MITOCHONDRIA IN CARDIAC MUSCLE CELLS
OF THE CANARY AND SOME OTHER BIRDS

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ABSTRACT

The fine structure of mitochondria from the ventricular myocardium of canaries, sparrows, zebra finches, quail, and geese has been studied. The first three of these birds have very fast heart rates, the quail being intermediate, and the goose has a relatively slow rate. The canary heart has a unique form of mitochondrion containing large, parallel arrays of *zigzag* or angled cristae. Other cristae, continuous with the zigzag ones and also occupying large parts of the mitochondrial volume, are named *retiform* because of the hexagonal network which they form, sometimes in a single plane and sometimes three dimensional. These two types of cristae appear to be interconnectible. It is possible that there is a direct functional significance in these peculiar forms, but, in any case, the relative constancy of dimensions in these arrays is probably related to specific properties of the molecules of which the cristal membrane is composed. It is also demonstrated that this membrane is composed in part of approximately 30-A particles which are believed to be protein molecules. This unusual mitochondrial morphology is not seen either in the other fast bird hearts or in the slower ones, so that there is neither a simple correlation with heart rate nor probably with the separate parts of the cardiac cycle. Although none of the other four hearts shows more than an occasional angled crista, there does seem to be a rather gross correlation between heart rate and mitochondrial size and complexity of crista structure, but no correlation with presence or absence of zigzag forms. The cristae of quail heart mitochondria are disposed in unusually large close-packed whorls.

An interpretation of mitochondrial fine structure was proposed by Palade (7) over 10 years ago. He visualized the organelle as a pair of membranes separated by a narrow space and completely enclosing a matrix of variable density. The latter is penetrated by shelf-like infoldings (cristae) of the inner member of the membrane pair. At about the same time, Söjstrand (14, 15) presented an interpretation of the general form of mitochondria which differed from Palade's in that he believed the membranes of the cristae were not continuous with the inner limiting membrane. Reports on mitochondrial morphology subsequent to 1953 are in most cases incidental to other descriptions and far too numerous to detail here. These observations have served primarily to demonstrate that all mitochondria conform to the general pattern put forth in Palade's model, but they have also made it evident that variations occur in parameters such as the size, shape, and number of cristae; matrix density; and numbers of "dense bodies." As the descriptions have multiplied, the probability that these variables may, in a general way, be related to function has become evident. For example, cristae may be longer and more closely packed in mitochondria of cells with a high metabolic rate, etc.

It has been known for a long time that the striated muscles have unusually large numbers of mitochondria and that cardiac muscle illustrates

this condition to advantage. From the reports of a number of investigators (6, 10, 13, 18), it is evident that cardiac sarcosomes vary in dimension but are generally large, that they have very large numbers of long, closely packed cristae, and that the foliate or shelf-like cristae are often fenestrated and may occasionally be arranged in whorls.

In the course of a comparative investigation of cardiac muscle cells, some unusual mitochondrial forms were observed. They are reported here in the belief that they may help to elucidate the underlying molecular configuration of the enzyme-bearing membranes and that they may focus attention on some functional aspects of mitochondria not ordinarily considered. Recently, Revel, Fawcett, and Philpott (11) described mitochondrial modifications of a similar nature, and Smith (17) has shown some related forms in an invertebrate muscle. The present paper supports and extends the views of these authors.

This investigation is part of a larger study to relate the elaboration of organelles in cardiac muscle cells to the speed of contraction. The observations reported here lend some support to this correlation between mitochondrial organization and speed of muscle contraction.

MATERIALS AND METHODS

The animals used in these studies were 29 canaries (*Serinus canarius*), 24 sparrows (*Spizella arborea*), 6 quail (*Colinus virginianus*), 18 zebra finches (*Taeniopygia castanotis*), and 2 geese (*Anser anser*). The structures described here were best preserved when the birds were anesthetized with nembutal or killed by simultaneously crushing the spinal cord and occluding all head and neck circulation with a heavy clamp. The thorax was quickly opened and all chambers of the heart immediately filled by injection with ice-cold fixative. The pressure of the fixative was adjusted so that the ventricles were slightly distended and the perfusion continued until the muscle stopped beating. Under these circumstances, the hearts were always beating well when fixation began

and were stopped in a few seconds. The tissue was then placed in a pool of fresh, ice-cold fixative, the atria were removed, and the ventricles were cut into small pieces. The fixative used in these studies was an ice-cold 1 per cent osmium tetroxide solution buffered at pH 7.7 with veronal acetate and containing 10^{-3} M $CaCl_2$ and 0.06 M sucrose. The tissues were fixed for 30 minutes, rapidly dehydrated in a graded series of methanol, and allowed to return to room temperature in absolute methanol. They were then embedded in Maraglas as recommended by Freeman (4). Sections were cut on a Porter-Blum or Huxley-Cambridge ultramicrotome and stained with lead, using the lead citrate method of Reynolds (12). Thin sections were examined in an RCA EMU 3E and micrographs were taken at magnifications up to $\times$ 50,000 and subsequently enlarged.

[A wide variety of related procedures were tried, involving variation of the temperature, pH, ionic strength, type of buffer, and duration of the fixation; fixation with $KMnO_4$; killing by cervical dislocation, decapitation, or anesthetization with nembutal; cardiac arrest with elevated K^+ or nembutal; dehydration in methanol or ethanol; embedding in Epon 812 or Maraglas. Many of the procedures incorporating these variations yielded tissues useful in some way, and several of them gave excellent general preservation, although always differing in some details from each other. The method described above was selected for this study because it provided for the best preservation of the structures described in this report.]

OBSERVATIONS

Canary

The most striking mitochondria seen in the ventricular myofibers of the canary are illustrated in Figs. 1 and 2. They are characterized by two forms of cristae, both of which are tubular but differ markedly in their disposition within the mitochondrion. Both forms may be found in a single organelle where, though confined to separate domains, they merge at the boundaries, or all

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Abbreviations for Figures

E, endothelial cell.
Er, erythrocyte.
R, retiform cristae.

SR, sarcoplasmic reticulum.
Z, zigzag cristae.

FIGURE 1 Section from the ventricular myocardium of the canary. Both giant and small mitochondria are present illustrating *zigzag* and *retiform* arrays of cristae. In most cases both forms are present in a single mitochondrion. $\times$ 27,000.
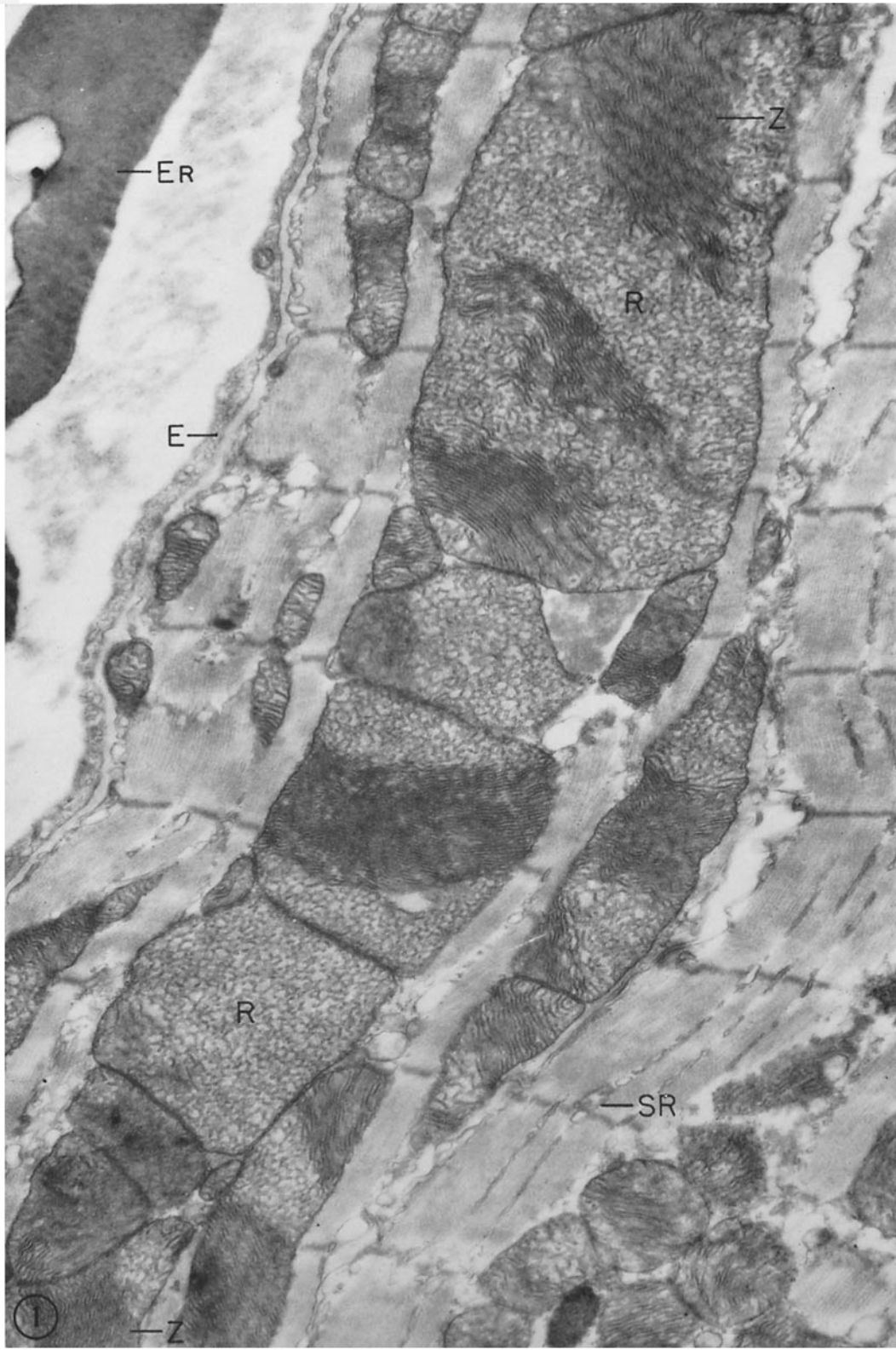
of the cristae in a single mitochondrion may be of either type. For the sake of brevity, the two forms will be referred to as *zigzag* and *retiform*.

