Dystrophic Cardiac Calcinosis in Mice

Genetic, Hormonal, and Dietary Influences

Gordon J. Eaton, PhD, R. Philip Custer, MD, Frederic N. Johnson, BS, and Karen T. Stabenow, MS

Mice of five inbred strains (BALB/c, C3H, C3Hf, DBA/2, and C57BL/6) of both sexes, mated and virginal, were examined for the incidence, severity, and location of dystrophic cardiac calcinosis (DCC) at various ages. Three hybrids, B6C3F1, C3B6F1, and CC3F1 of both sexes, all mated, were likewise studied. Excepting DBA/2, females of the inbred strains acquired the lesion at a much earlier age than males; DCC appeared in young DBA/s mice of both sexes. DCC in BALB/c mice was almost exclusively epicardial and occurred with equal frequency and severity in mated males and females, with higher incidence but lesser extent in virginal females. The occurrence was highest, the degree most severe, and the location exclusively myocardial in C3H and C3Hf mated females, irrespective of parity, whereas virginal females of these strains were entirely free of disease even after administration of exogenous progesterone. Involvement of males, also myocardial, was relatively minimal, especially in C3Hf mice. Over half the DBA/2 mice were affected, regardless of sex or mating; calcinosis appeared in the epicardium and/or myocardium, predominantly in the myocardium. Strain C57BL/6 was completely devoid of the lesion, as were the two hybrids thereof, B6C3F1 and C3B6F1. The hybrid of BALB/c and C3H showed a high incidence of minimal involvement, exclusively myocardial and limited to breeding females, indicating dominance of the C3H gene(s). Renal calcinosis was uncommon among BALB/c mice but was frequently found in C3H, C3Hf, and DBA/2 strains. Pulmonary calcinosis was rare and limited to C3H and C3Hf female breeders. Mated C3H females fed increasing amounts of fat showed a concomitant rise in incidence and severity of the cardiac lesions. Progression of the lesion from necrotic myocardial fibers to fibrocalcific masses is illustrated, as is formation of the renal deposits. (Am J Pathol 90:173–186, 1978)

ROUTINE AUTOPSIES performed on aging mice, mostly retired breeders, in the Laboratory Animal Facility of The Institute for Cancer Research, disclosed a high incidence of calcific deposits in the heart in certain inbred strains and their hybrids. The lesion was usually inconsequential but sometimes reached such extensive proportions that it led to congestive heart failure. The site of cardiac involvement was

From The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania.

Supported by Public Health Service Grant CA-06927 from the National Cancer Institute, by Grant RR-05539 from the Division of Research Resources, and by an appropriation from the Commonwealth of Pennsylvania.

Accepted for publication September 16, 1977.

Presented in part at the Twenty-sixth Annual Meeting of the American Association for Laboratory Animal Science, Boston, Massachusetts, November 1975.

Address reprint requests to Dr. Gordon J. Eaton, The Institute for Cancer Research, 7701 Burholme Avenue, Philadelphia, PA 19111.

variable: in BALB/c it was confined to the epicardium, in C3H it was confined to the myocardium and in DBA/2 there was concurrent distribution. Calcinosis elsewhere, irrespective of strain, was limited to the kidneys and lungs, notably the former, and usually correlated well with the degree of cardiac involvement.

A genetic susceptibility to dystrophic cardiac calcinosis (DCC) has been proposed for BALB/c,[1] C, C3H, and DBA/2.[2,3] The stress of pregnancy has been suggested as another determining factor in pathogenesis, since the incidence and severity are greater in retired breeder females than in males and such disparity does not occur between the sexes of virginal mice.[4] However, nulliparous BALB/c and Tac:(SW) mice of both sexes injected with progesterone or estrone failed to develop cardiac lesions.[5] The only significant hormonal influence was demonstrated by injecting virginal C3H females with hydrocortisone on both short- and long-term regimens; necrosis of myocardial fibers was followed by calcification.[6,7] Other means of experimentally producing dystrophic cardiac calcinosis have been reviewed by Rings and Wagner,[4] among which were dietary influences, of which high-fat, low-protein, hypolipotropic intake appeared to be the most significant.[8] Myocarditis as a precursor state was suggested by Bellini et al [9] from their study of BALB/c mice infected with Moloney sarcoma virus.

This study provides conclusive evidence of genetic susceptibility to DCC in certain strains of mice, with complete freedom in at least one strain. Sex differences are demonstrable in several strains in which the influence of pregnancy is clearly shown. Support is lent to the relevancy of high fat intake.

