INCREASED NUMBERS OF ANNULATE LAMELLAE IN MYOCARDIUM OF CHICK EMBRYOS INCUBATED AT ABNORMAL TEMPERATURES

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ABSTRACT

Annulate lamellae have been observed in the myocardium of 18-day-old chick embryos maintained at the normal temperature of 100°F and at 90°F during the last week of incubation. An increased number of annulate lamellae was observed in heart muscle of embryos incubated at 90°F. This is probably caused by a persistent production of these organelles, since annulate lamellae are present in greater frequency than in 11-day-old embryos incubated at 100°F. In the hypertrophic hearts of 18-day-old embryos incubated at 90°F, the annulate lamellae were associated with a net increase of protein content and an elevated concentration of myocardial glycogen. It is suggested that the increased number of annulate lamellae is a sequela of reduced environmental temperature during incubation.

INTRODUCTION

During the past decade, several publications have dealt with the ultrastructural appearance, source, mechanism of production, and function of annulate lamellae. In this period, annulate lamellae have been found in somatic and germ cells in both invertebrate and vertebrate organisms. They have a complex architecture and appear similar in ultrastructure to the nuclear envelope. Despite the presence of these lamellae in a wide variety of species, little has been established with respect to their function.

McCulloch (1952) found that *Arbacia* egg cytoplasm contained a birefringent fibrillar element that appeared on electron micrographs as parallel fibrils and that was termed "coarse fibrous component." Serial sections of the coarse fibrous component of McCulloch were shown by Lansing et al. (1952) to indicate that the component in reality consists of sheets, rather than fibers, composed of double membranes separated by cross-striations. Afzelius (1955) described, in sea urchin oocytes, an irregular extranuclear membrane identical in fine structure to the nuclear membrane. Palade (1955) described structures of similar appearance in rat spermatids and named them "fenestrated cisternae." Rebhun (1956 *a*, *b*; 1960) described identical intracytoplasmic, basophilic "periodic lamellae" in oocytes of the clam *Spisula* and ovotestis of the snail *Otala*. Swift (1956) coined the descriptive term "annulate lamellae" for similar structures observed in clam oocytes, snail ovotestis, and acinar cells of amphibian larval pancreas. This latter term has become the most widely accepted one.

Similar structures have been reported by Ruthmann (1958) and Kaye et al. (1961) in spermatocytes of crayfish; by Merriam (1958, 1959) in sand dollar eggs, by Barer et al. (1959, *a*, *b*; 1960) in

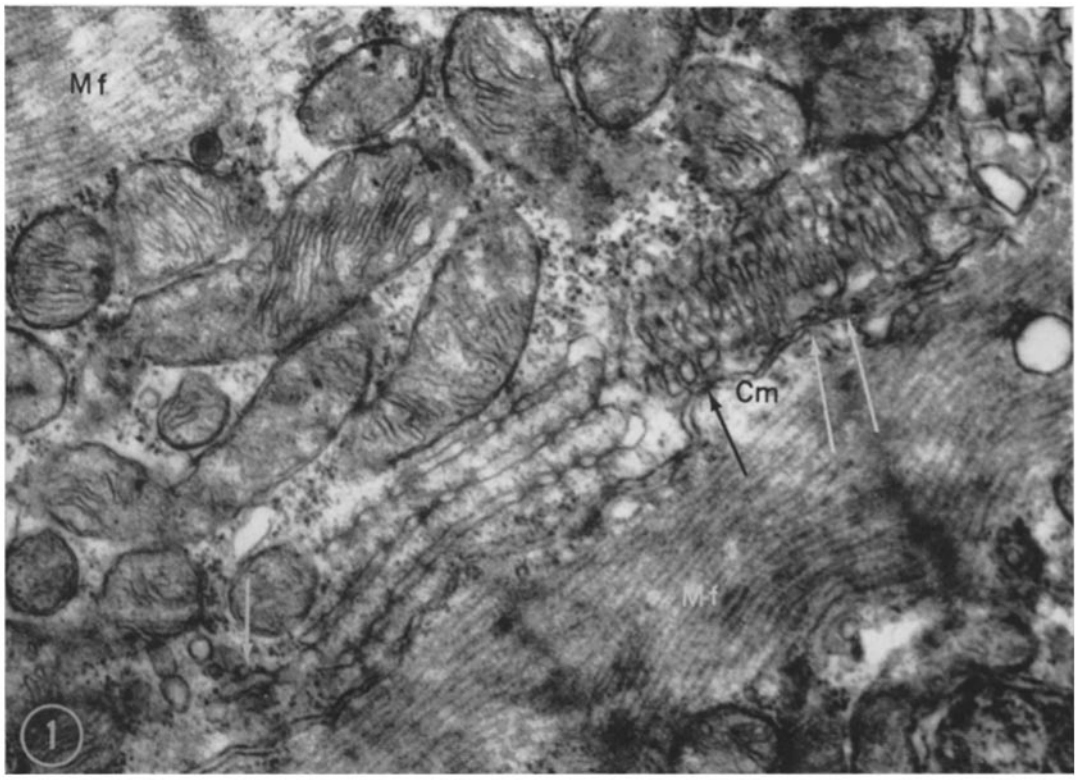

FIGURE 1 Myocardium of chick embryo incubated at 100°F. The rarely observed annulate lamellae are cut in 2 planes of section. Three lamellae in transverse section (left, center) display periodic constrictions with several free margins forming a dilated vesicle. One lamella (lower left arrow) has several attached ribosomes. Granular material is present between lamellae. The sarcoplasm contains many mitochondria, vesicles, and free ribosomes. In the oblique plane (upper right), the lamellae are contiguous with the cell membrane (*Cm*) at several points (arrows). Perhaps this arrangement is fortuitous. Myofibrils (*Mf*) are present along two borders. × 16,000.