The zigzag cristae are long slender tubules 150 to 175 A in diameter which arise in the usual manner by invagination of the inner one of the membrane pair limiting the mitochondrion. The tubules are packed in hexogonal or staggered array as illustrated in Fig. 3 *a*. The cristae in a single row, such as that indicated by the lines *A* and *B* in the figure, appear to be interleaved, that is, they arise from opposite sides of the mitochondrion as indicated by the numbers *1* and *2* in the diagram. Also they may be interleaved in the plane *C–D*.

Although the cristae pursue a straight or gently curving course over-all, they do so in a zigzag line (Figs. 3 *b* and 5). At the outset a crista is inclined at about 30° to the main course it will follow. After a 500- to 600-A segment it bends abruptly 60° (making an angle of 120° with the first segment), passing through its main course again at an angle of 30°. After another 500 to 600 A, it makes another 60° turn (again, 120° from the previous segment) to run parallel to its original direction, and so on. All of the *zigs* and *zags* are in a single plane. The cristae of row *C–D* of the diagram (Fig. 3 *a*) will zig and zag in phase, but those in row *A–B* (a tier) will be out of phase by a few Angstroms (constant and always in the same direction for a given mitochondrion). Sections passing through this plane have the appearance seen at the top of Fig. 2 where the in-phase segments of contiguous cristae make parallel, diffuse, dark lines 120° from this principal course.

An indication of the great extent of the zigzag arrays may be seen in Figs. 8, 10, and 11. Fig. 9 illustrates, in the upper left, a grazing section of a single tier, and in the lower left the plane of the section is tilted so as to pass through several tiers. The great restriction of the matrix space is evident.

Although the zigzag pattern may be perfectly regular, two apparent variations are seen. There seem to be angles greater than 120°, and some zigzag portions appear to be continuous with straight ones. Stereoscopic micrographs of thick sections and careful scrutiny of usual micrographs show that in many cases the angles greater than 120° (up to 180°) are probably an illusion, caused by the direction from which the array is viewed. However, this is not true in every case, for, as in the lower part of Fig. 2 and the central part of Fig. 10, the cristae seem to be straight for a short distance.

As indicated by the arrows in Figs. 5 and 9, short tubules are sometimes seen at the angles where adjacent cristae connect in the *A–B* plane. These cross-pieces are illustrated in the diagram (Fig. 3 *b*). They are believed to occur at every other angle between adjacent cristae and at alternate angles successively along the tier. It is difficult to demonstrate more than very short segments of them in micrographs, because in most planes of section they will be obscured by the compactness of the array of cristae. However, it is improbable that they are as regular as in Fig. 3 *b*. They may, in fact, be unstable or transient, since the retiform array described below requires their regular distribution. In fact, it is these connecting segments which provide anastomoses between the zigzag and retiform cristae and enhance the parallel between these forms and an extremely fenestrated, foliate crista, as seen in Fig. 14 and as was reported earlier for the shrew, *Blarina brevicauda* (16), and also reported recently by Smith (17) in blowfly muscle.

Although relatively easily inferred from the micrographs, the cross-section, diagrammed in Fig. 3 *a*, is also difficult to illustrate. Efforts to section a small Permoplast model have confirmed the belief that sections thin enough and passing through a plane which might display this pattern should be extremely rare. A small field is seen in the mitochondria in Figs. 13 *a* and *b*.

The retiform cristae (Figs. 1 and 2) are also tubules with a 150- to 175-A outside diameter. They are obviously highly anastomotic and much less regularly arranged. However, in Figs. 11 and 12 and in the diagram of Fig. 13 *d*, continuity between the two systems is seen by way of a regular hexagonal pattern.

Gradations in the *apparent* regularity of the *retiform* cristae can be seen, from that in Figs. 10 to 12 and Fig. 16, where their disposition results