Materials and Methods

Mice of both sexes and of varying age, totaling approximately 1000, represented five inbred strains (BALB cAnNIcr, C3H HeNIcr, C3HfB HeNIcr, C57BL 6NIcr, and DBA/2AnIcr) and three hybrids, B6C3F1, C3B6F1, and CC3F1. All were from the breeding colonies of the Institute for Cancer Research (accredited by AAALAC) and were housed in stainless steel, shoebox-sized cages bedded with pine shavings. Most were fed Old Guilford Diet OG96W (7.5% fat) (Emory Morse Co., Old Guilford, Conn.) *ad libitum*, and all were given acidified drinking water (pH 2.5 to 2.7). Groups of C3H female breeders, the most susceptible to DCC, were tested separately on three commercial diets of varying fat content, viz, Wayne Sterilizable Lab-Blox (4.3) (Allied Mills, Inc., Chicago) and the standard OG96W (7.5) and the Old Guilford OG911R (10.9) (Emory Morse Co., Old Guilford, Conn); we realized that Wayne differed from OG in ingredients other than fat and that the two OG diets varied in proportion of components other than fat.

Most animals were subjected to monogomous mating throughout their reproductive lives. Appropriate numbers of virgins, eg, 20 to 41, from susceptible strains were also included in the study; virginal males were not tallied separately, since they did not differ in incidence of DCC from male breeders.

Five virginal C3H females were given 2.5 mg of progesterone intraperitoneally daily for

10 days; a similar group received a 5-mg dose. They were killed by cervical dislocation at monthly intervals.

The age at onset of DCC in the various strains was estimated by observing progressively younger mice until the lesion was no longer found. The majority, however, were maintained until they appeared ill from whatever cause, usually mammary tumors in C3H and C3Hf female breeders and frequently pulmonary tumors in BALB/c mice of both sexes.

Serum levels of calcium, phosphorus, cholesterol, and triglycerides were determined in male and female breeders of strain C3H chosen at random (courtesy of Dr. William Foster, Clinical Laboratories of the Jeanes Hospital).

Pooled samples of hearts and livers from C3H females with DCC were passaged twice through primary mouse tissue culture. The culture material was inoculated into young random-bred Swiss mice which were bled 28 days later and assayed for antibodies against the standard battery of murine viruses (Microbiological Associates, Bethesda, Md.). Cardiac tissue containing lesions and surrounding muscle were examined under the electron microscope for evidence of viruses (Microbiological Associates, Bethesda, Md.). Lesions were cultured for bacteria and fungi, and the colony was always under surveillance for ectoparasites and endoparasites as previously reported.[10]

Autopsies were performed under a Bausch and Lomb StereoZoom dissecting microscope. The extent of cardiac and renal calcinosis was scored initially on the fresh tissues as 0 (absent), +1 (slight), +2 (moderate), or +3 (marked). Location in the heart was specified as epicardial and/or myocardial.

Tissues were fixed in 10% neutral formol and sectioned in paraffin at 5 μ. All were stained with hematoxylin and eosin; selected ones were stained with alizarin red S, phosphotungstic acid-hematoxylin (PTAH), periodic acid-Schiff reagent (PAS), and Masson trichrome to demonstrate calcium, myofibrils, myocardial necrosis, and fibrosis, respectively.

Results

Gross Pathology

With the dissecting microscope (× 10 to 30), even minimal foci of calcinosis could usually be detected. In the BALB/c strain, gradients from fine flecks to extensive crusts were visible in or just beneath the epicardium, but the subjacent muscle appeared normal; the surface of the right ventricle was most frequently involved. Hearts of most C3H and C3Hf female breeders had pronounced white flecking throughout the myocardium, especially of the left ventricle; the incidence and extent were far less in males; and this flecking was absent in virginal females. A composite was often noted among DBA/2 mice, although muscle alone was more commonly affected. It was remarkable how closely the findings at autopsy fitted with those of the histologic examination.

Some hearts graded +2 and virtually all graded +3 were estimated as being larger than normal by an experienced prosector, but unfortunately they were not weighed. Congestive heart failure was noted in the most markedly affected mice, evidenced by edema of soft tissues, accumulation of fluid in serous cavities, and dusky red discoloration of the various organs, along with marked hepatosplenomegaly.

The only other organs showing calcinosis were the kidneys and lungs, the former being the more common, often grossly evident by white dots or streaks just above the corticomedullary junction. As for the lungs, gross nodules were found in only 1 case, in which calcium deposits were so heavy that a granulomatous reaction was provoked.

Histopathology

A heart scored as +1 displayed a relatively sparse scattering of calcific foci (Figure 1), occasionally limited to a few solitary fibers. At the other extreme, +3 indicated richly distributed, often very large lesions, sometimes so destructive that one wondered how the heart could possibly function (Figure 2). Based entirely on the examiner's impression, +2 was reserved for intermediate grades (Table 1).