insect spermatocytes; by Stay (1965) in abnormal oocytes of the *Cecropia* moth; by Wischnitzer (1960) and Kessel (1963 *a*, *b*) in salamander oocytes; by Gay (1955), King and Devine (1958), Okada and Waddington (1959), Waddington and Okada (1960), and Mahowald (1962; 1963) in *Drosophila* eggs; by Afzelius (1957), Pasteels et al. (1958), Kane (1960), and Gross et al. (1960) in sea urchin eggs; by Balinsky and Devis (1963) in oocytes of the toad; and by Kessel (1964 *b*; 1965) in echinoderm and tunicate oocytes. Merriam (1959), Hsu (1963), and Kessel (1964 *a*; 1965) noted intranuclear as well as cytoplasmic annulate lamellae in oocytes of several species. Ross (1962) observed annulate lamellae in adrenal cortical cells of fetal rats. Hruban et al. (1965 *a*, *b*) reported their occurrence in hepatocytes of rats treated with β-3-furylalanine and more rarely in pancreatic acinar cells in animals treated with azaserine. Annulate lamellae have been also observed in transplantable animal tumors. Epstein (1957) noted annulate lamellae in Sarcoma 37 cells; Schulz (1957) noted them in mammary carcinoma cells of the rat; Wessel and Bernhard (1957) reported their presence in Ehrlich and Yoshida ascites tumor cells; and Binggeli (1959) observed annulate lamellae in chemically induced chicken fibrosarcoma cells. They were also reported by Epstein (1961) in HeLa cells, by Hoshino (1963) in two of four Yoshida ascites hepatoma strains, and more recently in Sarcoma I cells by Chambers and Weiser (1964 *a*, *b*). Annulate lamellae have also been noted in our laboratory in an ethionine-induced hepatoma. To our knowledge, the only

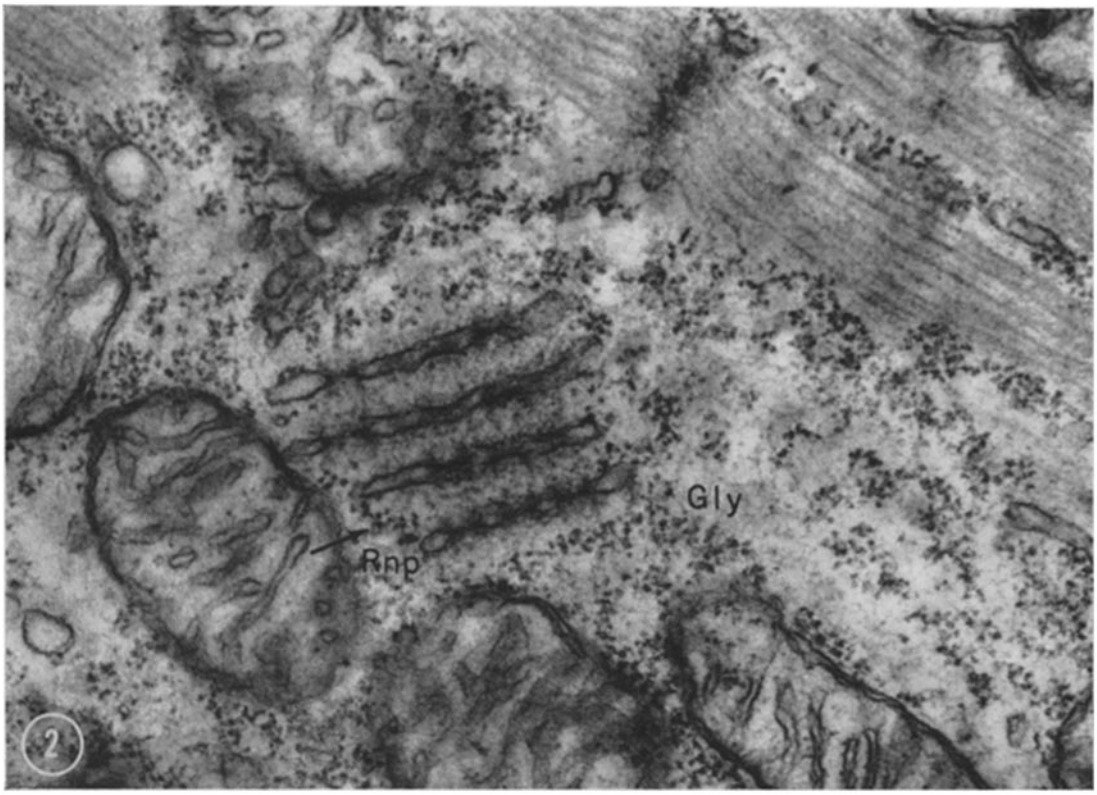

FIGURE 2 Four lamellae in myocardium of chick embryo incubated at 90°F, illustrating their periodic constrictions and dilated vesicular free ends. Dense interlamellar granularity is prominent. Free ribosomes (*Rnp*) are associated with the free margins of the lamellae. A background containing considerable lightly staining glycogen (*Gly*) is present. × 55,000.

known occurrence of annulate lamellae in birds has been reported by Harrison (1962) in sea gull adrenals.

In the present study, increased numbers of annulate lamellae were observed in the myocardial cells of chick embryos incubated at an abnormally low temperature. Associated with these annulate lamellae were increased concentrations of myocardial glycogen. This study grew out of our observations on the development of cardiac enlargement in late chick embryos following their incubation at reduced temperatures (Leighton et al., 1964).