FIGURE 2 Enlargement of the mitochondrion in the upper right of Fig. 1. Note the many Y-shaped pieces in the retiform array, suggesting that the hexagonal pattern persists but is no longer constrained to a single plane. × 60,000.
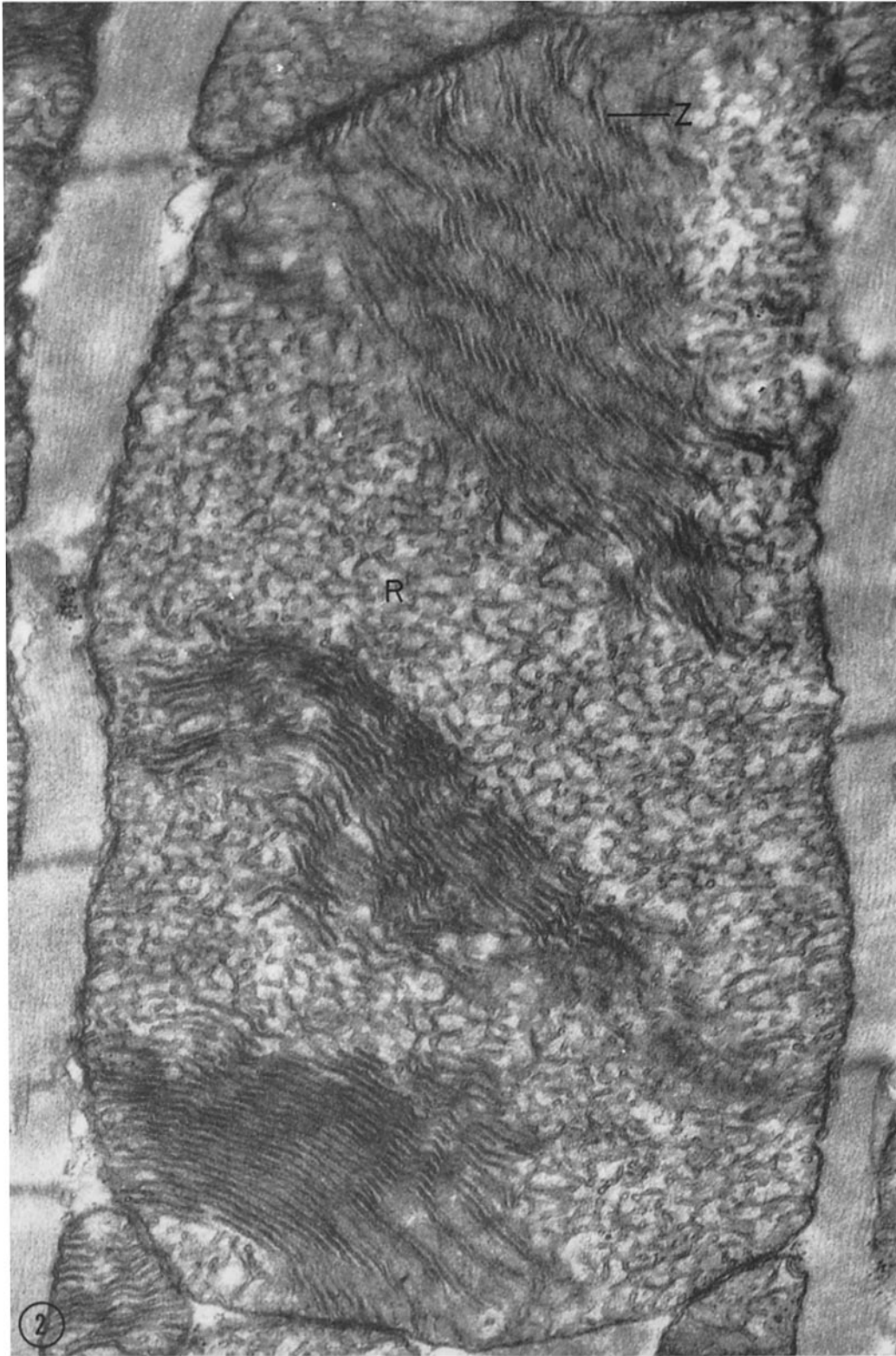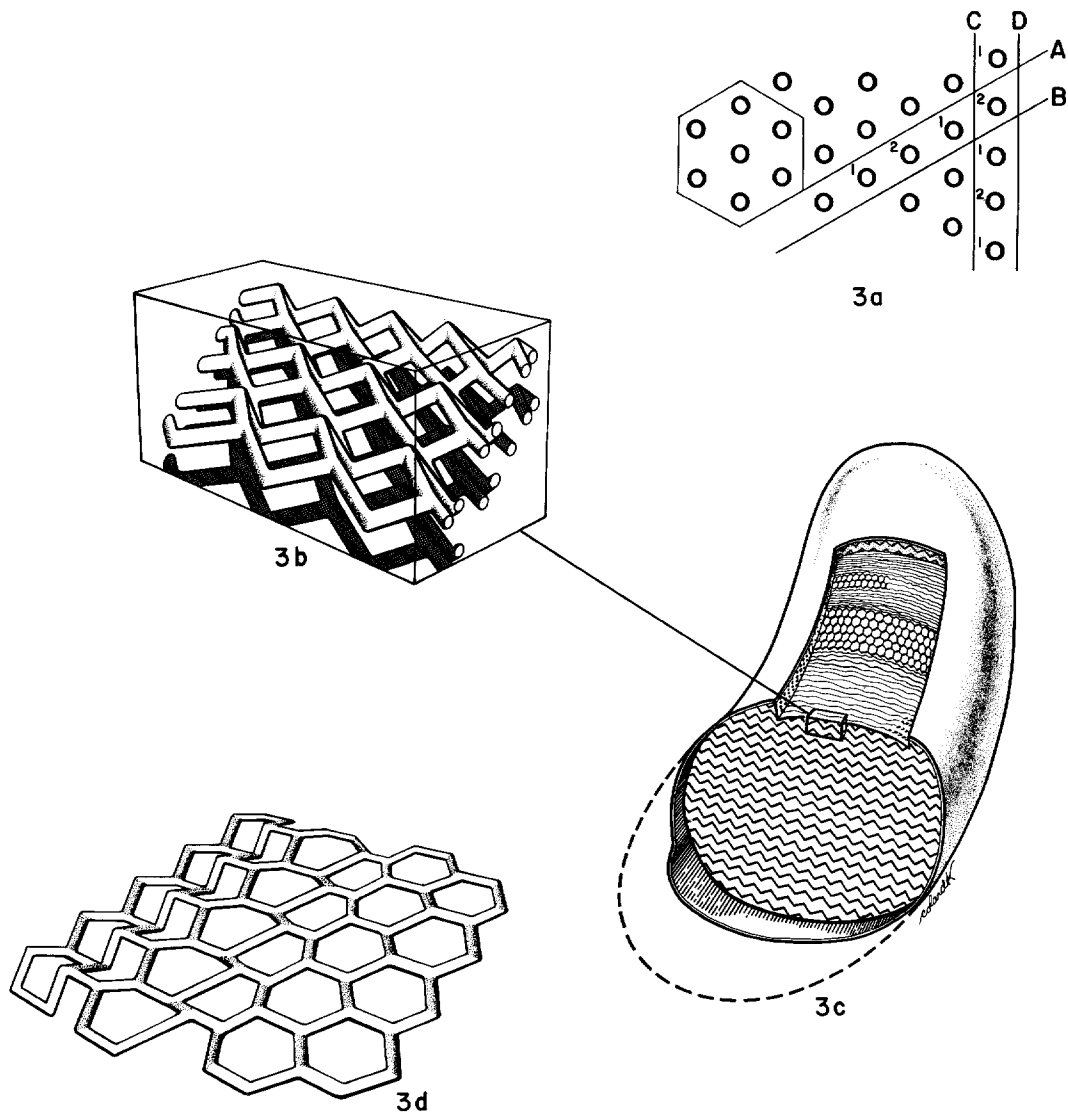
FIGURE 3   Diagram illustrating the disposition of cristae in canary heart mitochondria. 3 a   This drawing represents a cross-section of a field of zigzag cristae. 3 b   In this three-dimensional view of a segment of a zigzag array, two tiers of cristae are shown. 3 c   The disposition of zigzag and retiform arrays within a mitochondrion is shown in this diagram. 3 d   This drawing is a schematic representation of the development of a hexagonal pattern from zigzag cristae. The sequence of events may be more easily visualized if one imagines a force pulling on the crista farthest to the right and the zigzags rotating about their long axes into the plane of the hexagons.

FIGURE 4   This mitochondrion is similar to that shown in Fig. 1 but displays the zigzag pattern to better advantage. The area enclosed in the rectangle has been enlarged in Fig. 5. × 63,000.
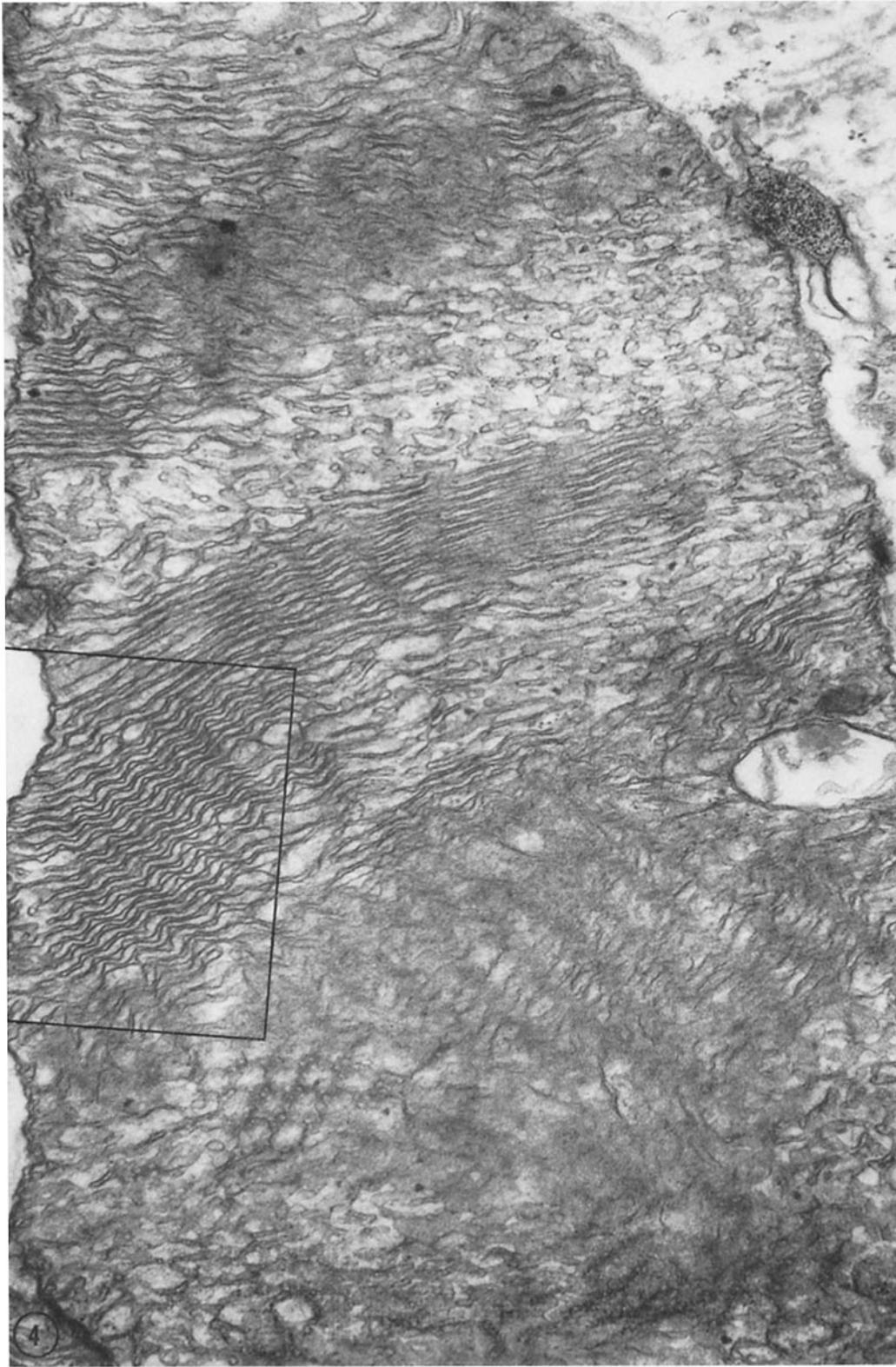
in regular hexagons, to that in Figs. 4 and 11, where they are less regular but still evident, to that in Fig. 8 and finally that in Fig. 2, where the hexagons are not evident in any single plane. The probable explanation of the continuity between the two systems, the gradations in form, and the possible functional implications will be discussed below, but it should be pointed out here that many Y-shaped figures with 120° angles between limbs can be seen in Figs. 1 and 2.