In earlier stages of epicardial calcinosis, necrotic muscle fibers could be detected in the granular to partially condensed calcific layer and immediately subjacent myocardium (Figure 3). Scarring followed, the underlying muscle thereafter being quite normal (Figure 4).

Development of myocardial lesions could be followed from incipient changes to the most massive ones; we observed every stage among many animals. Whatever the damaging influence may ultimately prove to be, the initial effect was on muscle fibers, often solitary ones that showed swelling, fading nuclei, and ruptured sarcolemma (Figure 5). The sarcoplasm of fibers in the general area was frequently frayed or vacuolated, with lysis or pyknosis of the nuclei (Figure 6). A single fiber in the field pictured had begun to acquire calcium in the form of barely detectable fine basophilic granules (Figure 7), resembling clusters of cocci within the sarcolemmal boundaries. The sarcoplasm of such fibers was PAS-positive, and myofibrils were lost (Figure 8). With extension of the myopathic foci, the calcific deposits were progressively more compact (Figure 9), thereafter losing their granular character and appearing as raggedly solid purplish-black blocks in hematoxylin-eosin preparations and brilliant orange-red when coated with alizarin red S. Such calcific masses frequently evoked a reaction of cells resembling so-called Anitschkoff myocytes (Figure 10) and, in time, resulted in a dense collagenous scar.

Support was given the gross diagnosis of cardiac hypertrophy by comparison with muscle fiber width of normal mice in the same age range, some being virtually twice as large.

Calcinosis of the *kidney* was commonly associated with the cardiac lesions, seldom appearing alone (Table 2) except in C3Hf males, in which cardiac involvement was noted in only 10% and renal deposits in 55%. The calcific plugs usually lay in the lumina of Henle's loops, and their

Table 1—Dystrophic Cardiac Calcinosis in Mice Maintained on 7.5% Fat Diet

Strain	No. Mice	Sex	Mated	Total affected		Severity								Site of lesion				No. of both
						0		+1		+2		+3		Epicardium		Myocardium		
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
BALB/c	58	♂	+	20	34	38	66	5	9	10	16	5	9	18	90	2	10	0
BALB/c	73	♀	+	24	33	49	67	4	5	14	19	6	8	24	100	0		0
BALB/c	20	♀	–	15	75	5	25	7	35	8	40	0		15	100	0		0
C3H	41	♂	+	16	39	25	61	10	24	4	10	2	5	0		16	100	0
C3H	149	♀	+	132	89	17	11	19	13	56	38	57	38	0		132	100	0
C3H	41	♀	–	0		0		0		0		0		0		0		0
C3Hf	20	♂	+	2	10	18	90	2	10	0		0		0		2	10	0
C3Hf	44	♀	+	40	90	4	10	24	53	10	23	6	14	0		40	90	0
C3Hf	30	♀	–	0		30	100	0		0		0		0		0		0
DBA/2	35	♂	+	19	54	16	46	12	34	4	11	3	8	5		19		5
DBA/2	47	♀	+	37	79	10	21	13	28	14	30	10	21	5		28		12
DBA/2	20	♀	–	13	65	7	35	5	25	5	25	3	15	11		2		1
C57BL/6	26	♂	+	0		26	100	0		0		0		0		0		0
C57BL/6	61	♀	+	0		61	100	0		0		0		0		0		0
B6C3F1*	20	♂	+	0		0	100	0		0		0		0		0		0
B6C3F1	20	♀	+	0		0	100	0		0		0		0		0		0
C3B6F1†	15	♂	+	0		0	100	0		0		0		0		0		0
C3B6F1	15	♀	+	0		0	100	0		0		0		0		0		0
CC3F1‡	5	♂	+	0		0	100	0		0		0		0		0		0
CC3F1	10	♀	+	7	70	3	30	7	70	0		0		0		7	70	0
CC3F1	20	♀	–	0		20		0		0		0		0		0		0

* B6C3F1 = C57BL/6 × C3H.
† C3B6F1 = C3H × C57BL/6.
‡ CC3F1 = BALB/c × C3H.

Table 2—Comparative Cardiac, Renal, and Pulmonary Involvement (%)

Strain	Sex	Heart	Kidney	Lung
BALB/c	♂	34	4	0
	♀	33	5	0
C3H	♂	39	32	0
	♀	89	25	0.2
C3Hf	♂	10	55	0
	♀	90	59	0.2
DBA/2	♂	63	53	0
	♀	79	22	0

sites were denuded of epithelium (Figure 11). Uncalcified and partially calcified necrotic tubular epithelium was found, presumably representing stages in the formation of the densely calcific casts (Figure 12).