MATERIALS AND METHODS

White Leghorn embryonated eggs were maintained at the normal temperature of 100°F until the 11th day of incubation. At this time, the eggs were divided into three groups. One-third remained in the 100°F incubator, a second group was transferred to a 90°F incubator, and the last third to a 108°F incubator. The eggs were then kept at their respective temperature until harvesting on the 18th day. 1-mm cubes of posterior left ventricular myocardium were taken from six embryos in each of the three 18-day-old groups and immediately fixed in 1% phosphate-buffered osmium tetroxide at pH 7.4. A similar sample of heart tissue was removed from two 11-day-old chick embryos incubated at 100°F. The cubes of tissue were kept in cold fixative and refrigerated for 1 to 2 hr. Rapid dehydration through a series of cold ethanols was followed by treatment with propylene oxide. The tissue was then embedded in Epon 812 and Vestopal W. Thick sections were prepared and stained with toluidine blue. Equal numbers of blocks were sectioned from hearts for each of the three test temperatures. Sections displaying silver or gold interference colors were obtained from a Porter-Blum MT-1 ultramicrotome equipped with glass knives. Ultrathin sections were stained with a 2% solution of uranyl acetate followed

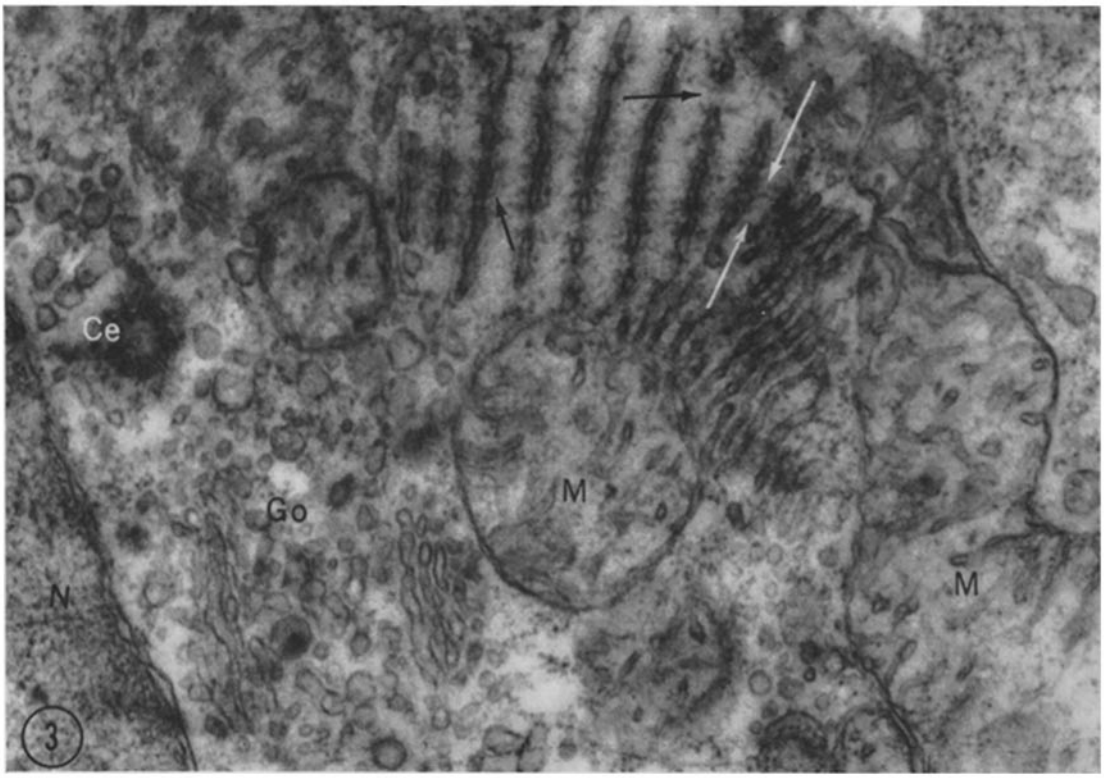

FIGURE 3 Annulate lamellae in heart of 90°F incubated chick embryo, showing at least 17 double membranes adjacent to a nucleus (*N*). Interlamellar granularity and small tubular channels are present (arrows). Mitochondria (*M*) are slightly swollen; a Golgi apparatus (*Go*) and centriole (*Ce*) are situated between the nucleus and annulate lamellae. × 40,000.

by lead hydroxide or lead citrate according to the method of Karnovsky (1961) or Reynolds (1963), respectively. Thin sections were examined with Phillips 100B and RCA EMU-3F microscopes.

OBSERVATIONS

Chick embryos incubated at 90°F for 7 days appeared underdeveloped grossly, but, despite this, had hearts with an average weight that was 50 mg greater than that of hearts of embryos incubated at 100°F. These hearts exhibited muscle hypertrophy, which was associated with a net increase of cardiac protein. There was also a concomitant increase in diastase-digestible, periodic acid Schiff (PAS)–positive material in the histologic sections of the 90°F hearts. Chemical analysis of the 90°F hearts showed a glycogen concentration that was twice that of the hearts of embryos incubated at 100°F. Both the 90° and 108°F chick hearts displayed many mitoses by light microscopy, whereas mitosis was rarely observed in myocardial cells of embryos incubated at 100° F. A detailed chemical and histologic analysis of the cardiovascular system of chick embryos incubated at the 3 different temperatures will be reported.

