

## **Amiodarone and its desethyl metabolite: tissue distribution and morphologic changes during long-term therapy**

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**ABSTRACT** The pharmacokinetic characteristics of amiodarone suggest extensive tissue deposition. We confirmed this by measuring tissue concentrations of the drug and of its major metabolite, desethylamiodarone, in human tissues. These were obtained at autopsy (n = 9), surgery (n = 7), or biopsy (n = 2) from 18 patients who had been treated with amiodarone for varying periods of time. High concentrations of amiodarone were found in fat (316 mg/kg wet weight in autopsy specimens, 344 mg/kg wet weight in biopsy specimens). Amiodarone and desethylamiodarone concentrations (mg/kg wet weight, autopsy samples) were also high in liver (391 and 2354), lung (198 and 952), adrenal gland (137 and 437), testis (89 and 470), and lymph node (83 and 316). We also found high concentrations of amiodarone (306 mg/kg wet weight) and desethylamiodarone (943 mg/kg wet weight) in abnormally pigmented ("blue") skin from patients with amiodarone-induced skin pigmentation. These values were 10-fold higher than those in unpigmented skin from the same patients. These high concentrations were associated with lysosomal inclusion bodies in dermal macrophages in the pigmented skin. The inclusion bodies were intrinsically electron dense and were shown to contain iodine by energy dispersive x-ray microanalysis. Lysosomal inclusion bodies shown by electron microscopy to be multilamellar were seen in other tissues. These tissues included terminal nerve fibers in pigmented skin, pulmonary macrophages, blood neutrophils, and hepatocytes and Kupffer cells. These characteristic ultrastructural findings occur in both genetic lipidoses and lipidoses induced by other drugs, e.g., perhexiline. We conclude that during therapy with amiodarone, widespread deposition of amiodarone and desethylamiodarone occurs. This leads to ultrastructural changes typical of a lipidosis. These changes are seen clearly in tissues associated with the unwanted effects of amiodarone, e.g., skin, liver and lung.

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AMIODARONE is recognized as an orally effective agent in the treatment of atrial and ventricular arrhythmias refractory to conventional therapy. It is a benzofuran derivative containing two iodine atoms per molecule. In man, only one major metabolite has been identified, desethylamiodarone.<sup>1</sup> During long-term therapy this compound reaches similar plasma concentrations to amiodarone, and both compounds show long terminal elimination half-lives reflecting a com-

paratively low clearance and an exceptionally large volume of distribution.<sup>2-3</sup> The pharmacologic and toxicologic effects of desethylamiodarone are not well defined.

Unwanted effects occur commonly during therapy with amiodarone.<sup>3-4</sup> Thyroid disturbance is well recognized,<sup>4-6</sup> as is the almost uniform development of corneal microdeposits.<sup>7</sup> Photosensitivity is common (occurring in up to 75% of patients after 2 years' therapy in northwest England),<sup>8</sup> whereas a small number of patients develop slate-blue pigmentation affecting light-exposed skin.<sup>9</sup> A number of recent reports document pulmonary toxicity.<sup>10-12</sup> Peripheral neuropathy has been noted in several patients.<sup>13, 14</sup> Abnormalities of plasma concentrations of hepatic enzymes occur in 15% to 50% of patients,<sup>4, 15</sup> and more severe hepatic

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disorders have been recognized,<sup>16, 17</sup> including fatal hepatitis.<sup>14</sup>

We have investigated the tissue distribution of amiodarone and desethylamiodarone in man and have noted ultrastructural changes in various organs during therapy with amiodarone. These observations throw light on the association between the drug's unwanted effects and the accumulation of amiodarone and its desethyl metabolite in the body.

## Methods

**Study population.** The clinical details of the 18 patients from whom tissue samples were obtained are shown in table 1. There were 12 men and six women (mean age  $\pm$  SD  $59 \pm 16$  years, see table 1). The duration of treatment with amiodarone ranged from 11 days to 53 months and the total cumulative dose varied from 9 to 810 g. Daily dose varied both within and between

patients, but the range for long-term dosing was 200 to 800 mg/day. Patients 2, 4, 12, and 13 had received no amiodarone for 3 to 41 days before tissue was obtained. The patients had received a median of six (range 0 to 11) other drugs in the 2 years before sampling. Amiodarone was the only drug taken by all patients.

**Tissue samples.** In nine patients, samples were obtained at autopsy 1 to 4 days after death. In seven patients, biopsy specimens were obtained during surgery (thoracotomy in four and laparotomy in three). All samples were taken during routine surgery or were part of clinically necessary biopsies. In two patients (17 and 18) with amiodarone-induced skin pigmentation, elliptic skin specimens were obtained from affected skin of either the face or the dorsum of the hand.

Unaffected skin was obtained from the trunk or upper arm in the same patients. Similar samples were also obtained from one patient at autopsy (No. 9). In patient 5, pulmonary macrophages were examined after bronchoalveolar lavage and a small lung specimen for histologic examination was also obtained during bronchoscopy.

**TABLE 1**  
**Clinical features**

Patient	Age (yr)	Sex	Cardiac diagnosis	Duration of therapy	Mean maintenance dose (mg)	Total cum. dose (g)	Time without therapy (days)	Cause of death	Samples obtained at	Body wt. at time of samples (kg)	Unwanted effects
1	34	F	VF, "normal heart"	53 mo	384	610	—	—	Thoracotomy	51	Photosensitivity, goiter (euthyroid), corneal deposits <sup>A</sup>
2	41	F	WPW, RT	22 mo	367	250	14 days	—	Thoracotomy	59	Nausea, bradycardia, corneal deposits <sup>A</sup>
3	60	M	VT, IHD	33 days	600	20	—	—	Thoracotomy	81	—
4	17	M	Ebstein's WPW, RT	12 wk	280	25	3 days	—	Thoracotomy	57	Anorexia
5	64	M	WPW, IHD, RT	28 mo	400	340	—	—	Laparotomy Skin biopsy	55	↑ AST, pulmonary fibrosis, blue pigmentation
6	67	F	AF, Pacer, SSS	4 wk	340	10	—	—	Laparotomy	52	—
7	78	F	WPW, RT	48 mo	540	810	—	—	Laparotomy	47	Hypothyroid ? Hepatitis
8	52	M	SVT, IHD, aneurysm	44 wk	253	79	—	Ischemic CM	Autopsy	67	—
9	70	M	VT, IHD	28 wk	514	100	—	Ischemic CM	Autopsy	72	Photosensitivity, hepatitis
10	59	M	VT, IHD	41 days	644	26	—	VT	Autopsy	72	—
11	76	M	VT, VF, IHD	11 days	800	9	—	VF	Autopsy	78	—
12	73	F	Recurr. VF, MVD	21 mo	400	240	6 days	Mitral stenosis	Autopsy	58	Hypothyroidism
13	53	M	VT, IHD	20 mo	405	230	41 days	VT	Autopsy	NA	Nausea
14	74	M	VT, IHD	14 days	600	11	—	VF	Autopsy	70	—
15	67	M	Recurr. VF, IHD	29 wk	286	60	—	Sudden death	Autopsy	54	—
16	48	F	AF, complex congenital	9 wk	228	13	—	Congestive failure	Autopsy	41	—
17	63	M	VT, IHD	44 mo	402	530	—	—	Skin biopsy	62	Blue pigmentation
18	65	M	VT, IHD	39 mo	402	476	—	—	Skin biopsy	73	Blue pigmentation, ↑AST, corneal deposits <sup>A</sup>