In the lung, flecks of calcium, often laminated after the fashion of so-called psammoma bodies, were relatively rare findings (Table 2) and apparently formed within capillaries of the alveolar walls rather than being embolic (Figures 13 and 14). Larger flecks were frequently extruded and lay free in alveolar spaces. In only two instances was pulmonary calcinosis extensive; in one instance it was sufficient to provoke a marked granulomatous reaction (Figure 15).

Genetics

The anatomic pattern of the cardiac lesion was strain-related: BALB/c was epicardial, C3H and C3Hf were myocardial, and DBA/2 was epicardial and/or myocardial. Of the five inbred strains include in this study, only one (C57BL/6) was completely free of DCC.

F_1 hybrids of both sexes derived from reciprocal crosses between strains C57BL/6 (zero incidence) and C3H (high incidence) were completely free of calcinosis. F_1 hybrids of crosses between strains BALB/c (epicardial distribution) and C3H (myocardial distribution) developed myocardial but not epicardial lesions (Table 1).

Sex

The incidence and severity of cardiac lesions were far greater in breeding females of strains C3H and C3Hf than among male breeders of the same strains. The same may be said for hybrid CC3F_1 for incidence, although the numbers were small and the involvement slight (Table 1). No significant sex difference in either incidence or severity was noted among BALB/c and DBA/2 mice.

Virginal females of strains C3H and C3Hf and hybrid CC3F$_1$ remained lesion-free until they were bred (Table 1). A single pregnancy was sufficient to result in DCC. There did not appear to be any correlation between the severity of the process and parity. Females which bore six litters were no more severely affected than those having but one litter during the standard 8-month breeding regimen practiced in this facility. Lack of breeding experience afforded no protection among BALB/c or DBA/2 mice; in fact, the incidence in BALB/c virgins more than doubled that of breeding females. C3H virginal females that received progesterone intraperitoneally did not develop DCC.

Age

In general, the severity of DCC increased with age in breeders of the four affected inbred strains. There was a striking difference in age at onset between males and females of strains BALB/c, C3H, and C3Hf; calcium appeared in females relatively early (Table 3). No significant difference was noted among DBA/2 mice; affected animals of both sexes were young.

Diet

The experiment relating to the influence of fat on C3H female breeders showed a progressive increase in the incidence and severity of cardiac calcinosis with augmented dietary fat ($P < 0.01$) (Table 4).

Serum samples collected from C3H mice, male and female breeders on 4.15% and 7.5% fat diets, showed no significant deviation from the norm in calcium, phosphorus, cholesterol, or triglyceride levels. Random blood samples obtained from various other mice were also normal.

Table 3—Estimated Age at Occurrence

Strain	Sex	Range (days)
BALB/c	♀	110–150
	♂	21–83
C3H	♀	292–465
	♂	81–89
C3Hf	♀	579–661
	♂	25–96
DBA/2	♀	58–67
	♂	55–87

Table 4—Effect of Dietary Fat on C3H Female Breeders

Dietary fat (%)	No. mice	Grade of DCC (% mice)			
		0	+1	+2	+3
4.3*	29	0	69	31	0
7.5†	40	0	5	45	50
10.9‡	15	0	0	30	70

$P < 0.01$.
* Wayne Sterilizable.
† Old Guilford 96W.
‡ Old Guilford 911R.

Infection

Serologic and electron microscopic studies failed to disclose any evidence of viral disease. Likewise, cultures for bacteria and fungi proved negative, as were the studies for parasites.

Discussion

A common denominator for sites of dystrophic calcification appears to be necrosis, irrespective of the tissue involved. With regard to the heart, Shen and Jennings [11] demonstrated striking calcium uptake following ischemic death of myocardial fibers; dense intramitochondrial granules were derived from calcium of the arterial blood. No uptake was observed in reversibly damaged fibers or in dead fibers excluded from arterial inflow. However, Wrogemann and Pena [12] presented evidence in support of another premise, ie, that, at least in the cases of muscular dystrophy, a mitochondrial overload of calcium resulting from a membrane defect may in itself bring about cell death. Whether such an overload would be visible by light microscopy, as in the dying cell in Figure 5, we do not know. Since the question is beyond the scope of our studies, we will not belabor the matter further on which came first and how.

We have demonstrated necrotic myocardial fibers in the susceptible strains of mice as well as necrotic tubular epithelium to account for the cardiac and renal calcific foci, respectively. Which cells are involved in the rare pulmonary lesions is not clear.