Annulate lamellae were found to be a normally present organelle in hearts of 11-day-old chick embryos incubated at 100°F. During subsequent embryonic development, these structures tended to disappear and were noted very rarely in heart tissue removed from 18-day-old chick embryos incubated at this control temperature (Fig. 1). On the other hand, intracytoplasmic annulate lamellae were frequently noted in the myocardium of 18-day-old chick embryos incubated at 90°F (Figs. 2 to 9). Their frequency in hearts of 18-day-old chicks incubated at 90°F appeared even greater than in hearts of 11-day-old chicks incubated at 100°F, though a quantitative analysis was difficult to make. This difference in frequency

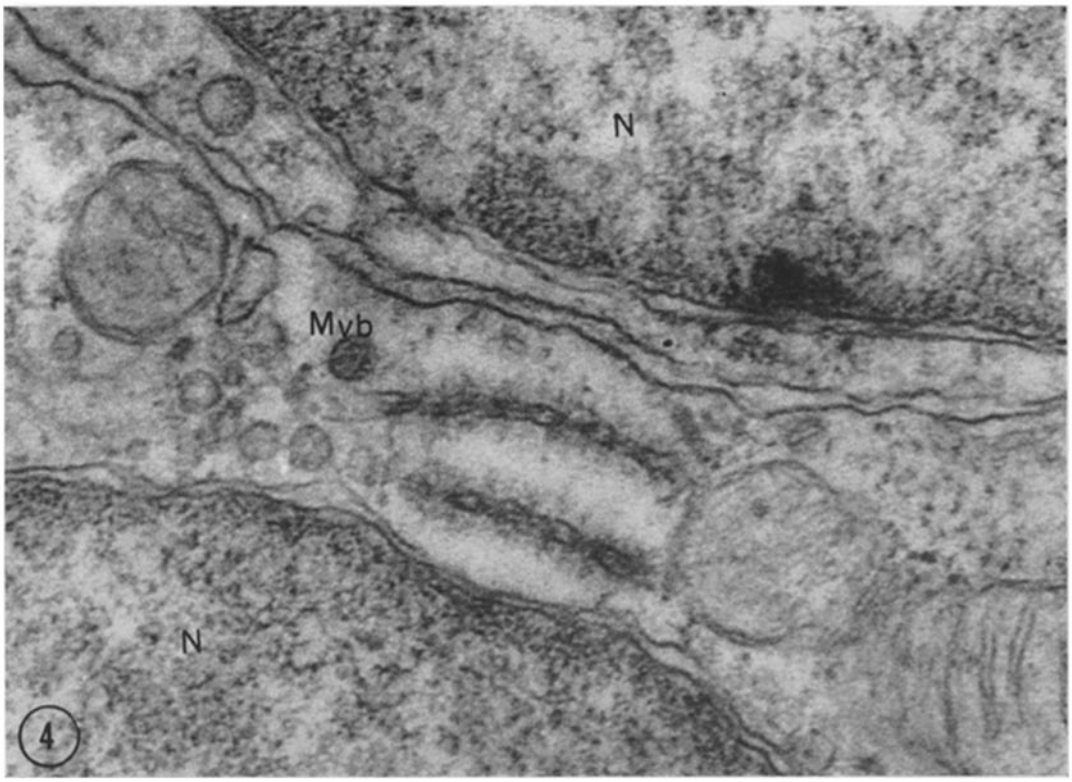

FIGURE 4 Myocardium of chick embryo incubated at 90°F. Annulate lamellae are present between two nuclei (*N*). The periodicity and granularity of the lamellae are evident. No connection can be seen between annulate lamellae and nuclear envelope. A structure which is adjacent to the upper lamella is interpreted as a multivesicular body (*Mvb*). × 48,000.

of annulate lamellae was impressive since the heart weights of the 11-day-old embryos incubated at 100°F, averaged 33 mg while those of the 18-day-old embryos incubated at 90°F averaged 225 mg. In spite of this 7-fold increase in cardiac weight, annulate lamellae were seen with greater frequency in the latter. We have noted excessive accumulations of intracellular glycogen (Figs. 2, 9) associated with annulate lamellae in the myocardium of 90°F incubated chick embryos. Accumulations of this particulate glycogen, which did not always stain with lead, were very frequently observed in the cytoplasm surrounding annulate lamellae. Although annulate lamellae appeared identical morphologically in the hearts of 18-day-old chick embryos incubated at 100° and 90°F, the investing cytoplasm in the 100°F hearts had no excess accumulations of glycogen (Fig. 1). On the other hand, annulate lamellae in the myocardium of control 11-day-old embryos incubated at 100°F were associated with abundant glycogen and appeared similar to annulate lamellae of the myocardium of 18-day-old embryos incubated at 90°F. Annulate lamellae were not observed in myocardial cells of chick embryos incubated at 108°F.

Annulate lamellae within myocardial cells of chick embryos incubated at 90°F displayed the usual ultrastructural characteristics that differentiate them from other cellular organelles. The double membranes or lamellae exhibited the characteristic periodic constrictions in register with adjacent parallel membranes (Figs. 1 to 9). These periodic constrictions impart a beading effect, the hallmark of annulate lamellae. Tubules extending from one lamella to another have been reported (Swift, 1956; Ross, 1962; Chambers and Weiser, 1964 *b*). These can be seen in Figs. 3 and 7 and attest to a rather complex structure. In many of the thin sections of myocardium from 90°F