VF = ventricular fibrillation; WPW = Wolff-Parkinson-White syndrome; RT = reentry tachycardia; VT = ventricular tachycardia; IHD = ischemic heart disease; AF = atrial fibrillation; SSS = sick sinus syndrome; SVT = supraventricular tachycardia; MVD = mitral valve disease; CM = cardiomyopathy; AST = aspartate transaminase; NA = not available.

<sup>A</sup>Examined only in these patients.

Where appropriate, tissue samples for measurement of amiodarone and desethylamiodarone concentrations were dissected free of fat. They were stored at  $-20^{\circ}\text{C}$  in stoppered glass tubes until analysis.

**Measurement of drug and metabolite concentrations.** The analytical technique is described in full elsewhere.<sup>18</sup> Tissue samples (10 to 100 mg) were digested with a proteolytic enzyme with the exception of fat, which was digested with crude lipase. The analytes were extracted from the homogeneous digest into an organic solvent, and their concentrations were measured by high-performance liquid chromatography. The limit of sensitivity for this method was 0.1 mg/kg wet weight with a 100 mg tissue sample. No endogenous sources of interference in the assay have been identified. The assay is highly specific for amiodarone and for desethylamiodarone; other drugs do not cross-react significantly.

#### Histologic examination

**Light microscopy.** Tissue samples were fixed in 10% formalin for a minimum of 12 hr, postfixed in formol sublimate, and embedded in paraffin wax. Five-micrometer sections cut in single batches were stained by standard techniques. Appropriate control sections were included.

**Electron microscopy.** Samples were received fresh, fixed in 2.5% glutaraldehyde in sucrose-cacodylate buffer (pH 7.3), and postfixed in osmic acid (1% aqueous osmium tetroxide). The samples were embedded in araldite. Sections of 60 to 90 nm thickness were cut on an ultratome (Ultracut, Reichert Jung) with glass knives. Sections were stained with uranyl acetate and lead nitrate. In some cases paraffin-wax embedded tissues were used. The wax was removed, and small blocks were processed for electron microscopy (JEOL JEM-100S, Tokyo). In one patient (No. 5) skin sections were prepared without heavy metal exposure. Buffy coat specimens were prepared by centrifugation of EDTA-treated blood samples for 10 min at 3000 rpm. The plasma was discarded and carefully replaced by buffered glutaraldehyde. After 30 min the buffy coat "plug" was removed, fixed again in buffered glutaraldehyde, and processed for electron microscopy.

**Energy-dispersive x-ray analysis.** Energy-dispersive x-ray analysis of skin biopsy specimens and bronchoalveolar lavage samples was performed on a modified Corinth 500 transmission electron microscope (A.E.I. Ltd.), with a CORA system (LINK Ltd.); 0.5  $\mu\text{m}$  araldite-embedded sections were mounted on 200 mesh carbon/Formvar-coated copper grids. The probe size was adjusted so that it equaled the size of the granule being analyzed.

## Results

### Amiodarone and desethylamiodarone tissue concentrations

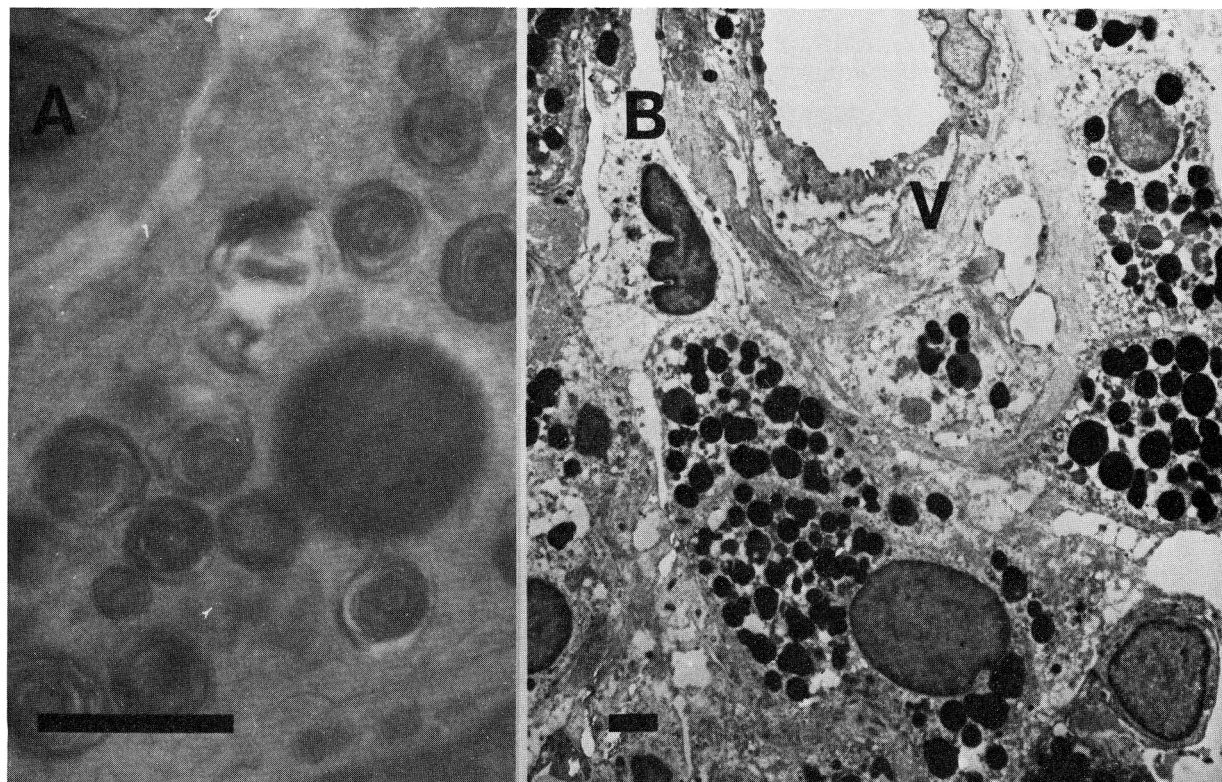
**Autopsy vs biopsy samples.** It was necessary to demonstrate comparability of autopsy and biopsy results, but a direct comparison of these results for individual tissues was not possible because patients could not be matched for dose and duration of therapy. However, inspection of the results for tissue concentrations in both groups (tables 2 and 3) showed that they were of the same magnitude, with overlap between the two groups, suggesting that results from the autopsy material can be regarded as an accurate reflection of the tissue concentrations during therapy with amiodarone. Furthermore, in patients 9 and 12, concentrations of amiodarone and desethylamiodarone were compared in liver biopsy specimens taken around the time of death with those in autopsy specimens obtained 3 and 4 days later, respectively. Concentrations of amiodarone (patient 9: premortem 1170 mg/kg wet weight, autopsy 1040 mg/kg wet weight; patient 12: premortem 706 mg/kg wet weight, autopsy 702 mg/kg wet weight) and of desethylamiodarone (patient 9: premortem 5900 mg/kg wet weight, autopsy 6830 mg/kg wet weight; patient 12: premortem 4300 mg/kg wet weight, autopsy 3970 mg/kg wet weight) were similar between the two sets of samples. Over this period, amiodarone and desethylamiodarone concentrations were not affected by the time after death.