Conclusive evidence of a *genetic influence* has been presented. Since the F_1 hybrids of crosses between strains BALB/c and C3H developed myocardial but not epicardial lesions, the gene(s) predisposing to an epicardial location would be recessive and the myocardial one(s) would be dominant. Further, in the DBA/2 strain where both sites were involved, the myocardial incidence was distinctly higher. It is not yet known whether a single major gene or several genes are involved; segregation

studies of F_2 progeny to determine this are in progress. A *maternal influence* is ruled out by the negative results of reciprocal crosses between C57BL/6 and C3H strains (Table 1).

The relationship of *sex* among C3H and C3Hf strains, as well as the $CC3F_1$ hybrid, was likewise clear-cut; female breeders were highly susceptible, female virgins were exempt, and the incidence in males was low irrespective of breeding activity. *Hormones* associated with pregnancy were naturally suspected, but exogenous progesterone administered to C3H virginal females had no effect. This confirms the findings of Ashburn et al[5] in BALB/c and random-bred Swiss mice. The only hormones known to be capable of inducing DCC are cortisone and adrenocorticotropic hormone, which initially produce myocardial necrosis.[6,7] *In vitro* studies have demonstrated that hydrocortisone can alter cell permeability and induce intercellular electrolyte imbalance, resulting in cell death,[13] perhaps through mitochondrial calcium overload.[12] Rings and Wagner[4] suggest that genetically determined higher secretion of adrenocorticosteroids in DBA/2 mice might bring about the necrosis. Sparks et al[6] implicate the pituitary gland further by showing that myocardial necrosis and calcification induced by either hydrocortisone or ACTH were potentiated by concomitant administration of growth hormone and prevented by prolactin.

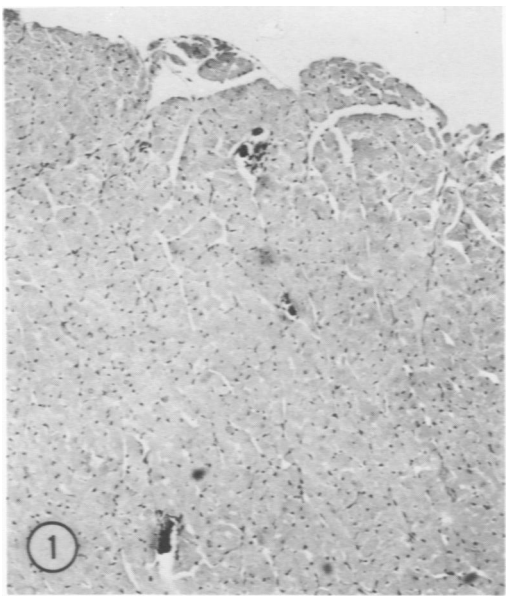
Our finding of increased incidence and severity of cardiac lesions in C3H female breeders with progressively higher fat intake is strongly suggestive of *diet* as a contributing etiologic factor. Much the same results were reported by Rings and Wagner[4] on an even lesser span of fat in the diet of DBA/2 mice. Furthermore, Ball and Williams[8] greatly increased myocardial necrosis and calcification, as well as epicardial calcification, far beyond the spontaneous incidence and severity in DBA mice with a 28% fat, 8% protein, and 57.5% carbohydrate diet, further implementing the cardiac damage by adding a lipotropic supplement (betaine). A dietary influence appears to be well established.

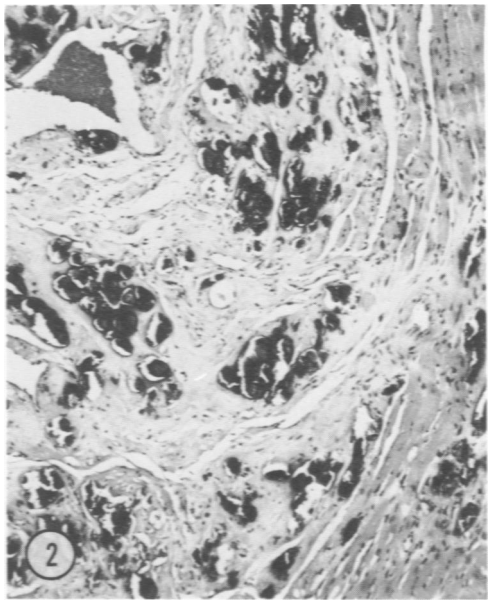
Thus, a genetic background for DCC is secure, with age, sex, parity, and diet as conditioning factors.

