vesiculation with the presence of small, myelin figures, to the presence of numerous, large vacuoles with larger, distinct myelin figures. In some myo-

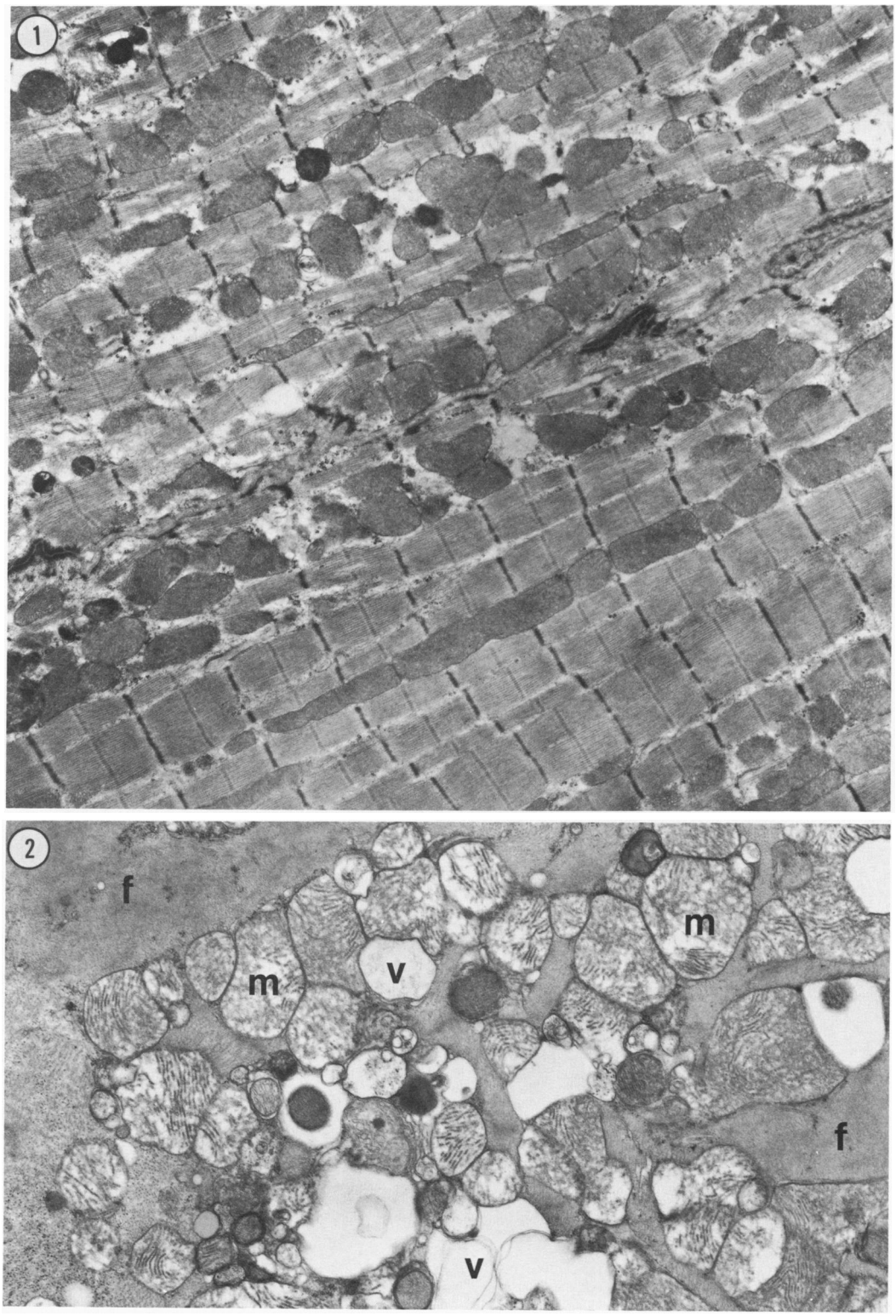
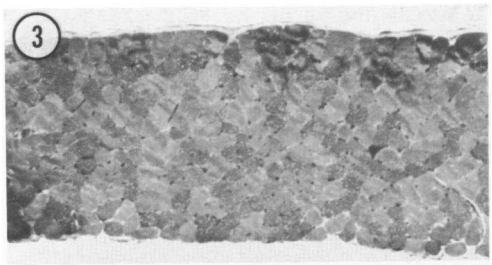
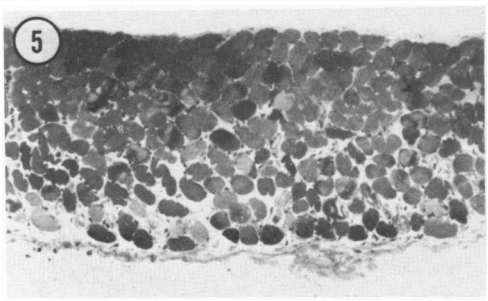
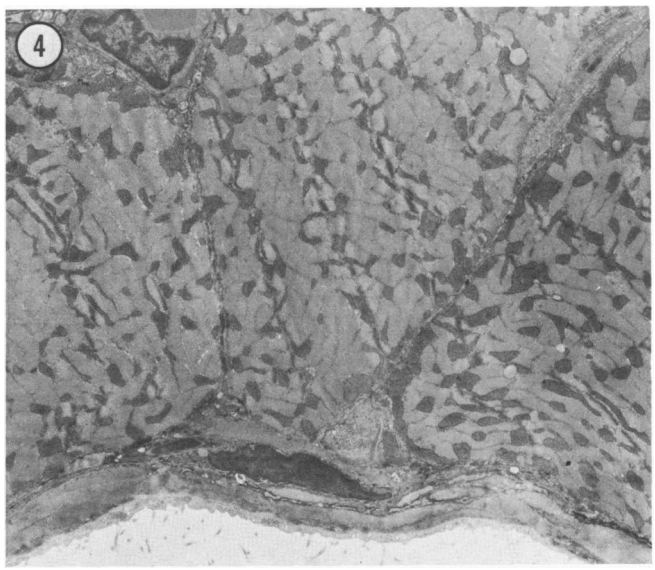