**Tissue distribution of amiodarone and desethylamiodarone (tables 2 and 3).** Blood samples were collected in six patients undergoing biopsy and in eight of the nine patients studied at autopsy. Postmortem blood was collected from the left ventricle in five patients (see table 3) and in most instances was lysed.

TABLE 2

Tissue concentrations of amiodarone (A) and desethylamiodarone (DA) (both mg/kg wet weight) in biopsy samples

Patient	Plasma		Skin		Fat		Skeletal muscle		Heart		Liver		Spleen		Lymph node		Pancreas	
	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA
1	2.1	2.4	8.3	24	470	125	23	46	47	95								
2	0.2	0.3	3.5	14	154	45	5.4	13	12	24								
3	1.4	1.3	4.6	9.3	117	30	8.3	12	12	47								
4			8.9	20	320	42	8.7	15	22	77								
5	3.2	3.7	34	45	856	216	57	71			1640	8150						
6	0.4	0.8	29	10	156	41	11	23			51	370						
7	0.6	0.9			333	65	16	30			286	1486	41	170	57	230	46	123
17			17	72														
18			35	46														
Mean	1.3	1.6	17.5	29.9	344	80.5	18.5	30.0	23.3	60.7	659	3335						
SEM	0.5	0.6	4.7	7.9	97.8	25.6	6.8	8.2	8.2	15.8	495	2429						



**FIGURE 1.** Electron micrographs of pigmented skin from a patient with amiodarone-induced skin pigmentation (No. 17). *A*, Structure of multilamellar bodies occasionally seen. The appearance can be contrasted with the electron-dense relatively homogeneous granules seen more commonly (*B*). *B*, Perivascular location of the granules in papillary dermal macrophages (*v* = vessel). Scale bar = 2  $\mu$ m.

In biopsy samples the mean ( $\pm$  SEM) concentrations of amiodarone and desethylamiodarone were  $1.3 \pm 0.5$  and  $1.6 \pm 0.6$  mg/liter, respectively. For autopsy samples the results were  $1.9 \pm 1.0$  and  $3.0 \pm 0.3$  mg/liter, respectively. In the autopsy samples the lysing process probably contributed to the lower ratio of amiodarone to desethylamiodarone concentrations because of the higher concentrations of the latter compound in red cells than in plasma.<sup>19</sup> In addition, post-mortem changes in blood concentrations depending on the sampling site cannot be ruled out.<sup>20</sup>

High concentrations of amiodarone and desethylamiodarone were found in tissue (tables 2 and 3); the highest concentrations were found in liver, with mean concentrations (amiodarone/desethylamiodarone, mg/kg wet weight) of 659/3335 in biopsy specimens ( $n = 3$ ) and 391/2354 in autopsy samples ( $n = 9$ ). The range of concentrations of amiodarone and of desethylamiodarone in a particular tissue was wide, reflecting differences in dose and duration of therapy and the time since therapy had been discontinued (patients 2, 4, 12, and 13). However, the concentrations of both of the compounds were consistently highest in liver, lung, adrenal gland, testis, spleen, and lymph node

and were lowest in skeletal muscle, thyroid gland, skin, and brain. This pattern was seen in all of the patients.

The ratio of amiodarone to desethylamiodarone concentrations was lower in tissues than in plasma (table 4), ranging from 0.13 to 0.72. In fat, however, the ratio of amiodarone to desethylamiodarone was 4.67. Mean concentrations in fat were as follows: biopsy specimens, amiodarone 344 mg/kg wet weight, desethylamiodarone 80.5 mg/kg; autopsy specimens, amiodarone 316 mg/kg wet weight, desethylamiodarone 76.5 mg/kg.

*Drug and metabolite concentrations in pigmented and unpigmented skin.* Concentrations of amiodarone and desethylamiodarone were compared in normal and pigmented skin from the three patients with amiodarone-induced cutaneous pigmentation (Nos. 5, 17, and 18) (table 5). In all three patients the concentrations of both compounds were much higher in pigmented than in unpigmented skin.