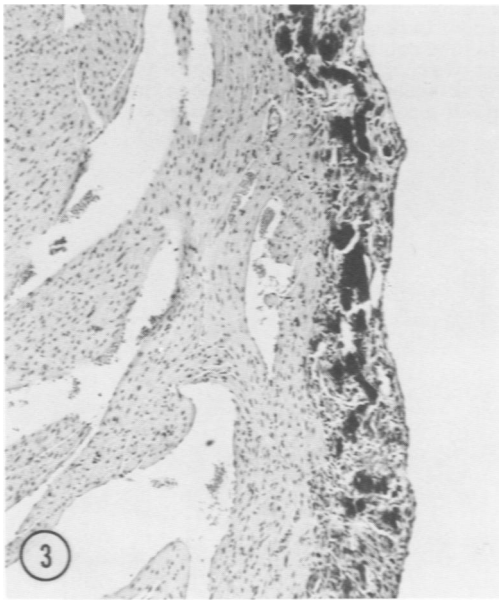
References

1. Frith CH, Haley TJ, Seymore BW: Spontaneous epicardial mineralization in BALB cStCrl mice. Lab Anim Sci 25:787, 1975
2. DiPaolo JA, Strong LC, Moore GE: Calcareous pericarditis in mice of several genetically related strains. Proc Soc Exp Biol Med 115:496–497, 1964
3. Nabors CE, Ball CR: Spontaneous calcification in hearts of DBA mice. Anat Rec 164:153–161, 1969
4. Rings RW, Wagner JE: Incidence of cardiac and other soft tissue mineralized lesions in DBA 2 mice. Lab Anim Sci 22:344–352, 1972

5. Ashburn AD, Williams WL, Cobb FR: Cardiovascular, hepatic and renal lesions in mice receiving cortisone, estrone and progesterone. Yale J Biol Med 35:329–340, 1963
6. Sparks LL, Rosenau W, MacAlpin RN, Daane TA, Li CH: Production of dystrophic calcification of cardiac muscle in mice by hydrocortisone. Nature 176:503–504, 1955
7. Lostroh A, Li CH: Deposition induced by hydrocortisone of calcium in heart tissue of female C3H mice. Nature 176:504, 1955
8. Ball CR, Williams WL: Spontaneous and dietary-induced cardiovascular lesions in DBA mice. Anat Rec 152:199–203, 1965
9. Bellini O, Casazza AM, DiMarco A: Histological and histochemical studies of myocardial lesions in BALB/cCr mice: Lab Anim Sci 26:329–333, 1976
10. Eaton GJ: Intestinal helminths in inbred strains of mice. Lab Anim Sci 22:850–853, 1972
11. Shen AC, Jennings RB: Kinetics of calcium accumulation in acute myocardial ischemic injury. Am J Pathol 67:441–452, 1972
12. Wrogemann K, Pena SDJ: Mitochondrial calcium overload: A general mechanism for cell-necrosis in muscle diseases. Lancet 1:672–674, 1976
13. Van der Schoot HCM: Cardiopathia in mice. (cited by Rings and Wagner [4])







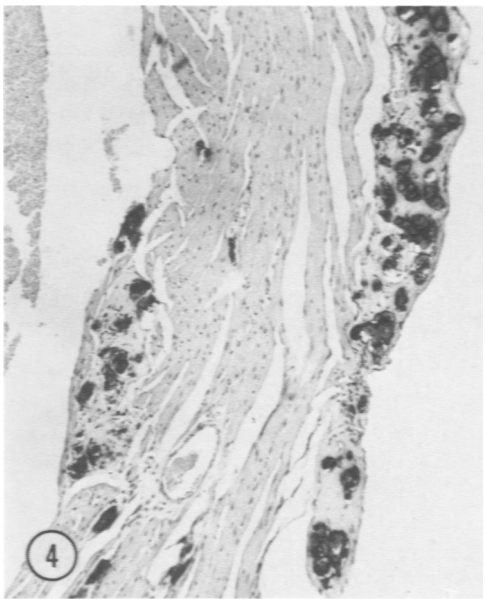
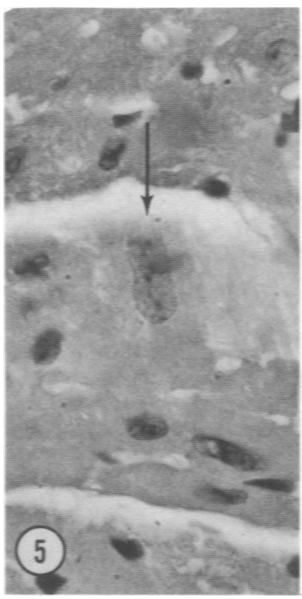
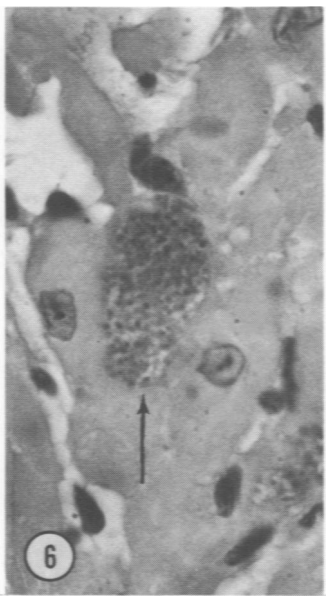
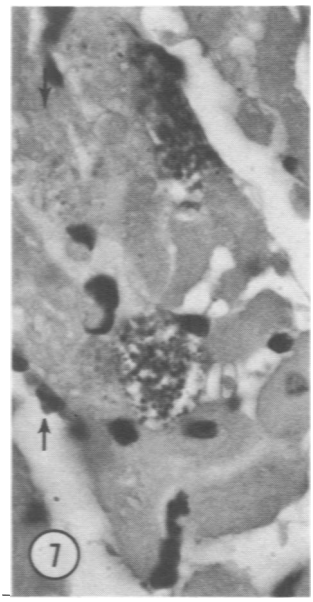