Figure 1—Portions of two cardiac myocytes are demonstrated. The more highly damaged cell (*above*) is characterized by cytoplasmic vacuolization and edema and myofibrillar disorganization. The second myocyte (*below*) demonstrates minimal damage. (×8900) **Figure 2**—Highly damaged cardiac myocytes were characterized by clusters of membranous whorls and vacuoles (*v*), mitochondria with fragmented cristae (*m*), and myofibrillar disorganization (*f*). (×13,300)







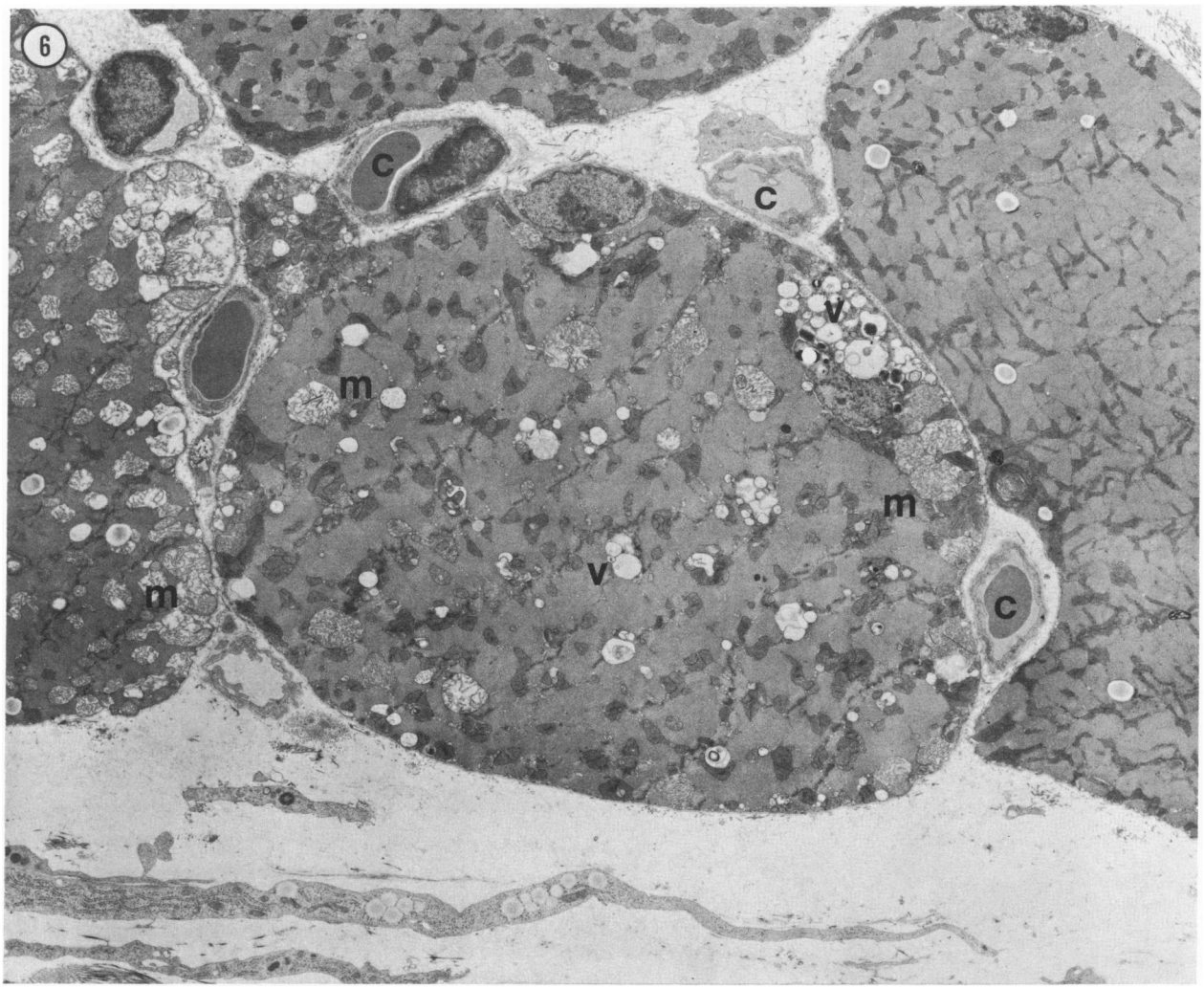


Figure 3—Light micrograph, control diaphragm. The abdominal cavity is *below*, and the thoracic cavity is *above*. Red, white, and intermediate fibers are densely packed and distributed uniformly across the entire thickness of the diaphragm. (×73) **Figure 4**—Electron micrograph, control diaphragm. The abdominal surface (*below*) has an intact, thin mesothelium with small microvilli projecting into the peritoneal space. (×4600) **Figure 5**—Light micrograph, Adriamycin-treated diaphragm. Dramatic swelling and tissue disruption is apparent at the abdominal surface (*below*). A clear gradient of damage typically was evident, extending approximately halfway across the diaphragm. (×73) **Figure 6**—Electron micrograph, Adriamycin-treated diaphragm, just adjacent to the abdominal surface. Only fragments of the mesothelium are evident (at bottom). The myocytes at the top and right appear relatively unaffected, whereas the myocytes at the *left* and *center* demonstrate extensive Adriamycin damage, ie, clusters of vacuoles (*v*) and mitochondria with fragmented cristae (*m*). Capillaries (*c*) remain consistently intact even in zones of extensive damage. (×4600)