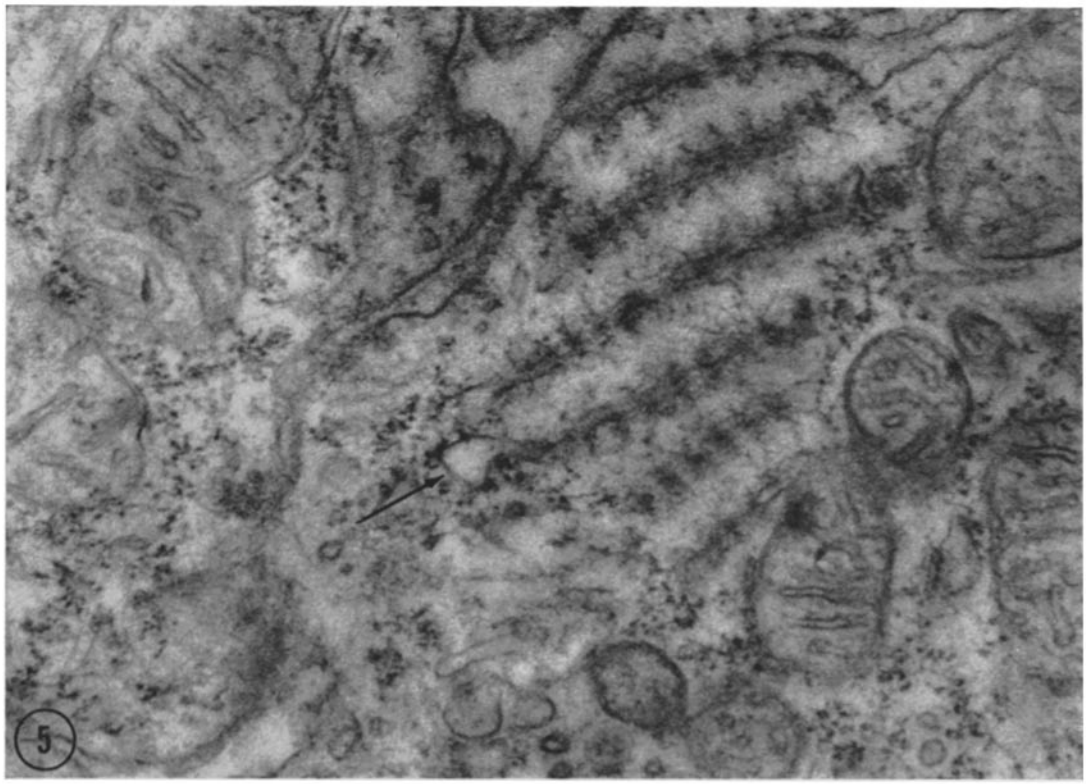

FIGURE 5 Myocardium of chick embryo incubated at 90°F. The dilated, vesicular free margin of an annulate lamella has ribosomes attached to it (arrow). There is a suggestion of interlamellar connections. Small granules are also associated with the lamellae. × 48,000.

incubated chicks, electron-opaque cross-striations radiated from a point of periodic constriction from one lamella to another. These gave the appearance of tubular interconnections between parallel lamellae arrayed in stacks (Figs. 2 to 8). These cross-striations appeared, in part, to be dense granular material.

When the annulate lamellae were sectioned in a face-on view, a hexagonal arrangement of the annuli could be noted. Each annulus circumscribed a diaphragm composed of dense, granular, amorphous material filling a pore. Rebhun (1961) has described this as a "pillowcase-like structure." The annulus wall itself consisted of several microcylinders (Figs. 8, 9). These microcylinders or subannuli have been previously observed (Merriam, 1958, 1959; Ross, 1962; Chambers and Weiser, 1964 *b*). Wischnitzer (1958) reported that an average of 8 microcylinders form each annulus of the nuclear envelope.

A continuity between the ends of the annulate lamellae and the endoplasmic reticulum exists (Pasteels et al., 1958; Epstein, 1962; Rebhun, 1961). Usually there is also a dilated vesicle present at the free margin of the lamellae (Rebhun, 1956 *b*). Although the rough endoplasmic reticulum is poorly organized in myocardium, we observed a continuity of lamellae with small fragments of endoplasmic reticulum and slightly dilated vesicles, with or without attached ribosomes, at the free ends of the lamellae (Figs. 1, 2, 4, 5, 7). The number of lamellae or double membranes ranged from 2 to 21.

Ribosomes were seen adherent to some lamellae near their free ends (Figs. 1, 7). Others were loose between lamellae, or adherent to the dilated terminal vesicles (Figs. 2, 5). This pattern is in agreement with that reported by Kessel (1965) and that of Chambers and Weiser (1964) who noted ribosomes between and attached to lamellae. In contrast, Swift (1956) observed ribosomes only on the contiguous endoplasmic reticulum and

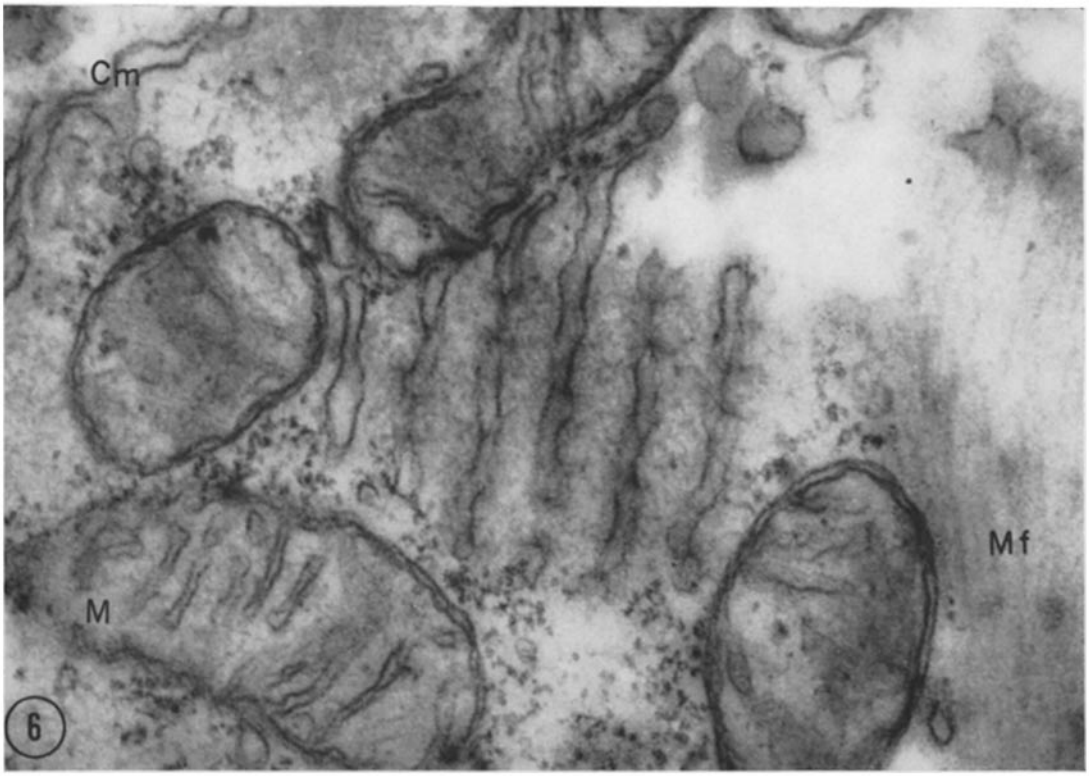

FIGURE 6 Six "smooth" lamellae in myocardium of 90°F incubated chick embryo display fewer than usual periodic constrictions. This apparent reduction in the number of constrictions is probably due to the plane of section. × 66,000.