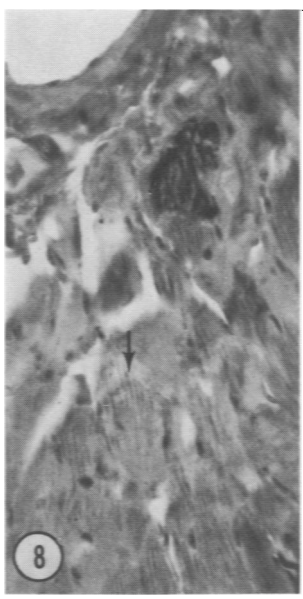
Figure 1—Heart. Minimal degree of dystrophic calcification limited to the myocardium. Typical of C3H male. (H&E, × 100) **Figure 2**—Heart. Advanced DCC with extensive myocardial scarring. Seen mostly in C3H and C3Hf female breeders. (H&E, × 100) **Figure 3**—Heart. Dystrophic calcification limited to the epicardium. Typical of BALB/c mice, both sexes. (H&E, × 100) **Figure 4**—Heart. Dystrophic calcification involving both epicardium and myocardium. Typical of DBA/2 mice, both sexes. (H&E, × 100)

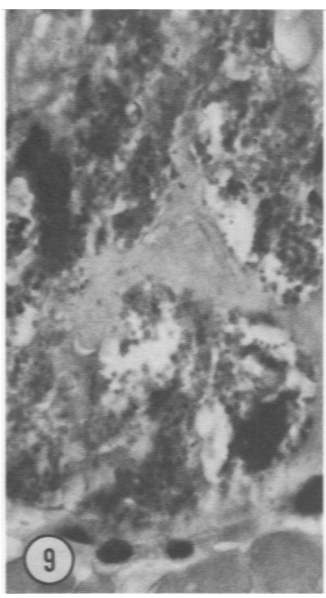
Figure 5—Heart. Central muscle fiber (*arrow*) is undergoing karyolysis and cytoplasmic dissolution prior to acquisition of calcium. (H&E, × 1000) **Figure 6**—Heart. Early calcific deposit within a necrotic muscle fiber (*arrow*); the fine granules resemble cocci. (H&E, × 1000) **Figure 7**—Heart. Increasing density of calcific foci. Note ragged appearance of adjacent degenerating fibers (between *arrows*). (H&E, × 1000) **Figure 8**—Heart. Muscle near a calcified fiber shows swelling, partial loss of myofibrils (longitudinal black filaments), and complete disappearance of cross-striation. (Phosphotungstic acid and hematoxylin, × 400) **Figure 9**—Heart. Calcium spreading through a necrotic area, mostly still in granular form, but with condensation into several solid concretions. Early scarring is noted in the center. (H&E, × 1000) **Figure 10**—Heart. Calcium in an older deposit has almost completely lost its granularity to form homogeneous masses. A cellular reaction resembling "Anitschkoff myocytes" (*arrows*) is evoked, along with a collagenous deposit. (H&E, × 1000)