#### Histology

*Skin.* In skin affected by amiodarone-induced cutaneous pigmentation there was a striking accumulation of dermal macrophages with a perivascular localiza-



**TABLE 3**  
**Tissue concentrations of amiodarone (A) and desethylamiodarone (DA) (mg/kg wet weight) in samples obtained at autopsy**

Patient	Plasma		Skin		Fat		Skeletal muscle		Heart		Liver		Spleen		Lymph node	
	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA
8			43	46	479	112	10	30			188	573	94	277	41	133
9	3.9	3.7 <sup>A</sup>	60	138	805	240	95	195	147	624	1040	6830	360	2050	272	1070
10	2.3	2.0 <sup>B</sup>			210	45	8	19	21	72	430	1520	114	385		
11	2.1	2.5 <sup>B</sup>	2.7	7.9	61	14	12	17	25	103	44	202	28	92	22	29
12	1.3	2.6 <sup>B</sup>	13	25	359	77			40	199	702	3970	153	893	71	337
13	0.4	3.2 <sup>B</sup>	8.3	26	305	53	6.6	31	12	85	69	1180	49	59		
14	1.2	4.6 <sup>B</sup>	10	6.5	61	8.7			13	44	24	42	11	50	8.7	8.9
15	2.1	2.5 <sup>A</sup>			243	87	7.5	33	35	161	890	6500				
16	2.0	3.1 <sup>B</sup>	15	39	321	52	17	29	27	64	131	365	95	342		
Mean	1.9	3.0	21.7	41.2	316	76.5	22	50.6	40.0	169	391	2354	113	519	82.9	316
SEM	0.4	0.3	8.0	17.1	75.6	23.2	12.2	24.2	15.7	67.5	131	905	39	239	48.6	197

<sup>A</sup>Collected before death.  
<sup>B</sup>Collected at autopsy from left ventricle or femoral vein (patient 12).

tion in the papillary dermis. Within these macrophages were many golden brown pigment granules, resembling lipofuscin. The staining characteristics of the granules were the same in samples from all patients with pigmentation (Nos. 5, 17, and 18). The pigment was positive in the periodic acid–Schiff test, both before and after diastase extraction, and Perl’s technique for ferric iron yielded negative results. Masson-Fontana and long Ziehl-Neelsen techniques were positive. The pigment was resistant to treatment with potassium permanganate and oxalic acid (melanin bleach stable). These findings suggest lipid deposition in secondary lysosomes with little melanin content. Unpigmented skin showed only occasional pigment granules.

Electron microscopy of pigmented skin (figure 1) showed 10 to 40 homogeneous granules per cell profile, 0.5 to 1.5  $\mu$ m in diameter, in the cytoplasm of papillary dermal histiocytes. Figure 1 shows that many of the bodies appeared electron dense and relatively homogeneous but that some (at higher magnification) were multilamellar with a single limiting membrane. Similar dense bodies and occasional multilamellar inclusions were seen in terminal nerve fibers and infrequently in vascular endothelial cells.

Energy-dispersive x-ray microanalysis was performed on sections from the same blocks. In figure 2 a typical energy spectrum obtained from an electron-dense granule (*top*) in pigmented skin is contrasted with that from background cytoplasm (*bottom*) in the dermal macrophages. There was a high concentration of iodine and phosphorus in the electron-dense granule, the high phosphorus content probably reflecting phospholipid accumulation. The high iodine concentration was consistent with local accumulation of

amiodarone or desethylamiodarone or other iodine-containing metabolites. Even without exposure to heavy metal during processing and when unstained, the granules appeared electron dense during routine transmission electron microscopy, further supporting the presence of an element with high atomic number, presumably iodine. The patient from whom the sample for energy-dispersive microanalysis was taken had never received iodine-containing contrast media.

*Lung.* Light microscopy of lung sections was performed in patients 8, 9, 10, 12, 15, and 16 (autopsy samples) and in patient 5 (transbronchial biopsy, araldite-embedded specimens only). In patient 5, amiodarone pulmonary toxicity was suspected because of a reduced carbon monoxide diffusion capacity, associat-

**TABLE 4**  
**Mean ratio of amiodarone to desethylamiodarone concentrations in human tissue**

Tissue	Mean ratio ( $\pm$ SD)	No. of cases
Plasma	0.72 (0.30)	14
Skin	0.71 (0.70)	15
Fat	4.67 (1.37)	16
Skeletal muscle	0.50 (0.17)	14
Heart	0.31 (0.13)	12
Liver	0.23 (0.13)	12
Spleen	0.32 (0.20)	9
Lymph node	0.46 (0.32)	6
Pancreas	0.44 (0.20)	7
Lung	0.22 (0.08)	7
Adrenal gland	0.51 (0.37)	7
Testis	0.29 (0.12)	4
Thyroid gland	0.28 (0.14)	8
Kidney	0.26 (0.11)	7
Brain	0.13 (0.05)	9

TABLE 3  
(Continued)

Pancreas		Lung		Adrenal gland		Testis		Thyroid gland		Kidney		Brain		Bile		Femoral nerve	
A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA	A	DA
64	114	183	623	103	292	113	357	12	44	37	102	3.0	25				
		600	2540	515	1890					215	960	36	209	150	420		
		180	793					12	40			8.1	44				
28	77			50	177	52	132	13	37	15	62	2.3	20				
38	136	224	1150	81	67			34	159	85	456	12	72			43	116
44	247	81	1140	42	372	167	1320	6.7	102	13	156	1.2	29	10	64	70	69
17	21	26	119	31	38	25	72	10	29	7.7	28	2.3	16			15	15
								15	92			3.7	49				
47	96	96	301	134	223			7.6	14	29	69	4.3	27				
39.8	115	198	952	137	437	89.1	470	13.8	64.5	57.4	262	8.1	54.4	80.1	242	42.7	66.7
6.7	30.8	71.9	302	64.5	246	31.9	290	3.1	17.3	28.1	129	3.7	20.2	70.0	178	15.8	29.1

ed with apical shadowing on the chest radiograph. In patient 12, large numbers and in patients 8 and 9 moderate numbers of intra-alveolar foamy macrophages were present. In patients 10, 15, and 16 similar cells were absent or sparse. In the araldite-embedded specimens from patients 5 toluidine blue staining showed the macrophages to be full of densely staining granules.

Electron microscopy was performed in lung specimens from patient 5 (figure 3). The granules seen by light microscopy in pulmonary macrophages were revealed to be multilamellar inclusions similar to those seen in some dermal macrophages and in terminal nerve fibers in pigmented skin. Macrophages in bronchoalveolar lavage specimens were of identical appearance. Energy-dispersive x-ray microanalysis showed that the multilamellar bodies in macrophages obtained by lavage contained considerable quantities of iodine. The patient had had an intravenous urogram 1 year before biopsy and we cannot exclude a contribution of iodine from the contrast medium used at that time.