never within lamellar arrays. Epstein (1957, 1961) reported only free ribosomes associated with lamellae.

Various structures have been found to be associated with or in close proximity to annulate lamellae (Rebhun, 1960; Ross, 1962; Chambers and Weiser, 1964 *b*). We observed a large stellate-shaped osmiophilic lipid body (Fig. 7), a Golgi complex (Fig. 3), an occasional aster (Fig. 3), and an infrequent multivesicular body (Fig. 4). In several micrographs (Figs. 6, 8, 9), the lamellae appeared to have smooth walls and to be partly lacking in the usually sharp periodic constrictions. Barer et al. (1960) noted this appearance in secondary spermatocytes of the locust. We agree with the view expressed by Barer et al. that this appearance is probably due to the plane of sectioning.

There is a striking structural similarity between cytoplasmic annulate lamellae and the nuclear envelope (Fig. 4). Although annulate lamellae were often in close proximity to the nucleus, we did not observe attachment to the latter (Figs. 3, 4). Nor did we note any blebbing of the outer layer of the nuclear envelope. The observation of annulate lamellae in several planes in the same thin section suggests that they can be rather large complex structures, certainly greater in size than most other organelles (Figs. 8, 9). In Fig. 8, annulate lamellae appear sectioned in 3 different planes. One plane is the frequently observed transverse section with periodic constrictions. Another plane shows a honeycomb-like structural pattern with hexagonally arranged annuli, each composed of numerous microcylinders. The latter surround a pore with a slightly electron-opaque central diaphragm containing granular material and representing a face-on view. A third plane is an unusual, oblique section showing the lamellae arranged as double membranes (Figs. 8, 9). Fig. 9, which is a higher magnification of a portion of Fig. 8, shows only the oblique and face-on views. Minute, bead-like, dense, granular material forms the membranes of the lamellae. However, the outer mitochondrial membranes and the

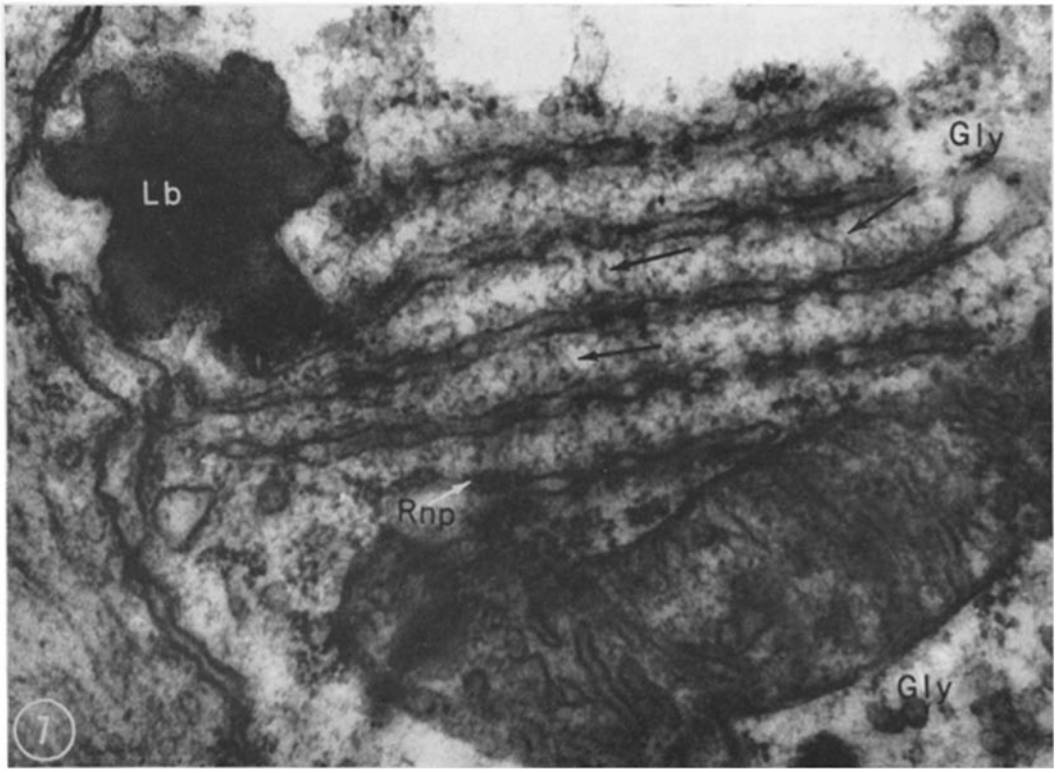

FIGURE 7 A large, scalloped, osmiophilic lipid body (*Lb*) in myocardium of 90°F incubated chick embryo is adjacent to the annulate lamellae. Small tubules connect the lamellae (arrows). Ribosomes (*Rnp*) are adherent to portions of the lamellar membrane surface. Glycogen (*Gly*) is present in the background (lower and upper right corners). × 78,000.

cristae also appear to consist of this material (Fig. 9). This can be interpreted as staining artefact.