The mitochondria bearing these kinds of cristae vary greatly in size and shape and they are sometimes disposed in a subsarcolemmal cluster and sometimes (Fig. 1) interposed between myofibrils. Fig. 1 also illustrates some of the varieties. They range from the small ones, 0.2 x 0.5 μ, to the large one (repeated in Fig. 2), 2.5 μ x 4.0 μ. (That the small ones are not just pieces of larger ones has been confirmed, in a few instances, by serial sections). Still other variants may be seen in Fig. 14. Such long, slender mitochondria usually have few retiform cristae. The one on the left in Fig. 14 is 0.5 x 9 μ.

It is difficult to estimate the relative abundance of these mitochondria, and only an impression can be gained from examining a large number of sections cut from many tissue blocks taken from 29 birds. The fact that measurements of relative mitochondrial volume made from thin sections are notoriously unreliable precludes more than an impression that these peculiar organelles account for about 20 per cent of the total mitochondrial volume.

Other more familiar types of mitochondria are observed in canary ventricles. In the lower right corner of Fig. 14 is a large mitochondrion with closely packed fenestrated cristae. Elsewhere may be seen mitochondria with simple tubular or foli-

ate cristae. Profiles of these seldom exceed 1.5 μ in length and 0.3 μ in width, but some of those whose cristae have abundant fenestrae are large (Fig. 15).

Some of the finer structural features of the membrane of these unusual mitochondria are illustrated in greater detail in Figs. 4 to 7. Figs. 4 to 6 are progressive enlargements of the same mitochondrion which was stained with lead. Fig. 7 is a section comparable to Fig. 6, but of an unstained mitochondrion. A distinct granularity is evident in all four of the micrographs, but at the lower magnifications it is difficult to decide whether the granules are in or on the membrane. At a magnification of 500,000 it is clear that the granules are part of the substance of the membrane. In the lead-stained section, the particles appear to have a 30- to 40-A diameter and are separated from each other (or held together) by a less dense band or line. In some areas the particles are seen close-packed in a row, and the center-to-center distance in these instances is 60 to 70 A, suggesting that the interval between particles is also of the order of 30 A along one axis of the array and that there are 9 to 10 particles per zig or zag. To reduce the possibility that these particles are staining artifacts, unstained sections were studied (Fig. 7). The bracketed areas in Fig. 7 show that the particles are present, but somewhat smaller (20 to 30 A) and much less conspicuous. A generalized mottling in the background of these preparations interferes with an accurate delineation of the particles.

Sparrow, Zebra Finch, Quail, and Goose

These birds were chosen for comparison with the canary because of the range of "speed" of cardiac contraction represented by their heart

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FIGURE 5 This enlargement of the mitochondrion in Fig. 4 shows more details of the zigzag pattern. The arrows indicate segments of cross-pieces which connect cristae within a tier. Small dense granules in or on the membrane can be seen faintly. The rectangle outlines the area enlarged in Fig. 6. × 108,000.

FIGURE 6 In this enlargement of the mitochondrion in Figs. 4 and 5 it can be seen that particles (arrows) about 30 to 40 A are in the substance of the membrane. Two areas in which the particles are closely packed are indicated by brackets. × 500,000.

FIGURE 7 Section of a mitochondrion similar to that in Fig. 6, but not stained with lead. Some of the particles in the membrane are indicated by the brackets. They show much greater density and more uniform size than the non-specific mottling in the background. The particles are approximately 20 to 30 A in diameter. × 500,000.
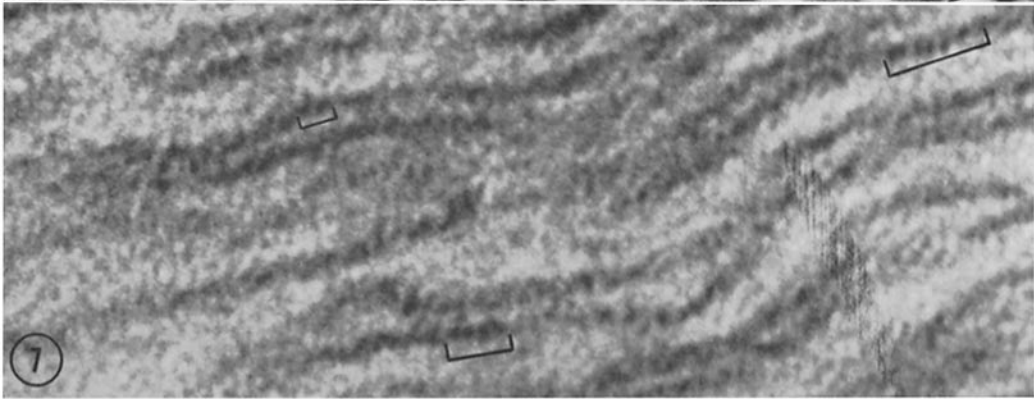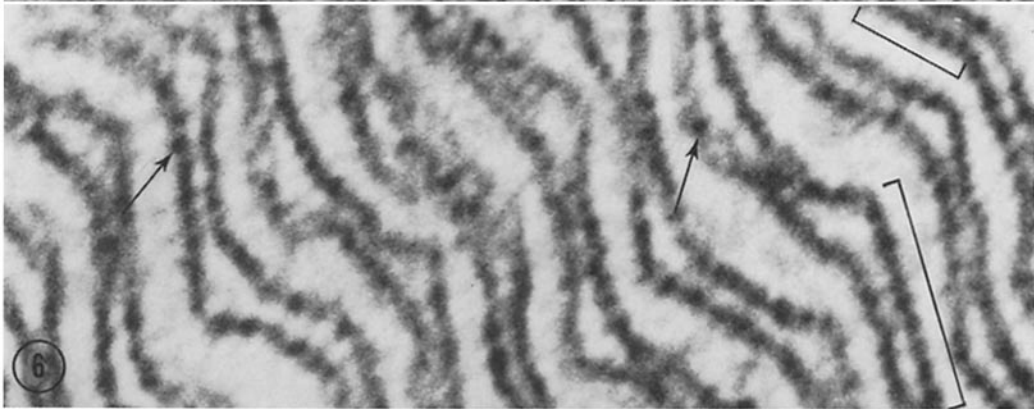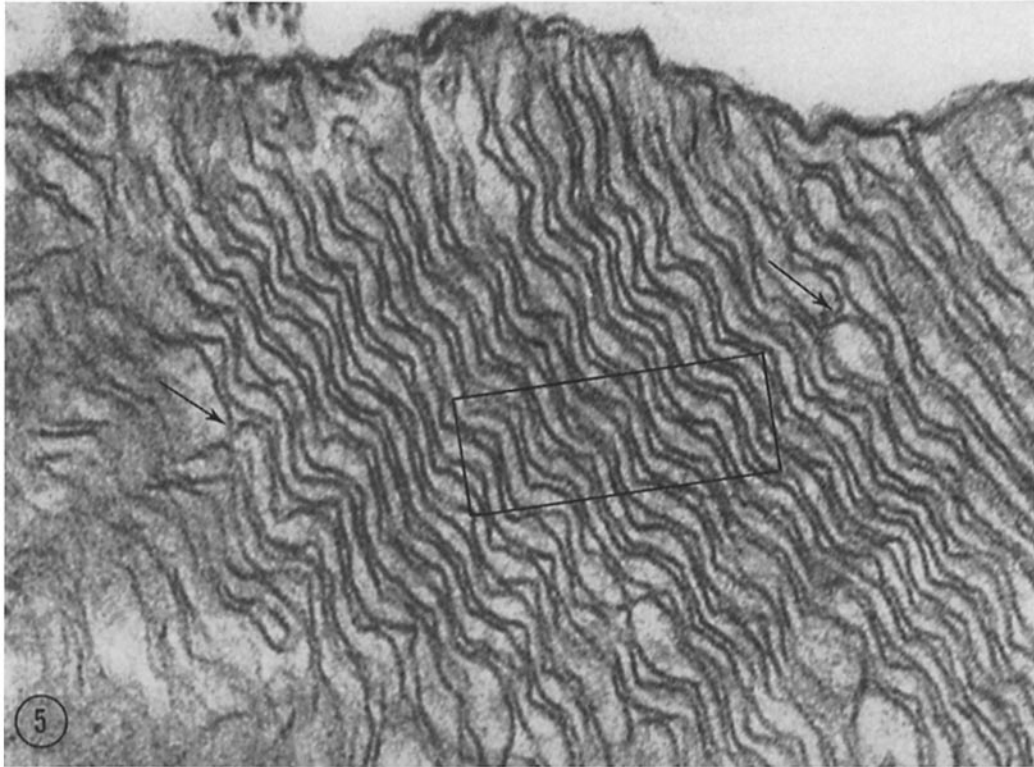
rates. The sparrow and the zebra finch, like the canary, have very fast rates—about 1,000 beats/minute. The quail has a much slower rate, about 500 to 600 beats/minute, and the goose heart rate is relatively slow at about 200 beats/minute. It seems reasonable to assume that the twitch velocities, recovery phases, etc., of these hearts are related to one another in the same way, although this has been confirmed with electrocardiograms only for the sparrow and canary.