*Peripheral neutrophils.* Electron microscopy of neutrophils was possible in patients 5 and 17. In patient 5, between five and 10 multilamellar bodies with a single limiting membrane were seen in each cell profile (fig-

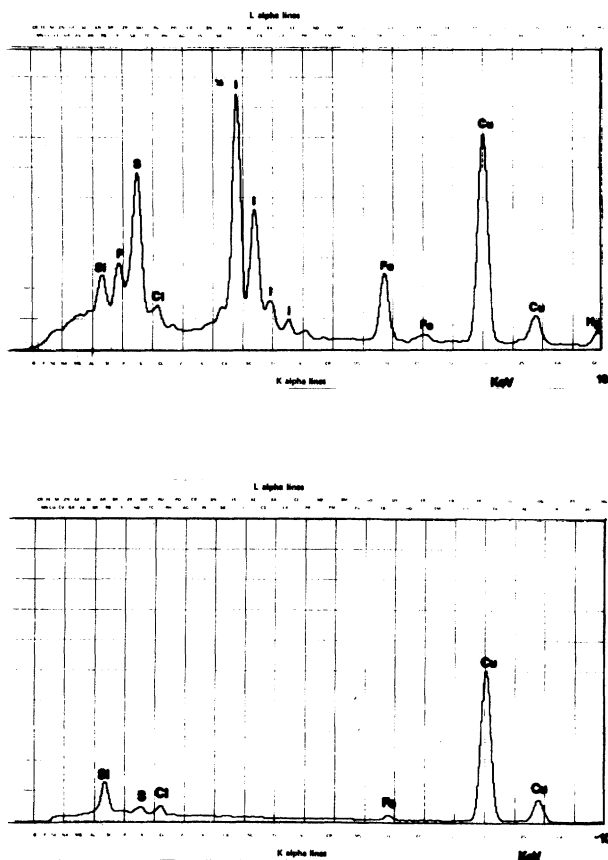
ure 4). These were very similar to those seen in other tissues. In patient 17 somewhat abnormal lysosomal bodies were seen but they did not have similar multilamellar contents.

*Liver.* In patient 9, liver biopsy was performed because of evidence of hepatitis with a low plasma albumin concentration. Light microscopy revealed preservation of normal lobular architecture with a mild inflammatory infiltrate, predominantly lymphocytic. Many hepatocytes were swollen with large lipid vacuoles. Kupffer cells were prominent with diastase-resistant, periodic acid-Schiff positive granules. Electron microscopy (figure 5) revealed that the vacuoles in hepatocytes and granules in Kupffer cells represented multilamellar bodies, similar in appearance to those seen in skin, neutrophils, and lung. Similar but less marked changes were seen in patient 5, in whom aspartate transaminase concentrations were persistently elevated to twice normal. Similar changes were also seen in patient 7, but interpretation was complicated by the presence of a lymphoma. In patient 12, changes of long-standing cardiac failure were the only abnormalities seen. In this case the aspartate transaminase concentrations were normal.

Energy dispersive x-ray analysis by the same technique as before consistently failed to reveal iodine in

TABLE 5  
Concentrations of amiodarone and desethylamiodarone in pigmented and unpigmented skin from the same patients

Patient	Pigmented		Unpigmented	
	Amiodarone (mg/kg wet weight)	Desethylamiodarone (mg/kg wet weight)	Amiodarone (mg/kg wet weight)	Desethylamiodarone (mg/kg wet weight)
9	297	699	60	138
17	119	539	17	72
18	502	1590	35	46



**FIGURE 2.** Energy-dispersive x-ray analysis spectrum from granule in macrophage from pigmented skin in a patient (No. 17) with amiodarone-induced skin pigmentation (*top*). *Bottom*, Spectrum from cytoplasm of macrophage. There is marked accumulation of iodine (arrow, I) in the granule, not seen in the cytoplasm. There is also more iron and sulphur than in the cytoplasm. The copper (Cu) is derived from the grid and the mercury (Hg) from the staining process.

any part of the hepatic sections. The high tissue concentrations of amiodarone and desethylamiodarone found in these samples are inconsistent with the failure to demonstrate iodine in the samples. If amiodarone was lost during electron microscopy processing, this was most probably caused by loss of lipid. Because iodine was demonstrated in other tissues processed in the same manner, this may indicate a different form of binding.

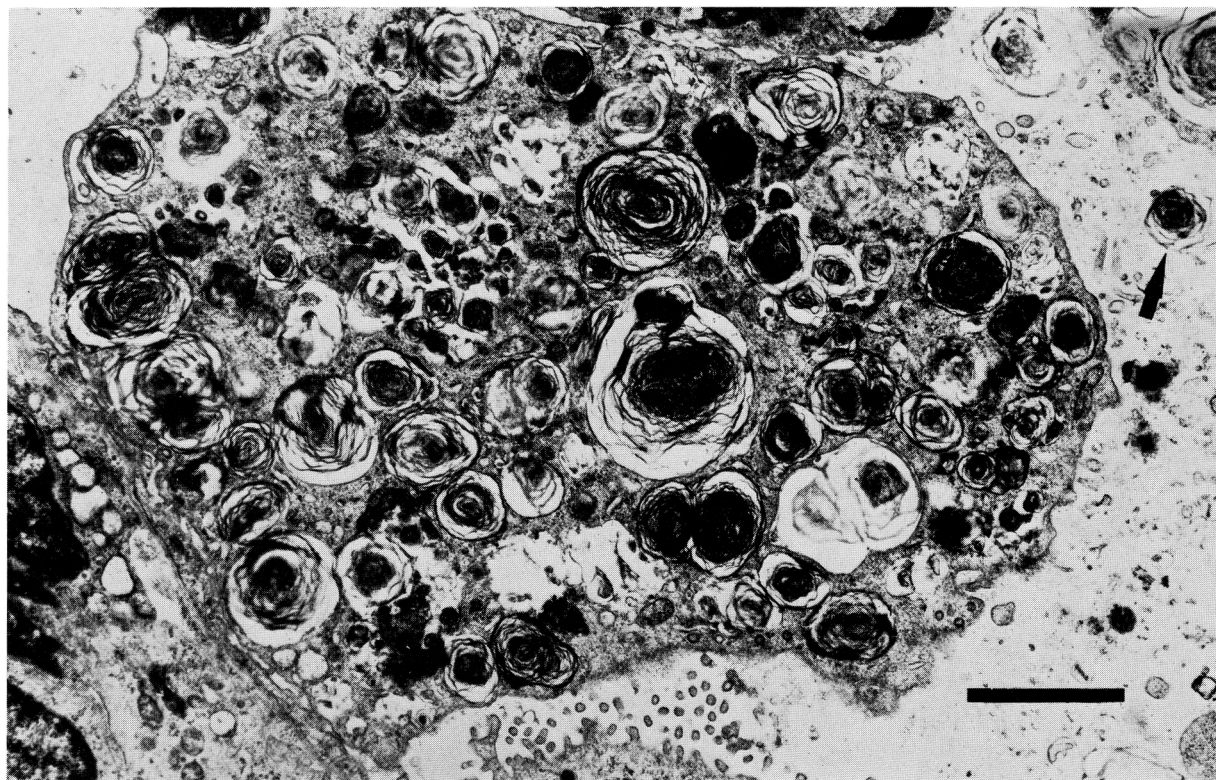
## Discussion

**Pharmacokinetic implications.** Data concerning the tissue distribution of amiodarone and desethylamiodarone in man are sparse, being restricted to isolated case reports<sup>11, 14, 17, 21, 22</sup> or to cardiac distribution.<sup>23</sup> Yet for a drug with a mean terminal elimination half-life of 53 days and a mean volume of distribution of several thousand liters,<sup>2</sup> tissue drug deposition is likely to be important. Indeed, studies in the rat show extensive tissue deposition of amiodarone, even after brief intra-