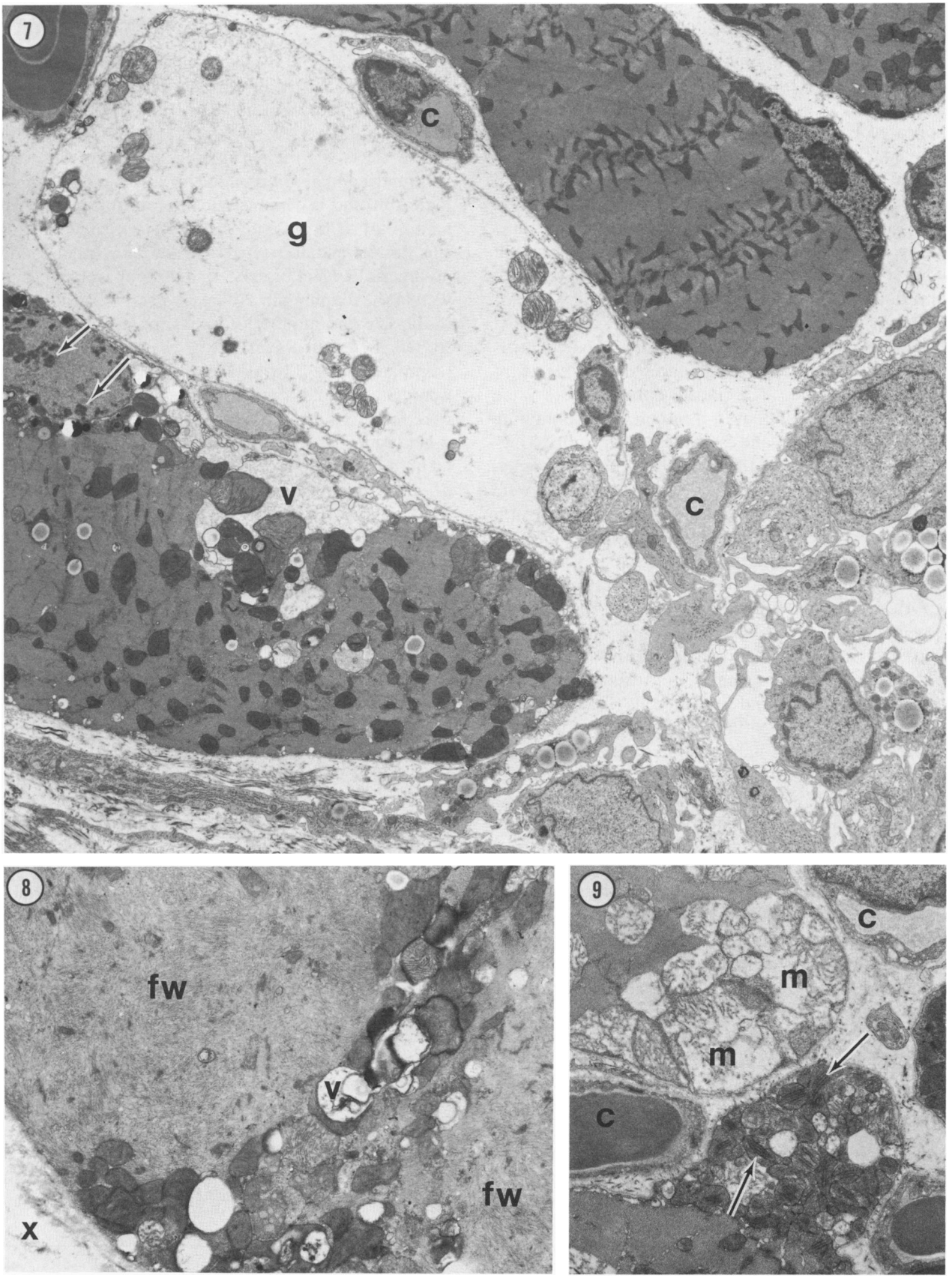


Figure 7—Variations in the extent of Adriamycin-related damage and its focal nature are demonstrated in this section taken near the abdominal surface. The myocytes at *top right* appear essentially normal, although immediately adjacent to a "ghosted myocyte" (*g*), ie, a highly damaged cell retaining an intact sarcolemma but containing only a few mitochondria and nuclei and lacking myofibrils. The myocyte (*bottom left*) reveals intermediate damage

cytes, the myofibrillar organization was lost (Figure 8), and the sarcoplasmic reticulum was very sparse, and evident only as small vesicles. Mitochondria at times appeared leached with loss of matrix density and fragmented cristae; but mitochondria were also found that had an electron-dense matrix which was associated with paracrystalline bodies and myelin figures (Figure 9).

Myofibrillar organization was variable after adriamycin treatment. In some myocytes the myofibrils were completely disorganized, which resulted in a feltwork appearance (Figure 8). Fragments of Z-lines were evident, and the remaining altered organelles accumulated at the sarcolemma. In other myocytes, the myofibrils were distorted by large, clear spaces, suggestive of intracellular edema. The most extreme cases were "ghosted myocytes," that is, a sarcolemma containing only a few organelles and essentially no myofibrils (Figure 7). Consistent with the variable nature of Adriamycin toxicity were myocytes in which myofibrils were totally disorganized but contained many intact mitochondria; conversely, myocytes were found that contained mostly degenerating mitochondria but that had maintained normal myofibrillar organization.

Other effects of adriamycin treatment included severe interstitial edema and myocytolysis. Furthermore, fibroblasts, histiocytes, and other connective tissue cells were found more frequently in Adriamycin-treated animals. Capillaries and small vessels consistently retained their normal features even in areas of the most severe drug effects (Figures 6, 7, and 9).