There is surprisingly little difference in the fine structure of the mitochondria in the hearts of these four birds. None of the hearts shows any of the unusual forms of mitochondria described in the canary heart. Figs. 17 to 20 illustrate typical fields of mitochondria. In a very general way, it can be said that the faster hearts tend to have larger mitochondria with more frequent examples of tubular cristae; cristae disposed in whorls (Fig. 19) were more common in mitochondria of the slower hearts. Isolated examples of zigzag or angled cristae were occasionally seen in the sparrow heart but only rarely in hearts of the other three birds. In the quail heart a somewhat uncommon mitochondrial pattern is seen. As illustrated in Fig. 19, the numerous and tightly packed cristae are foliate and slightly fenestrated with neither angles nor anastomoses. In most cases, the length of the cristae is much greater than the width of the mitochondrion, and the cristae are interleaved. Sometimes they are interleaved with the cristae directly opposite, in which case their great length is accommodated by pursuing an S-shaped course, but more commonly the cristae are interleaved with others originating only a few hundred Angstroms farther along the length of the mitochondrion. The swirls thus produced intricately fit together so that no regions of the mitochondrion are devoid of cristal membranes. It is interesting to note that the mitochondria are frequently packed in such a way that their cristae are aligned, from one mitochondrion to the next, as illustrated in the lower right (arrow) of Fig. 19. Other mitochondria in this tissue (not illustrated

here) do not differ significantly from Palade's model.

DISCUSSION

The very large mitochondria of the canary heart are extraordinary both in their size and in the complex arrays assumed by their cristae. The angles of the zigzag cristae resemble those reported in a variety of mitochondria by Revel, Fawcett, and Philpott (11), but in the canary and sparrow hearts the zigzag cristae are much longer, more numerous, and somewhat more regular. Zigzag cristae were described earlier in the ameba *Pelomyxa carolinenesis* by Pappas and Brandt (8), but the mitochondria are not unusually large and the array, though complex, is quite different from that reported here. It has been suggested (11) that the angles represent "pliable junctional regions" joining "relatively inflexible segments" and that these properties may have a molecular basis. This view of the relative flexibility of parts is evidently not true for the mitochondria described here, for the angles remain constant no matter what disarray the cristae as a whole may suffer. Furthermore, the fact that acute angles are never seen argues against flexibility at these points. However, the view that the angles reflect an underlying molecular configuration seems indisuptable. Furthermore, the recent demonstration that the structure of mitochondria remains essentially unchanged when the phospholipids are extracted from their membranes (3) leads to the conclusion that the proteins of the membrane are responsible for the integrity of structure. It is reasonable to assume, therefore, that the constancy of measurements of the segments and angles of zigzag cristae corresponds to some property of the membrane proteins. As a consequence of this argument, one might expect all cristae to have angles when preserved as described here; that they do not is amply confirmed by the observations on cristae in hearts of the sparrow, zebra finch, quail, and goose, these hearts being prepared in just the same way as the canary hearts. A possible explanation of this

FIGURE 8   This micrograph shows the extent of the zigzag array. Some of the cristae are over 2.5 μ long and the array is over 2 μ wide. × 38,000.

FIGURE 9   The section in this micrograph has grazed one tier of zigzag cristae on the left and is tilted slightly relative to the plane of the tier on the right of the mitochondrion. Arrows indicate cross-pieces between zigzag cristae. × 74,000.
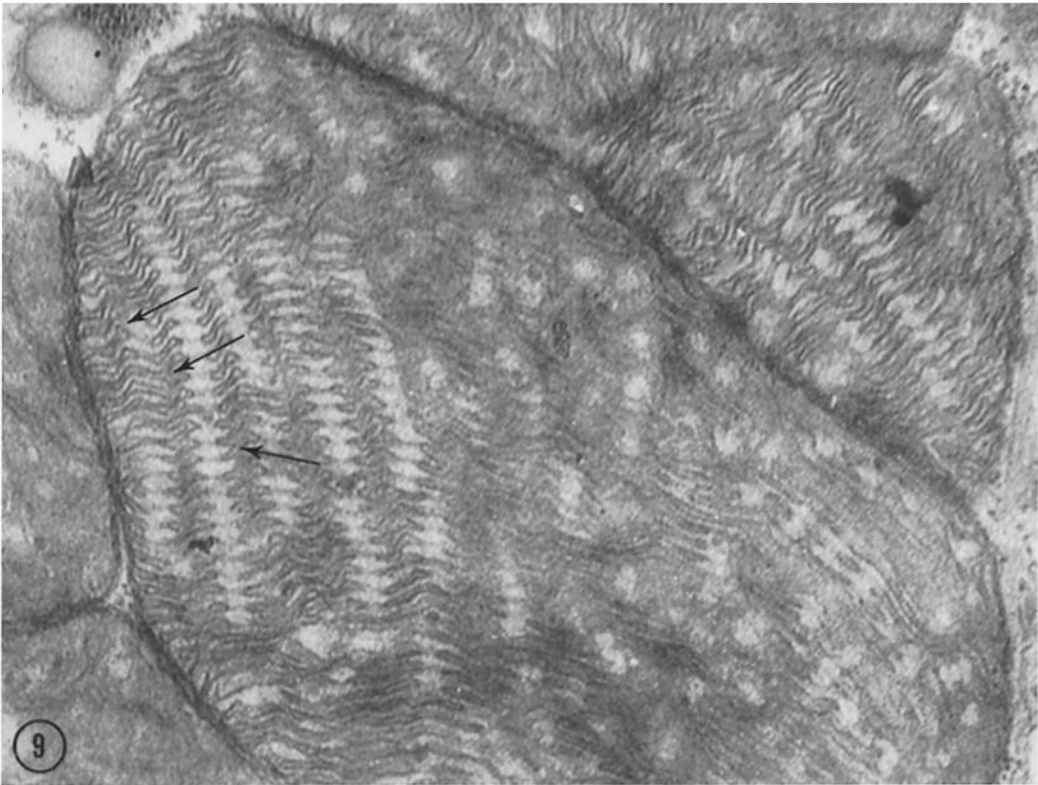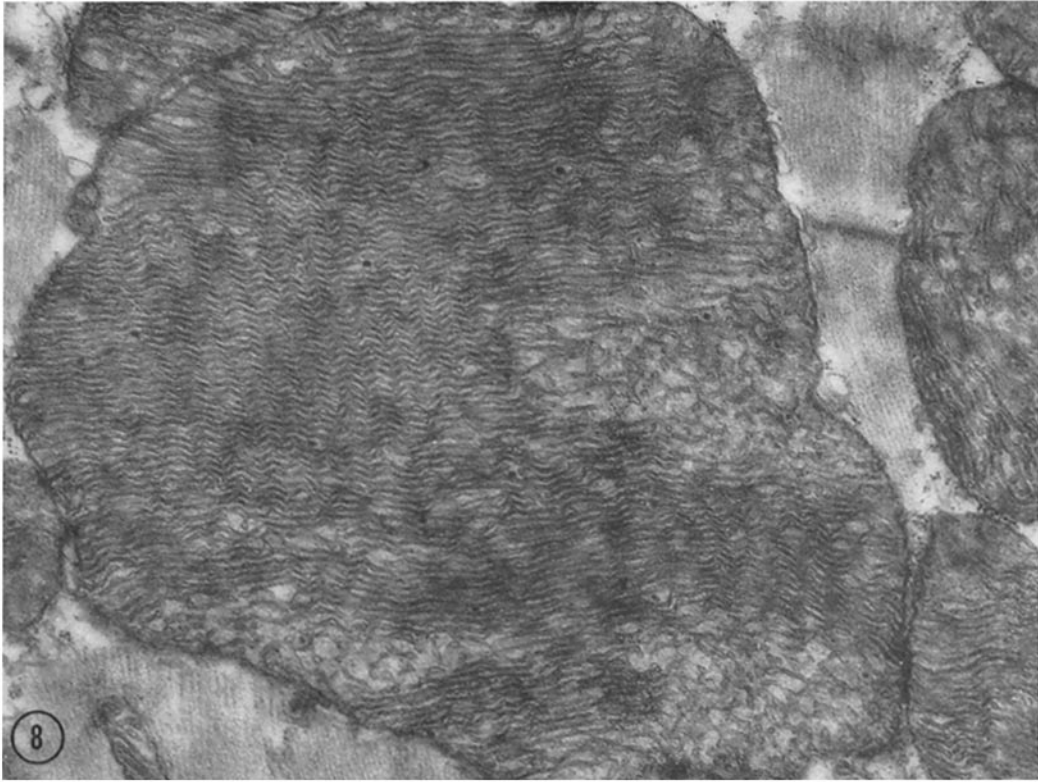
apparent enigma is that not all mitochondria, even within the same cell, are alike.