venous therapy.<sup>24</sup> Our results show that high concentrations of amiodarone and of its desethyl metabolite are indeed found in human tissue during long-term therapy. The highest concentrations of both amiodarone and desethylamiodarone were found in liver, lung, adrenal gland, testis, spleen, and lymph node and lowest in skeletal muscle, thyroid gland, skin, and brain. High concentrations of amiodarone were also found in fat. Although the dosage and duration of amiodarone therapy varied widely in the study population, the pattern of distribution was virtually identical in all cases. Relatively large amounts of amiodarone, and particularly of desethylamiodarone, were found in bile. This suggests that biliary excretion may be important and is consistent with the observation that renal elimination of both compounds is negligible.<sup>25</sup> The low concentrations of both compounds in brain suggest an active blood brain barrier. This observation is consistent with the minor central nervous system toxicity associated with the drug, particularly in comparison with other antiarrhythmic agents.

With mean values derived from our autopsy data, the tissue of a 70 kg man with a normal body distribution would contain 3 g of amiodarone and 7 g of desethylamiodarone. The major store for amiodarone is fat, which contains 41% of the total, followed by skeletal muscle with 22% (figure 6). These stores represent therapeutically significant amounts of the drug. Thus, for instance, a tissue content of 3 g of amiodarone would provide therapy for over 30 days on a maintenance dose of 200 mg daily, assuming a bioavailability of 40%. As well as providing an explanation for the persistence of clinical effects after cessation of amiodarone therapy, our results suggest that obesity may well influence the dosage requirements for the drug. The pharmacologic and toxicologic significance of the large amounts of desethylamiodarone are unknown.

**Amiodarone and desethylamiodarone distribution.** The patterns of distribution of amiodarone and desethylamiodarone were strikingly similar. Does this pattern give any clue about the mechanism responsible for this distribution? The high concentrations of amiodarone in fat are consistent with its lipid solubility, but desethylamiodarone is also highly lipid soluble. The high concentrations of both compounds in liver, lung, adrenal gland, and testis would in themselves be consistent with the lipid solubility of these compounds. However, the disproportionately high concentrations of desethylamiodarone in these tissues (table 4) suggest the possibility of another mechanism. The observation of high amiodarone and desethylamiodarone concentrations in pigmented skin in association with large num-



**FIGURE 3.** Pulmonary macrophage in lung biopsy specimen (patient 5). The cell is stuffed with multilamellar bodies, which in places are coalescent. Note a multilamellar body free in the alveolus (arrow). Scale bar = 2  $\mu$ m.

bers of macrophages laden with secondary lysosomes suggested that the tissue distribution could be related to varying uptake of drug and metabolite by macrophages. Liver, lung, spleen, adrenal gland, and testis all contain large numbers of macrophages or other cells that actively take up lipid or lipid-rich membrane fragments into lysosomes; we have shown that all these tissues accumulate amiodarone and desethylamiodarone in large amounts.

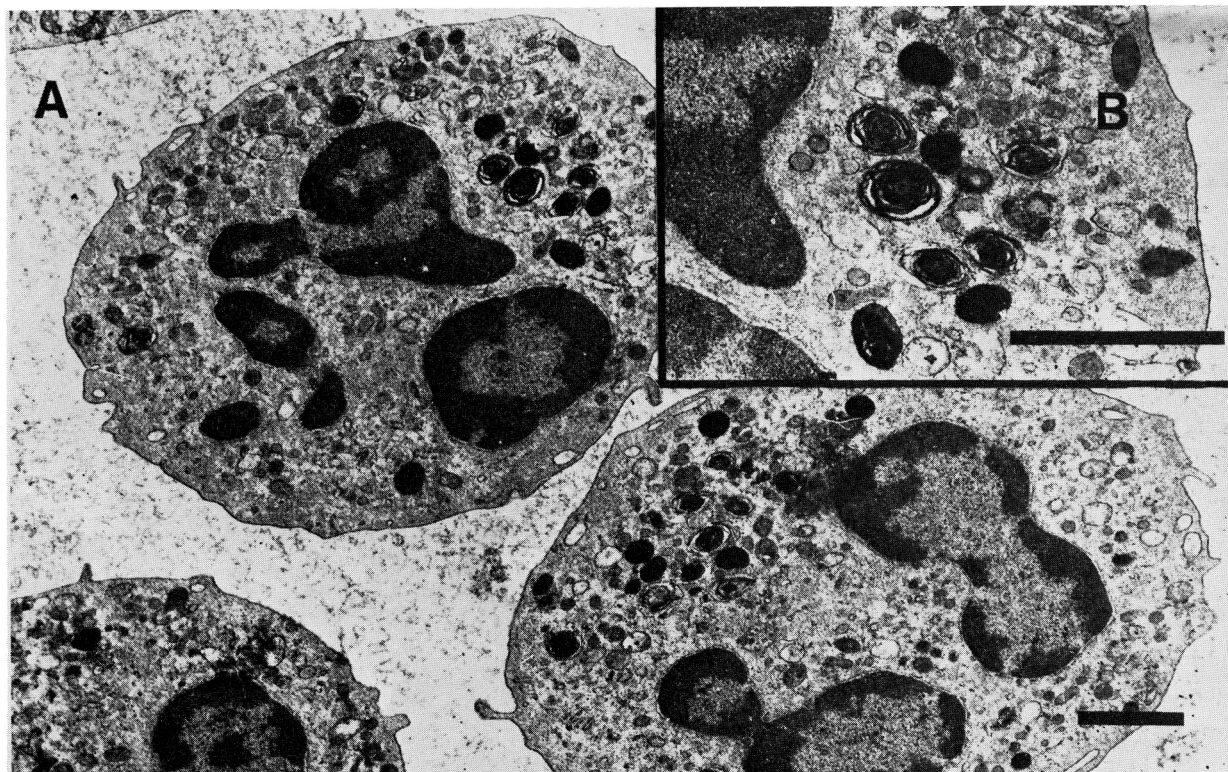
**Ultrastructure and drug and metabolite concentrations.** The ultrastructural changes seen in skin, i.e., iodine-containing, lipid-rich inclusions within macrophages, were associated with high concentrations of amiodarone and desethylamiodarone. Similar inclusions were seen in pulmonary macrophages, Kupffer cells, hepatocytes, and peripheral neutrophils. Electron microscopic and histochemical analysis of these bodies suggested they were of lysosomal origin.