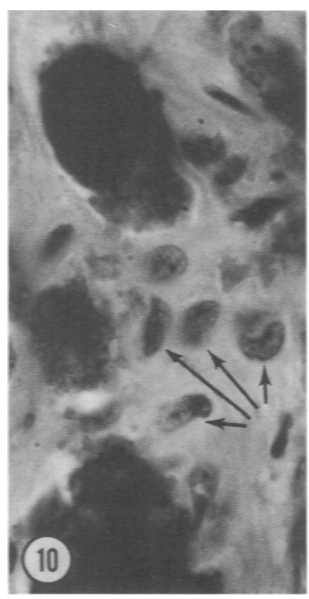

5

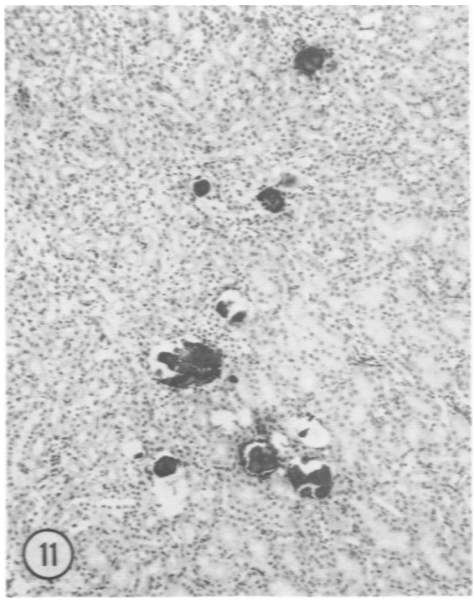

6

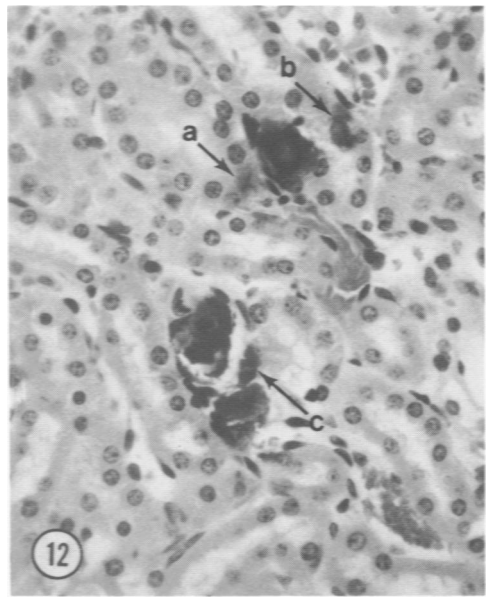

7

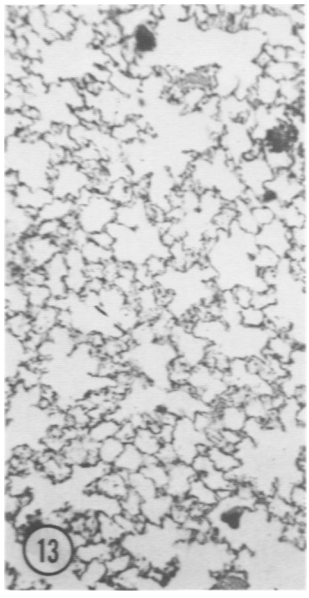

8

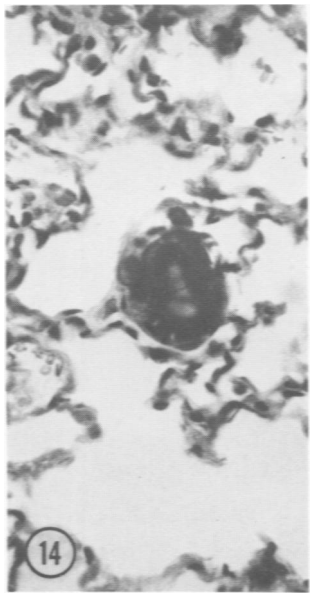

9


10









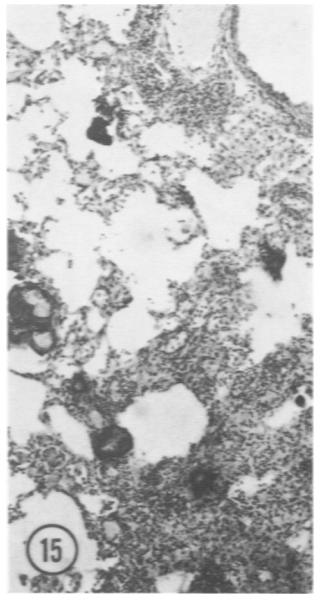


Figure 11—Kidney. Calcific casts lie almost exclusively in the limbs of Henle's loops. (H&E, × 100) **Figure 12**—Kidney. Progression of lesion: necrotic epithelial cell (*a*); necrotic cell acquiring calcium (*b*); exfoliating calcific cell (*c*). (H&E, × 400) **Figure 13**—Lung. Calcific bodies lie for the most part within alveolar walls, occasionally being extruded into air sacs. (H&E, × 100) **Figure 14**—Lung. Detail of a small concretion resembling a "psammoma body", presumably formed within a capillary of an alveolar wall rather than being embolic. (H&E, × 400) **Figure 15**—Lung. Solitary example of extensive calcinosis sufficient to provoke a marked granulomatous reaction. (H&E, × 100)