It has also been said (8) that close packing of zigzag cristae results in more membrane surface per unit volume of mitochondrion and that, since this surface carries organized enzyme systems, a more efficient mitochondrion is produced. Though the premise in this statement may be accurate, problems of diffusion in the large mitochondria of canary heart make it interesting to consider an alternate interpretation. The zigzag and retiform cristae are evidently continuous with each other, and a third form (hexagonal cristae), described and illustrated in Figs. 8, 10 to 12, 16, and 3 d, is interpreted as an intermediate form between the two. This appears not to be a static situation. As a consequence of the presence of connections between contiguous cristae within each tier (Fig. 3 a, $A-B$ plane) and their absence between tiers (Fig. 3 a, $C-D$ plane), expansion of the array leads to 90° torsion of the cristae in alternating directions (Fig. 3 d). This produces the regular hexagonal pattern seen in Figs. 10 to 12, and Fig. 16. As the system continues to expand, the absence of connections between tiers results in no external constraint upon the hexagons to remain in a single plane (i.e., the plane of the tier). The presence of numerous Y-shaped structures and occasional whole hexagons or parts of them in the fields of retiform cristae supports the view that the hexagons remain intact, but are no longer confined to a single plane. It is conceivable that to bring about this change some substance alters the permeability of the mitochondrion to water and causes it to swell. (A fall in pH with lactic acid accumulation consequent upon a sudden increase in metabolic rate might produce such an alteration.)

However inadequately substantiated this interpretation may be at present, it does fit the circumstances well. The enormous mitochondria with close-packed cristae can hardly function efficiently, since the channels available for the diffusion of substrate into, and of product out of the mitochondria are very long and restricted. On the other hand, they do concentrate large amounts of presynthesized enzymatic machinery in a small volume. In this concept, the conversion of close-packed zigzag cristae into open mesh retiform cristae may constitute an off-on system to accommodate sudden increase in energy utilization by a cardiac muscle already functioning close to capacity at 1,000 or more beats per minute in the steady state.

It is possible, of course, that the observations reported here are without such direct functional significance. But whether or not these forms are artifacts of the preparative procedures (which seems unlikely in view of the generally good state of preservation of the tissue) is considerably less important than the fact that they can be demonstrated repeatedly. Whether these forms exist *in vivo* or are only produced upon fixation, they clearly indicate that there is a stable repeating element in or around the membrane to account for the zigzags and hexagons. Furthermore, that unit is usually attached to one like itself at an angle of 120°.

The large mitochondrion illustrated in Fig. 15 is similar in appearance to those recently described in a blowfly by Smith (17). The cristae are very extensive and profusely fenestrated. They arise at many different points from the inner mitochondrial membrane. The juxtaposition of fenestrae in adjacent cristae, which Smith reported, has not been clearly seen in the canary heart, although Fig. 9

FIGURE 10 In this mitochondrion are several areas of cristae which are clearly zigzag in form. In the central part of the mitochondrion these same cristae appear straight, and they may be so at areas such as those marked with a star (*). Diffuse diagonal banding, however, in other areas marked with arrows, suggests that the tiers are viewed from above thus minimizing the angles. Although the transition from zigzag to retiform cristae usually occurs at a discrete boundary, several areas such as that outlined by the rectangle show transition within the zigzag array. × 38,000.

FIGURE 11 The mitochondrion on the right in this micrograph illustrates the planar hexagonal forms in the transition from zigzag to retiform cristae. The larger profile on the left shows a zigzag field surrounded by retiform and transitional hexagonal cristae. × 38,000.
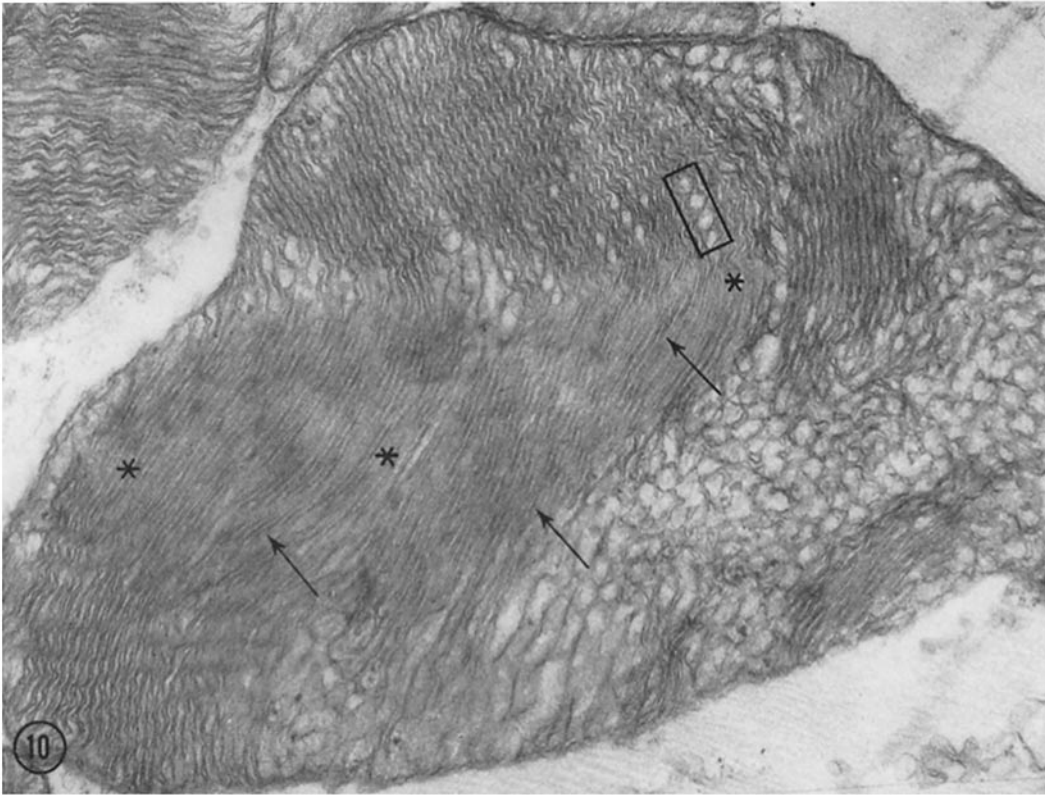
might permit such interpretation. It may be that the zigzag cristae are more closely related to the fenestrated ones than at first appears. One need only imagine enlargement of the fenestrae shown in Fig. 15 to understand how tubular cristae arranged in tiers could be produced.

The demonstration of a fine particulate component in the mitochondrial membrane may be related to the following interesting observations. According to Criddel *et al.* (1), the mitochondrial membrane contains a structural protein whose molecular weight is estimated to be 22,000. This figure suggests a 35-A diameter for a roughly spherical molecule. This dimension is in close agreement with that of the granule seen here. In the published micrographs of Stoeckenius (19) and Parsons (9) and in the as yet unpublished observations, in this laboratory, of negatively stained mitochondria, it can be seen that there is a "beading" of the membrane in addition to the elementary particle which is on a stalk and stands away from the membrane. These particles are also in the 35-A range. These observations tend to minimize the possibility that the granularity of the membrane seen in Figs. 5–7 is the result of coalescence of lipids under the influence of the electron beam, or of the addition of molecules to the membrane as suggested by Glauert, Dingle, and Lucy (5) for the saponin-treated membranes of Dourmashkin (2). In observations to be published soon with Fleischer and Green a similar beading of the membrane has been seen in sections of mitochondria from which 90 per cent or more of the phospholipid has been removed according to a method developed by Fleischer (3). These observations are in accord with our view that the membrane of canary heart mitochondria is composed in part of a 30- to 40-A spherical protein dispersed in a regular array in a film of unknown molecular composition.