Grading of the diaphragm by the Billingham 0–3 myocardial scale had to be accomplished in the following manner. The diaphragm at the thoracic side generally could not be distinguished from normal controls, Grade 0. However, cell death and tissue disruption was so dramatic nearer the abdominal surface that an expansion of the Billingham scale was required, ie, Grade 4. Grade 4 then represented extensive cell death and marked loss of tissue integrity and was characteristic of the surface. A mean pathology grade of 3.10 ± 0.54 ($n=5$) characterized the abdominal half of the diaphragm. Diaphragmatic tissue from saline-treated control mice had a score of 0.

Gastrocnemius

The gastrocnemius muscle in the mouse, like the diaphragm, is composed of red, white, and intermediate fibers. Red fibers are distinguished by Z-lines, large mitochondria, and lipid droplets adjacent to the mitochondria. White fibers have fewer, smaller mitochondria and a more highly developed sarcoplasmic reticulum.

The effects of adriamycin on the gastrocnemius were very mild, compared with those in heart and diaphragm. Figures 10 and 11 demonstrate the appearance in cross-section of control and Adriamycin-treated gastrocnemius. In animals treated with Adriamycin, we found no areas of edema or cell damage comparable to that seen in the diaphragm. However, a consistent increase in the size and number of lipid droplets was demonstrated in red fibers of Adriamycin-treated animals (compare Figures 12 and 13). Lipid droplets frequently were clustered and considerably larger than normal and were found adjacent to mitochondria. No changes in other organelles were apparent after Adriamycin treatment, although in a few myocytes there was evidence of some myofibrillar disorganization. When the observed changes in lipid droplets were incorporated into the criteria of the Billingham scale, a mean pathology grade of 0.52 ± 0.12 ($n=5$) characterized the Adriamycin-treated gastrocnemius.

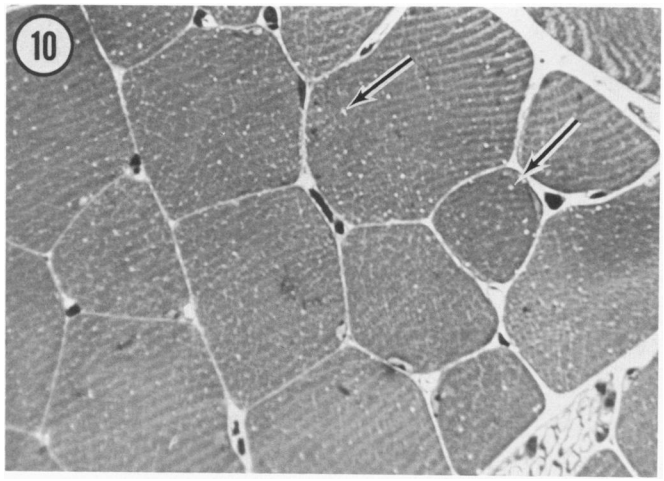
Drug Disposition

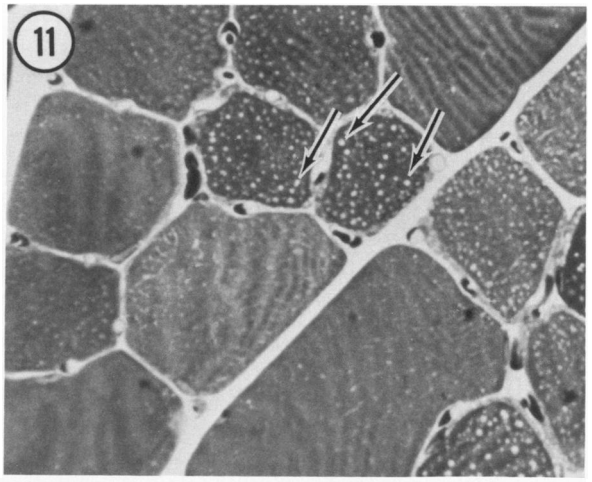
In order to explain the pattern of damage observed in diaphragmatic, cardiac, and skeletal muscle after intraperitoneal Adriamycin administration, we examined the pharmacokinetics and metabolism of the drug in these tissues. As shown in Table 1, the parent drug and its major metabolites can be detected in muscle for at least 24 hours after Adriamycin treatment by the intraperiotoneal route. Furthermore, the Adriamycin level in diaphragmatic muscle was, respectively, more than 7-fold and 50-fold higher than corresponding cardiac and skeletal muscle drug concentrations 2 hours after Adriamycin administration. A significant Adriamycin concentration differential between the diaphragm and heart and skeletal muscle persisted for 24 hours (Table 1). This marked difference in tissue Adriamycin levels may help to clarify the apparent difference between muscle types in the pattern of injury described in this investigation.