DISCUSSION

In this study, annulate lamellae have been observed in the myocardium of 18-day-old chick embryos. They occur normally, though with decreasing frequency, in hearts of 11- to 18-day-old chick embryos that have been incubated at the normal temperature of 100°F. However, their frequency appears substantially greater in hearts of 18-day-old embryos incubated at 90°F from the 11th to 18th day. Porter (1961) pointed out that annulate lamellae are "most prominent in cells that are actively growing and multiplying." We should like to add to this basic concept the possible sequelae of environmental alterations. Reduced environmental temperature may be one such influential factor. Annulate lamellae have been most frequently described in oocytes or spermatids of cold-blooded marine organisms. The rat spermatids described by Palade (1955, 1956) are at a lower than body temperature while in the scrotal sac. In our experimental model of reduced temperature of incubation, increased numbers of annulate lamellae were present in the heart of chick embryos incubated at 90°F. Thus, it would appear that a lower temperature of incubation alters in some manner the normal maturation of the heart and other organs.

Annulate lamellae were more frequently observed in hearts of 18-day-old chicks than in those of 11-day-old chicks. In addition, the heart weight was substantially greater in the 18-day-old chicks. Therefore, we feel that the annulate lamellae observed in hearts of 18-day-old embryos incubated at 90°F are the result of continued and recent production rather than persistence of these organelles.

It is of interest that in our experimental model

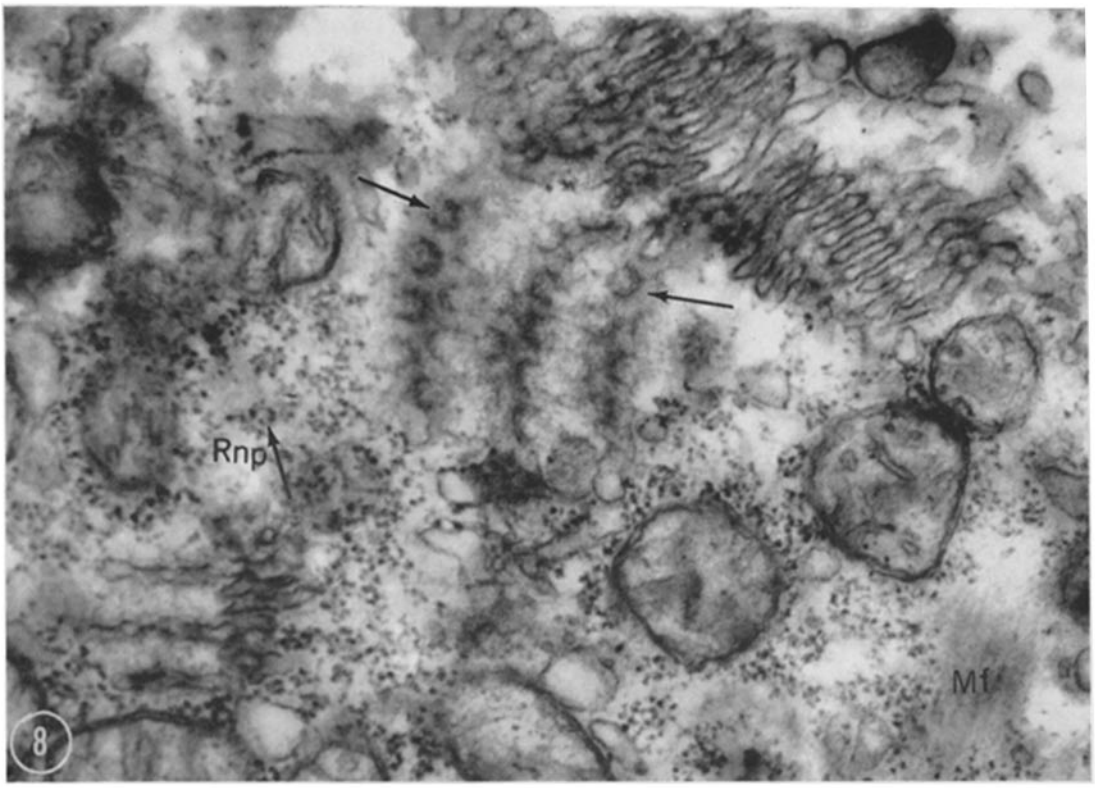

FIGURE 8 Annulate lamellae in myocardium of 90°F incubated chick embryo are sectioned in three planes. The plane of cross-section showing periodicity is at lower left. The face-on view showing pores and microcylinders (arrows) is in the middle. The oblique section is at upper right. × 23,000.

increased numbers of annulate lamellae occurred in the heart, an organ which usually has little rough endoplasmic reticulum. In addition, we have noted annulate lamellae in an ethionine-induced hepatoma which characteristically displays cells in which the rough endoplasmic reticulum is decreased in amount and/or altered. Since in the 90°F incubated chick embryos the total heart weight was increased and the protein concentration on chemical determination remained the same, net protein synthesis was increased. Since this net increase in protein was found in an organ having little rough endoplasmic reticulum, other mechanisms may be involved. Although we have not studied the mechanism of this net increase in protein content, the presence of annulate lamellae may be a morphological indication of abnormal protein synthesis in the heart. Physical agents such as reduced incubation temperature or toxic drugs could affect cellular environment and metabolism during embryonic or adult life. The presence of increased numbers of annulate lamellae and increased concentration of glycogen could represent manifestations of this derangement.

An alternative hypothesis to account for the net increase in protein synthesis might implicate the characteristic basophilia, associated with annulate lamellae, that can be removed by ribonuclease, as is characteristic of RNA. Afzelius (1957) has described toluidine blue-staining, RNA-positive, basophilic heavy bodies, possibly extruded from the nucleus and surrounded by a membrane, that resemble annulate lamellae. Rebhun (1956 *a*), Wischnitzer (1960), Gross (1960), Chambers and Weiser (1964 *b*), and Kessel (1965) have described ribosomes, heavy bodies, or central granules as being associated with, and conferring basophilia upon, annulate lamellae. On the other hand, Ruthmann (1958) and Barer et al. (1960) have reported that the material imparting basophilia to the annulate lamellae was nonparticulate RNA.