It is interesting to note the degree to which mitochondrial elaboration in heart muscle can be correlated with heart rate. The hearts of the canary, sparrow, quail, and zebra finch all beat very fast though the heart rate in the quail is a little slower than that of the other three birds. The goose heart rate is very much the slowest. Except for the bizarre mitochondrion of the canary heart, there seems to be poor correlation between the heart rate and the size, numbers, or complexity of organization of mitochondria among the four fast hearts, but mitochondria of the much slower goose heart have fewer and somewhat less complicated cristae. Preliminary examinations of a slow reptilian heart and of a hummingbird heart, suggest that this may be a real, though gross, correlation. Finally, it is clear that the notion that zigzag cristae *must* be present in fast muscle is not supported by these observations.

BIBLIOGRAPHY

1. CRIDDLE, R. S., BOCK, R. M., GREEN, D. E., and TISDALE, H., Physical characteristics of proteins of the electron transfer system and interpretation of the structure of the mitochondrion, *Biochem.*, 1962, 1, 827.
2. DOURMASHKIN, R. R., DOUGHERTY, R. M., and

FIGURE 12 The formation of planar hexagonal arrays from zigzag cristae leading to retiform cristae is well illustrated in this mitochondrion. × 40,000.

FIGURES 13 a AND b These mitochondria demonstrate the tubular appearance of cross-sections of the cristae. × 64,000.

FIGURE 14 The very long slender mitochondrion in this micrograph contains both zigzag and retiform cristae. The mitochondrion measures approximately 0.5 by 9.0 μ. In the lower right corner can be seen a part of a large mitochondrion with little or no evidence of zigzag or retiform cristae. × 23,000.
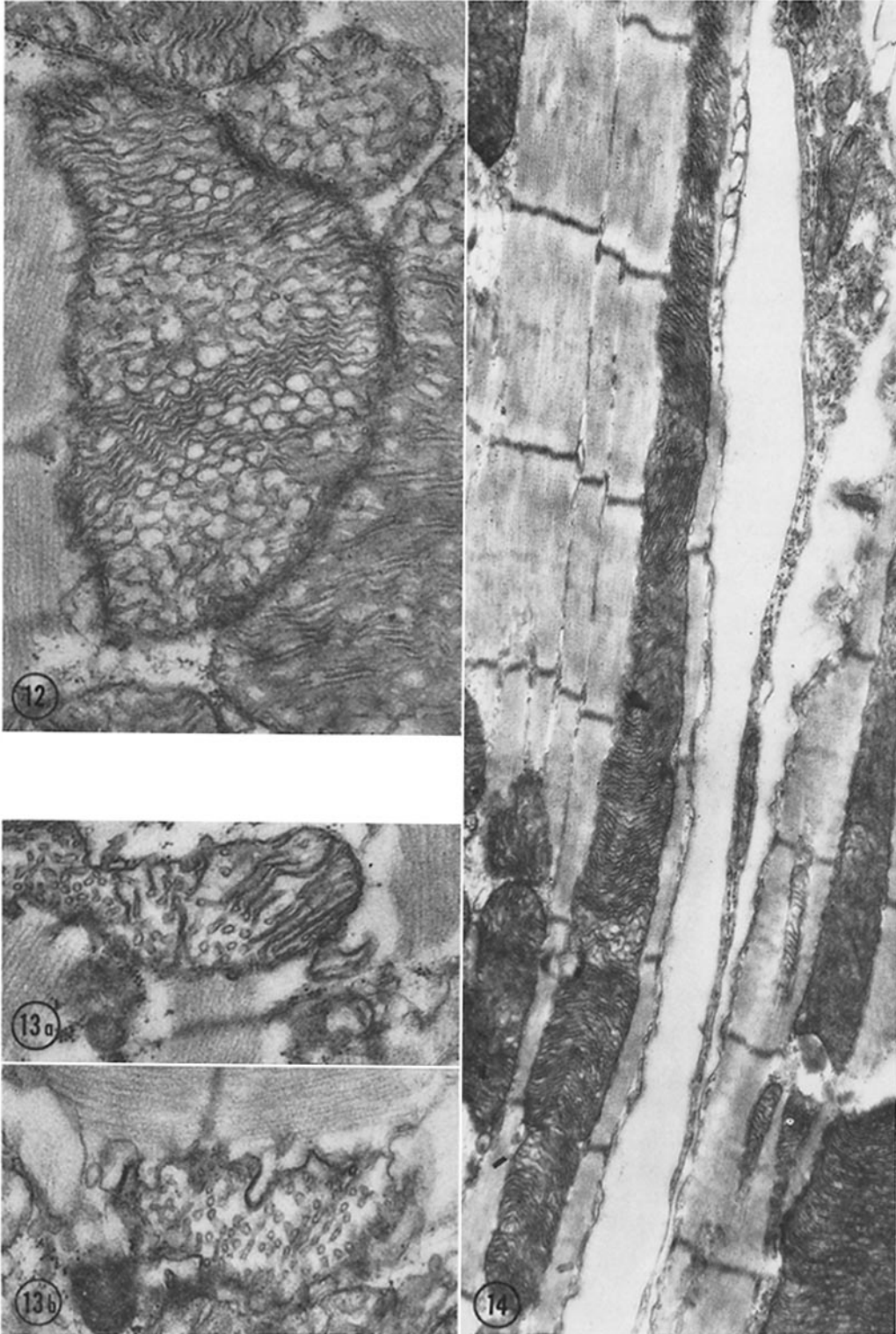
HARRIS, R. J., Electron microscopic observations on Rous sarcoma virus and cell membranes, *Nature*, 1962, **194**, 1116.

3. FLEISCHER, S., BRIERLEY, G., KLOUWEN, H., and SLAUTTERBACK, D. B., Studies of the electron transfer system. XLVII. The role of phospholipids in electron transfer, *J. Biol. Chem.*, 1962, **237**, 3264.

4. FREEMAN, J. A., and SPURLOCK, B. O., Maraglas epoxy embedding media, Electron Microscopy, Fifth International Congress for Electron Microscopy, Philadelphia, New York, Academic Press, Inc. 1962, **2**, 11.

5. GLAUERT, AUDREY M., DINGLE, J. T., and LUCY, J. A., Action of saponin on biological cell membranes, *Nature*, 1962, **196**, 953.

6. MOORE, D. H., and RUSKA, H., Electron microscope study of mammalian cardiac muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 261.

7. PALADE, G. E., An electron microscope study of the mitochondrial structure, *J. Histochem. and Cytochem.*, 1953, **1**, 188.

8. PAPPAS, G. D., and BRANDT, P. W., Mitochondria. I. Fine structure of the complex patterns in the mitochondria of *Pelomyxa carolinensis* Wilson (*Chaos chaos* L.), *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 85.

9. PARSONS, D. F., Negative staining of thinly spread cells and associated virus, *J. Cell Biol.*, 1963, **16**, 620.

10. PORTER, K. R., and PALADE, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.

11. REVEL, J. P., FAWCETT, D. W., and PHILPOTT, C. W., Observations on mitochondrial structure, *J. Cell Biol.*, 1963, **16**, 187.

12. REYNOLDS, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17**, 208.

13. SIMPSON, F. O., and OERTELIS, S. J., The fine structure of sheep myocardial cells; sarcolemmal invaginations and the transverse tubular system, *J. Cell Biol.*, 1962, **12**, 91.

14. SJÖSTRAND, F., and HANZON, V., Membrane structures of cytoplasm and mitochondria in exocrine cells of mouse pancreas as revealed by high resolution electron microscopy, *Exp. Cell Research*, 1954, **7**, 393.

15. SJÖSTRAND, F., and RHODIN, J., The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy, *Exp. Cell Research*, 1953, **4**, 426.

16. SLAUTTERBACK, D. B., The fine structure of shrew (*Blarina*) cardiac muscle, *Anat. Rec.*, 1961, **139**, 274.

17. SMITH, DAVID S., The structure of flight muscle sarcosomes in the blowfly *Calliphora erythrocephala* (Diptera), *J. Cell Biol.*, 1963, **19**, 115.

18. STENGER, R. J., and SPIRO, D., The ultrastructure of mammalian cardiac muscle, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 325.

19. STOECKENIUS, W., Some observations on negatively stained mitochondria, *J. Cell Biol.*, 1963, **17**, 443.

FIGURE 15 The large mitochondrion in this micrograph exhibits cristae with very many fenestrae. Note that as the size and proximity of the fenestrae increase the cristae assume a more tubular form. X 26,000.