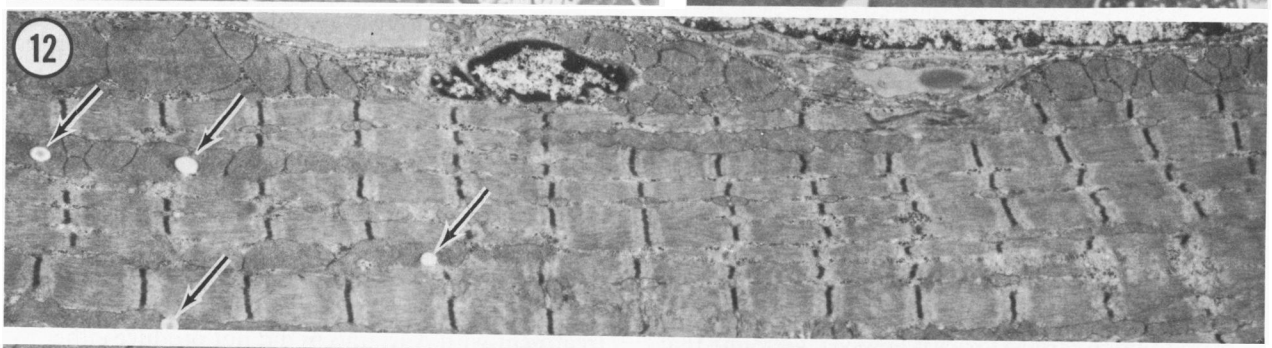
Discussion

In this study, we examined the effect of Adriamycin on mouse heart, diaphragm, and gastrocnemius mus-

consisting of cytoplasmic vacuolization (*v*) and characteristic granular clumping of chromatin (*arrows*). Greater numbers of connective tissue cells (*lower right*) were present more frequently in Adriamycin-treated animals. Capillaries (*c*) retained normal features. (×5300) **Figure 8**—Loss of myofibrillar organization associated with Adriamycin treatment. Large areas of cytoplasm appear as a filamentous feltwork (*fw*); vacuolization (*v*) and clumping of organelles is also evident. Extracellular space (*x*). (×9000) **Figure 9**—Two projecting areas of myocyte sarcoplasm are shown here, as well as at lower magnification in Figure 6. In Adriamycin-damaged myocytes, mitochondrial morphology varied between swollen mitochondria (*m*) with minimal matrix density and fragmented cristae to more electron-dense mitochondria, which frequently contained paracrystalline bodies (*arrows*). Capillaries (*c*) were consistently intact. (×8400)