Our results are consistent with the inclusion bodies containing amiodarone and desethylamiodarone. Evidence for this comes from the electron density of inclusions in skin sections observed in electron microscopy, even without heavy metal stains; high local concentrations of an electron-dense element, such as iodine, would produce this appearance. Furthermore, the en-

ergy-dispersive x-ray microanalysis findings of high local concentrations of iodine in the inclusion bodies, with none in the cytoplasm, strongly support this notion. Computed tomography of the abdomen has demonstrated high hepatic radiodensity in patients receiving amiodarone.<sup>26, 27</sup> These results also suggest high tissue concentrations of an element with large atomic number, presumably iodine.

We suggest, therefore, that during therapy with amiodarone high tissue concentrations of amiodarone and desethylamiodarone accumulate in some sites, associated with characteristic lipid-rich abnormal lysosomal bodies that contain iodine. At least some of this iodine is likely to be within the amiodarone or desethylamiodarone molecule.

**Significance of ultrastructural changes.** The ultrastructural changes we describe are not seen in normal tissue. This unusual appearance, quite strikingly similar from tissue to tissue, was associated only with amiodarone therapy, this factor being the only factor common to patients 5, 7, 8, 9, 12, 17, and 18, in which the electron microscopic abnormality (or its light microscopic equivalent) was seen. No other drug was common to this group of patients. Although there were slight variations from site to site, with multilamellar



**FIGURE 4.** A, Peripheral neutrophils (electron microscopy) in buffy coat (patient 5). Several abnormal multilamellar bodies with single limiting membranes are seen in each neutrophil. B, Higher magnification, showing that the most dense bodies have paler centers and a suggestion of multilamellar contents. Their apparent density may therefore be due to oversteining. Scale bar = 2  $\mu$ m.

bodies being less prominent in the skin, some were clearly multilamellar (figure 1, A). Slight variations in binding may explain these differences.

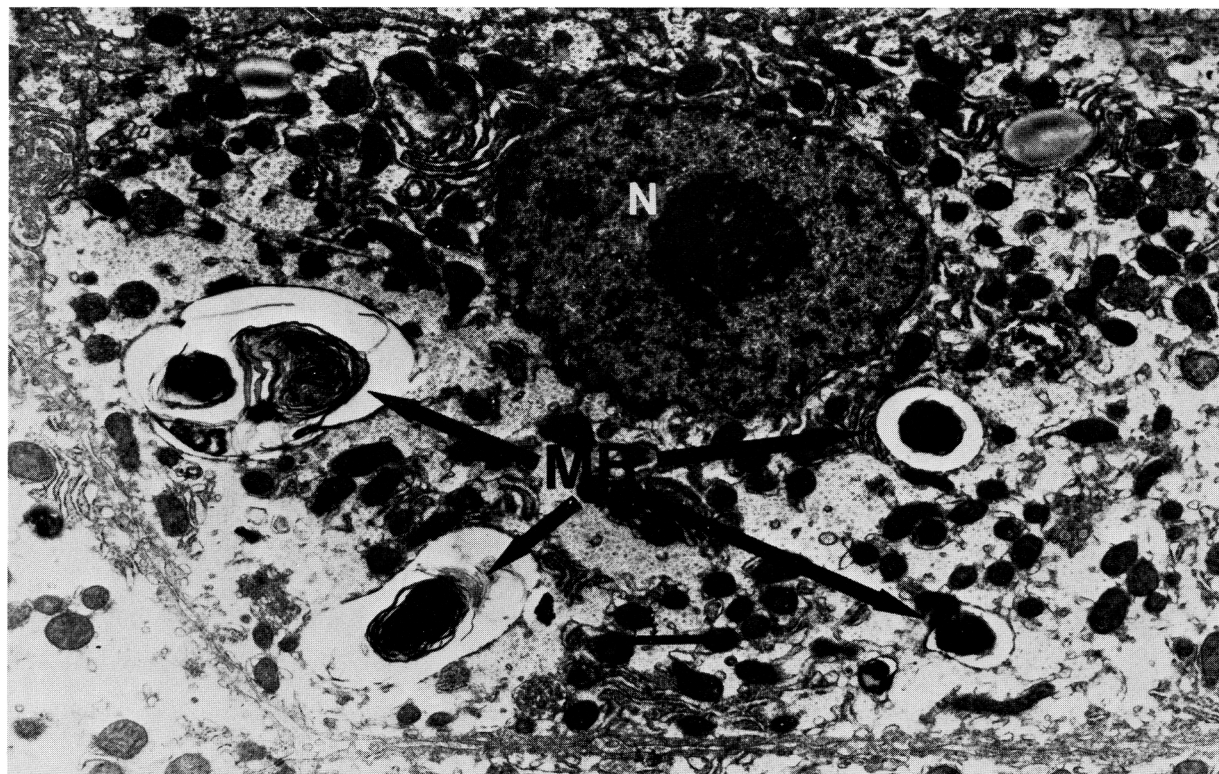
In our patients ultrastructural changes were associated most strongly with a clinically recognized unwanted effect. Other authors have recognized this association. The corneal deposits that develop in almost all patients receiving long-term amiodarone therapy are caused by multilamellar inclusions in corneal and conjunctival epithelial tissue<sup>28</sup> and are associated with high iodine concentrations.<sup>29</sup> Similar multilamellar bodies have been recognized in pulmonary macrophages, type II pneumocytes, and vascular endothelial cells in patients with pulmonary toxicity ascribed to amiodarone.<sup>10, 12</sup> We have confirmed these findings in patient 5 of this series and in other patients.<sup>30</sup> Multilamellar hepatic inclusions have also been seen by other authors.<sup>14, 16, 17</sup> Similar inclusions have been seen in peripheral nerve in patients with amiodarone-associated neuropathy.<sup>31</sup> Thus inclusions are seen in association with amiodarone-related adverse effects in some patients and probably in the corneal epithelium of most patients taking the drug. These findings, together with our data on tissue concentrations, again indicate that widespread

tissue deposition of amiodarone and desethylamiodarone leads to ultrastructural changes in many sites. These changes, some of which are associated with adverse reactions to amiodarone, are of the type seen in lipid storage disorders, both drug induced and genetically determined.