FIGURE 16 The large mitochondrion in this field shows several forms of cristae, evidently in a state of transition. The relatively open appearance in the upper left of this mitochondrion seems to be an oblique section of the hexagonal form. The sarcolemma of this myofiber is visible in the upper right of the figure. X 35,000.
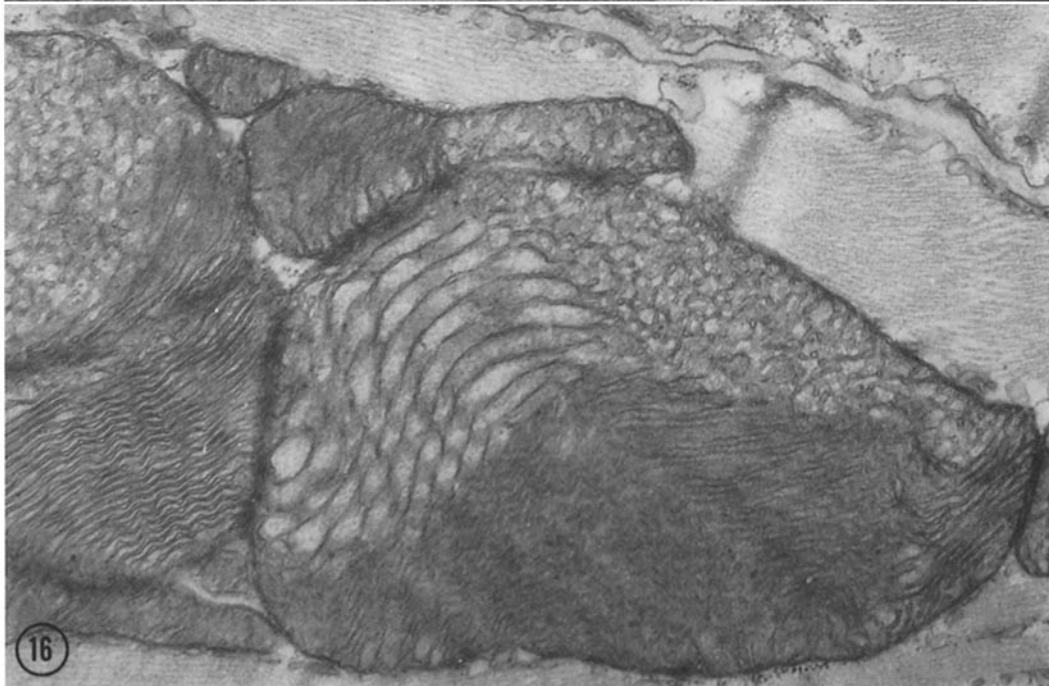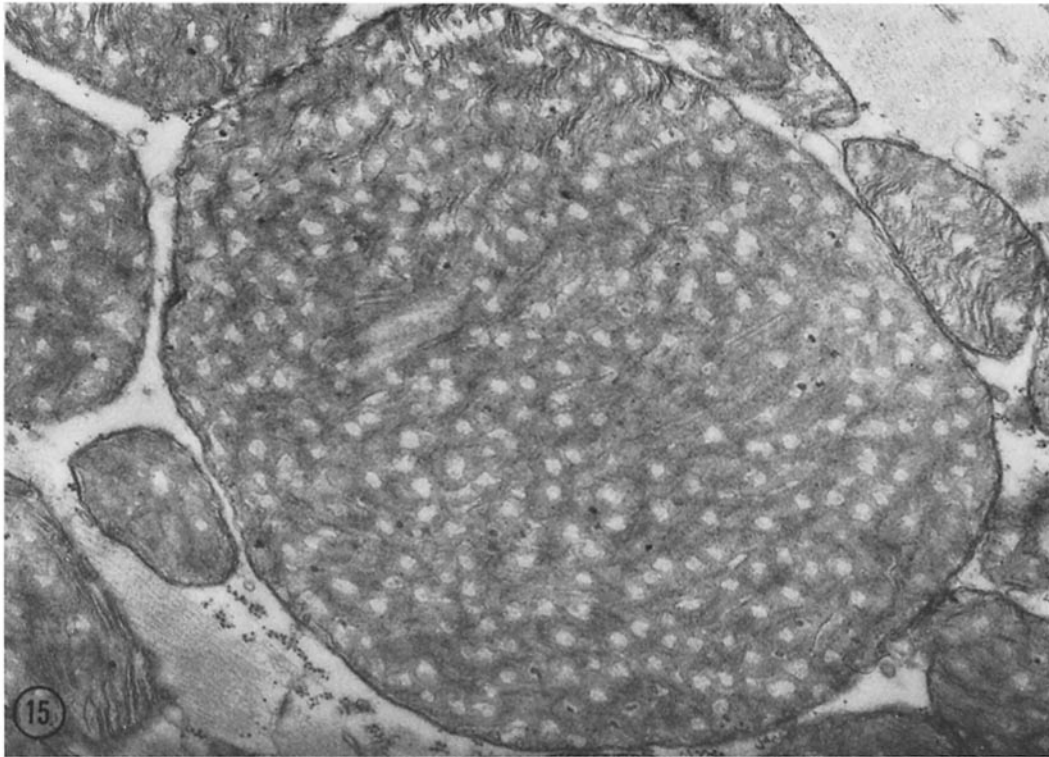
FIGURE 17 A typical field of mitochondria from the sparrow ventricular myocardium. Most of the cristae appear to be tubular, but angles (arrows) are infrequent. × 41,000.

FIGURE 18 This micrograph is similar to that in Fig. 14, but it is from the zebra finch. The few suggestions of angles in the cristae are indicated by arrows. × 40,000.
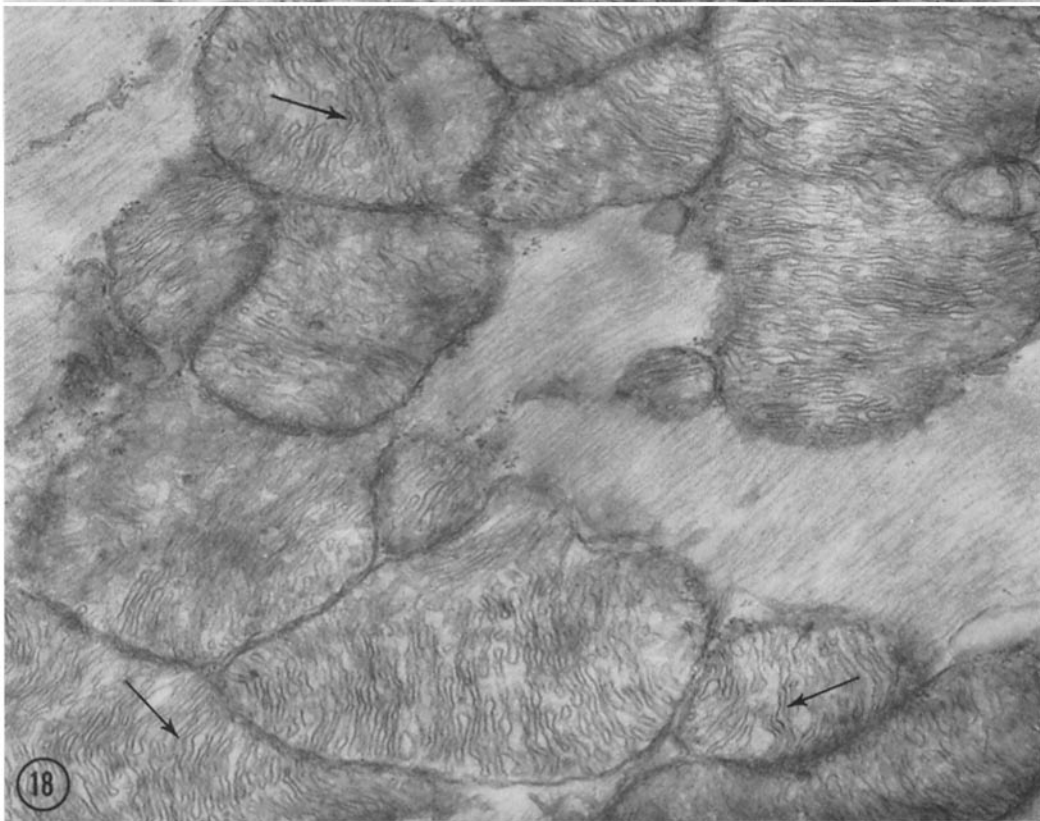
FIGURE 19   Mitochondria from the quail heart. Most of the cristae are evidently foliate and fenestrated and there are no angles or zigzag arrays. Notice the prominent whorls made by the cristae. At the arrow (lower right) the cristae of three contiguous mitochondria seem to form a continuous concentric array. Such images are common. × 23,000.

FIGURE 20   These mitochondria are from the ventricle of a goose. Some of the cristae may be tubular but the fenestrated foliate form (arrows) seems to predominate. Only rarely is there a suggestion of an angle in the cristae. × 25,000.