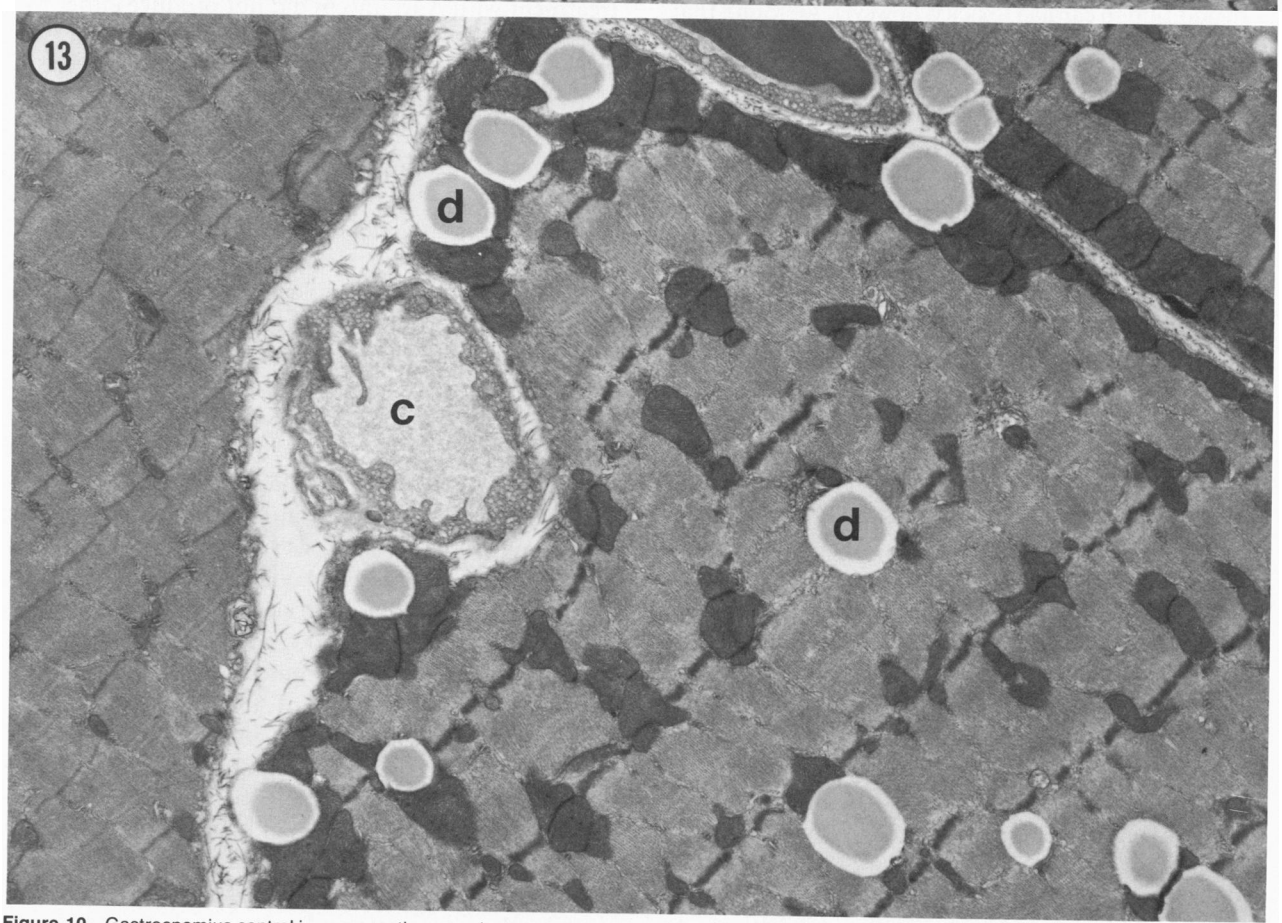


Figure 10—Gastrocnemius control in cross-section reveals morphologically normal muscle, including small clear spaces (*arrows*), most of which proved to be lipid droplets when viewed by electron microscopy (×400) **Figure 11**—Adriamycin-treated gastrocnemius in cross-section reveals a distinct population of myocytes (red fibers) with greater numbers and larger lipid droplets (*arrows*) in comparison with controls (×400) **Figure 12**—Gastrocnemius control of a red fiber demonstrates a few lipid droplets (*arrows*) in the sarcoplasm. (×5800) **Figure 13**—Red fibers in Adriamycin-treated gastrocnemius contain larger, and greater numbers of lipid droplets (*d*) than are in controls. A portion of a white fiber is seen at left. Capillary (*c*). (×9000)

Table 1—Adriamycin Distribution After Intraperitoneal Administration*

Hours after Adriamycin	Diaphragm†			Heart†			Skeletal Muscle†		
	A	A-ol	Ag	A	A-ol	Ag	A	A-ol	Ag
2	51.2 ± 7.6	1.7 ± 0.5	1.9 ± 0.5	6.8 ± 0.4‡	0.8 ± 0.05	0.08 ± 0.05	0.95 ± 0.21	ND	ND
24	15.6 ± 2.7	0.5 ± 0.1	0.7 ± 0.3	2.3 ± 0.1‡	0.33 ± 0.06	0.08 ± 0.05	0.80 ± 0.15	ND	ND

* Experimental animals were treated with adriamycin 20 mg/kg intraperitoneally. Mice were sacrificed at the times indicated, and tissues were processed for determination of Adriamycin concentration as described in the Methods. Data represent the mean ± SE of duplicate determinations on three experimental samples per time point.