Other drugs produce such ultrastructural changes in animals and in man.<sup>32</sup> The association of drug accumulation in body tissues with lipid-rich lamellar or crystalloid cytoplasmic inclusions in a variety of cell types suggests the development of a drug-induced lipid storage disorder.

Several drugs have been implicated in the production of a lipidosis in man. Chloroquine produces a corneal dystrophy with gross and ultrastructural features similar to those produced by amiodarone,<sup>29</sup> and in a patient with chloroquine-associated myopathy, multilamellar inclusions were found in muscle.<sup>33</sup> Similar changes were seen in hepatocytes in the rat.<sup>34</sup> Perhexiline produces adverse hepatic<sup>35</sup> and neural effects,<sup>36</sup> both of which are associated with the development of multilamellar inclusions in the affected tissue. 4,4'-DH (bis-diethyl-amino-ethoxy-hexestrol, Coralgil, a coronary vasodilator at one time used in the Far East),



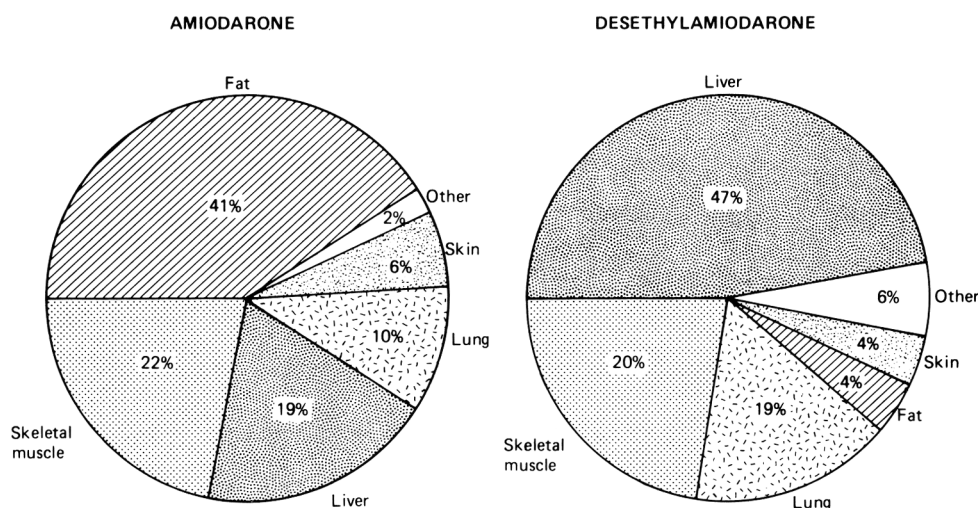


**FIGURE 5.** Hepatocyte (electron microscopy) from liver biopsy specimen (patient 9). Multilamellar inclusion bodies (MB) are shown in relation to the nucleus (N). The bodies are larger (up to 4  $\mu$ m) in liver than in neutrophils and skin (0.5 to 1.5  $\mu$ m). Scale bar = 2  $\mu$ m.

produced frequent “phospholipid fatty liver”<sup>37</sup> characterized by abnormal lipid metabolism and frequent multilamellar bodies in Kupffer cells and in hepatocytes. These changes were also seen in other tissues.<sup>38</sup> These common morphologic changes are paralleled by a tendency for all these compounds to accumulate in

tissues. 4,4’-DH can be found in tissue 18 months after the drug is discontinued, whereas the half-life of perhexiline in patients with neuropathy ranges between 9 and 22 days.<sup>39</sup>

Similar morphologic changes to those described with amiodarone and other drugs occur during the de-



**FIGURE 6.** Distribution of amiodarone and desethylamiodarone in tissues of 70 kg man, based on our postmortem mean data. The 3.1 g of amiodarone is distributed as shown on the left, with fat, skeletal muscle, and liver being the important stores. Most of the 7.5 g desethylamiodarone is in liver, smaller amounts are in skeletal muscle and lung, and fat is relatively unimportant. This suggests that different factors govern the distribution of the two compounds.



velopment of the genetic lipid storage disorders, e.g., sphingomyelin lipidosis (Niemann-Pick) and glucosyl ceramide lipidosis (Gaucher's disease), in which large quantities of lipid are laid down in tissue, usually of the reticuloendothelial system or the central nervous system. Deposition of lipids in these cases is caused by an inherited deficiency of enzymes essential for lipid metabolism. It has been postulated that drug-induced lipid storage disorders may be caused by similar enzymatic abnormalities in lysosomes, caused by the drug.<sup>32</sup> For example, chloroquine inhibits lysosomal  $\alpha$ -galactosidase; however, amiodarone therapy is associated with normal  $\alpha$ -galactosidase activity.<sup>40</sup>

These observations suggest that amiodarone produces a generalized lipid storage disorder. They are reinforced by the demonstration that the drug is a potent inhibitor of phospholipase,<sup>41</sup> a lysosomal enzyme; the effects of desethylamiodarone on this enzyme are not documented. Although the ultrastructural changes occur most clearly in association with adverse effects, a lipid storage disorder probably occurs in almost all patients treated with amiodarone for a long enough period. Corneal deposits occur in most patients treated with the drug,<sup>7</sup> and abnormalities in liver function tests, which are frequent in patients taking amiodarone<sup>4</sup> may well reflect early hepatic effects. Furthermore, we have shown that changes in pulmonary gas transfer occur in up to 30% of patients treated with a mean dose of amiodarone of 360 mg/day for 11.6 months<sup>30</sup>; these changes may well represent early pulmonary involvement in the lipid storage disorder. Indeed, some authors,<sup>42</sup> drawing on animal experiments and a single patient, suggest that the pulmonary reaction is a lipid pneumonia.

**Conclusions.** Our results show that during amiodarone therapy high concentrations of amiodarone and desethylamiodarone accumulate in tissue. The distribution of these compounds may be caused in part by the development of a generalized lipid storage disorder in which drug and metabolite accumulate in the lysosomes of many tissues. The corneal microdeposits seen with amiodarone and also some of the drug's more important adverse effects are related to this property. Similarities with other drugs suggest a mechanism that should be considered in the spectrum of drug-induced disorders. Our observations have also pointed to the value of routine monitoring of morphologic changes in tissues obtained at autopsy or where possible during life, after the introduction of a new drug.

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