† Concentrations of Adriamycin and its metabolites in diaphragm, heart, and skeletal muscle are expressed as drug equivalents per gram wet weight of A (parent Adriamycin), A-ol (Adriamycinol), and Ag (Adriamycin aglycone plus Adriamycinol aglycone). ND, not detected (<0.05 μg/g tissue).

‡ $P < 0.01$, compared with either diaphragmatic or skeletal muscle.

cle in an attempt to determine whether heart muscle is a specific target of drug-induced toxicity. We found that when Adriamycin is administered by the intraperitoneal route, typical features of Adriamycin cardiac toxicity are easily demonstrated. Drug treatment produced substantial vacuolation of the sarcoplasmic reticulum, mitochondrial disorganization, and interstitial edema; these findings have been reported previously in a number of other experimental animal models of Adriamycin cardiac toxicity, including studies from our laboratory in the mouse.[5,6]

In addition to cardiac toxicity, however, we found that treatment with intraperitoneal Adriamycin resulted in extensive damage to the diaphragm. In fact, a gradient of muscle injury was produced that probably reflects the penetration of the drug into myocytes from the abdominal toward the thoracic surface. As we have recently reported,[19] Adriamycin is a moderately lipophilic compound that diffuses in substantial concentration into three or four cell layers of the diaphragm after intraperitoneal administration. This probably explains in part the lesser muscle damage noted in subthoracic diaphragmatic myocytes.

Diaphragmatic muscle injury was characterized by vacuolation of sarcoplasmic reticular membranes, myofibrillar degeneration, interstitial edema, and the swelling and breakdown of mitochondrial membranes; muscle damage was focal, and examination of adjacent cells revealed different degrees of disorganization. On the whole, the toxicity score for diaphragmatic muscle was significantly higher than that for the heart ($P < 0.05$). Furthermore, as previously noted for the myocardium,[3] capillary injury could not be demonstrated in the diaphragm after drug administration. In short, intraperitoneal treatment with Adriamycin produced the same, albeit significantly more severe, spectrum of ultrastructural muscle injury in the diaphragm as produced by Adriamycin in the heart. These findings are important because they indicate that Adriamycin is capable of damaging noncardiac muscle, and that the drug damages the same intracellular organelles in the diaphragm as in the heart. Thus, it is entirely possible that the same enzymatic mechanism of drug activation could be involved in drug-induced muscle toxicity in both the heart and the diaphragm, because both tissues possess the enzymes thought to be responsible for metabolizing Adriamycin to its highly reactive drug intermediate.[12] Furthermore, it appears that a unique form of drug–tissue interaction does not need to be invoked to explain the cardiac toxicity of Adriamycin, because as we have shown, at the ultrastructural level a similar type of damage can be produced in noncardiac muscle.

An additional finding in this investigation was that treatment with Adriamycin did not produce either the type or degree of damage in gastrocnemius muscle as found in the heart or diaphragm. However, we did observe that Adriamycin administration was accompanied by an unexplained increase in the size and number of lipid droplets in the red fibers of the gastrocnemius. Previous studies have revealed that the concentration of Adriamycin in skeletal muscle is from 13% to 17% of that in the heart at the same time points after drug treatment[20,21]; we have made a nearly identical observation in these studies. Hence, it is likely that substantial alterations in the architecture of gastrocnemius muscle was not observed because the Adriamycin concentration in this muscle, compared with the heart or diaphragm, was so low. On the other hand, the extreme toxicity of Adriamycin for diaphragmatic muscle appears to be a clear reflection of the high drug levels found in this tissue. Thus, as suggested by the clinical studies of Legha and colleagues relating Adriamycin cardiac toxicity to plasma concentrations of Adriamycin in man,[22] our investigation indicates that the induction of Adriamycin-related muscle injury may be modulated specifically by the level of Adriamycin in the tissue.

Finally, our observation of significant diaphragmatic toxicity after intraperitoneal Adriamycin administration takes on added significance because of recent clinical trials aimed at treating human ovarian cancer by an intraperitoneal infusion of Adriamycin. In these studies, the major toxicity of Adriamycin administration by the intraperitoneal route which severely limits the maximum tolerable dose is clinical peritoneal and

diaphragmatic irritation,[23,24] a feature that could be explained by the severe local tissue toxicity that we have demonstrated in this study.

In summary, the present investigation has shown that Adriamycin produces substantial toxicity in noncardiac muscle, the features of which closely parallel the characteristic pattern of Adriamycin-induced damage to the heart.

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