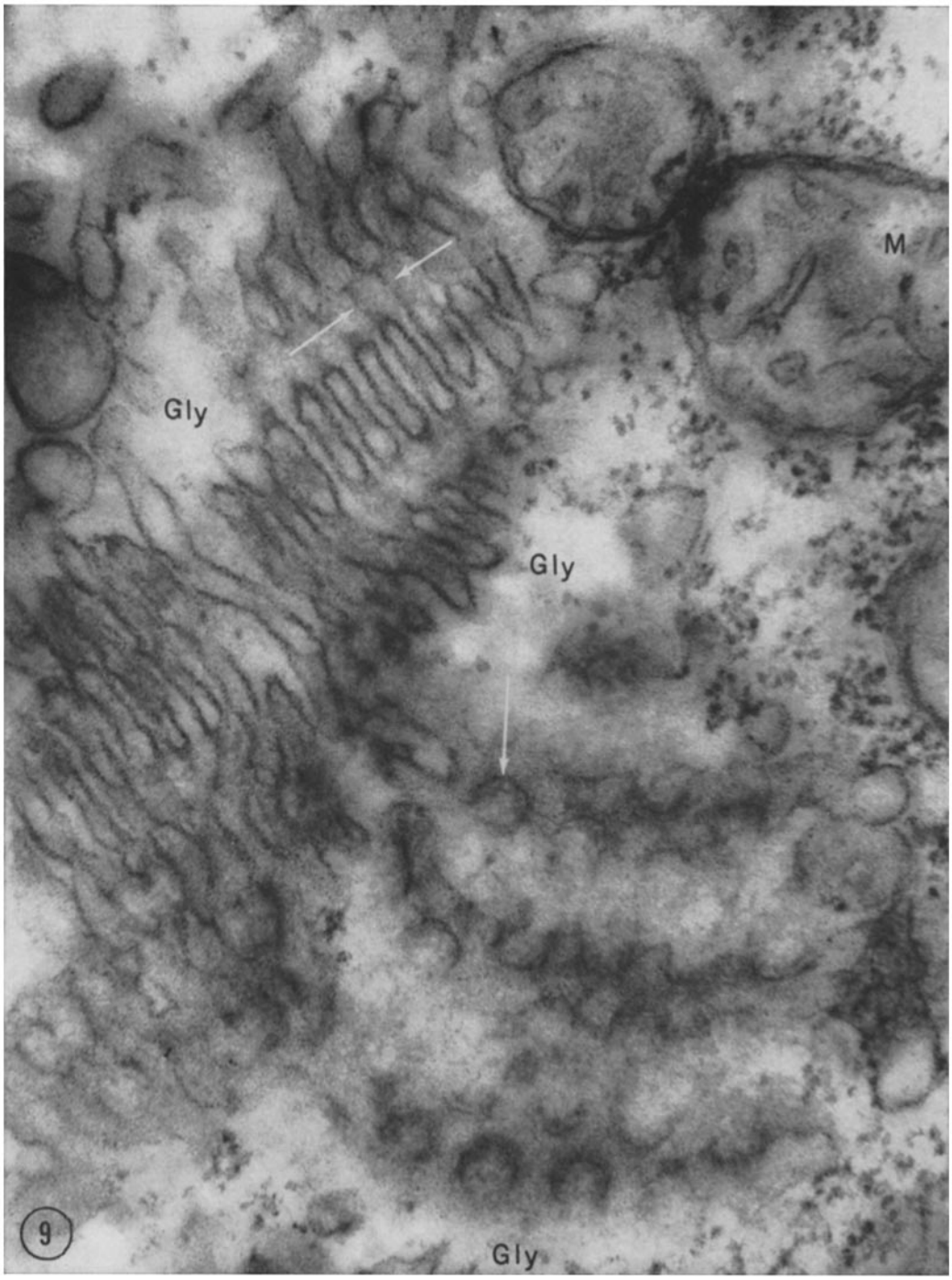

FIGURE 9 Higher magnification of the face-on view and oblique planes of section of annulate lamellae in myocardium of chick embryo incubated at 90°F shown in Fig. 8. Microcylinders (lowest arrow) form the wall of the annulus and have a dense peripheral rim of small granules. The oblique plane of section shows double membranes of the lamellae with similar small, dense granules forming their wall. Note also that the adjacent vesicles and the outer membranes and cristae of the mitochondria exhibit these same granular characteristics. This probably represents staining artefact. In oblique view, the region of the pore does appear to have a faint, less dense granular line across it which may represent a diaphragm or a pore rim (upper arrows). Abundant lightly staining glycogen (*Gly*) is present throughout the cytoplasm. × 102,000.

In our study, the dense granular material between lamellae was usually non-ribosomal (Figs. 1, 3, 6, 7).

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REFERENCES

AFZELIUS, B. A., *Exp. Cell Research*, 1955, **8**, 147.

AFZELIUS, B. A., *Z. Zellforsch.*, 1957, **45**, 660.

BALINSKY, B. I., and DEVIS, R., *Acta Embryol. et Morphol. Exp.*, 1963, **6**, 55.

BARER, R., JOSEPH, S., and MEEK, G. A., Fourth International Conference on Electron Microscopy, Berlin, 1959 *a*, 233.

BARER, R., JOSEPH, S., and MEEK, G. A., *Exp. Cell Research*, 1959 *b*, **18**, 179.

BARER, R., JOSEPH, S., and MEEK, G. A., *Proc. Roy. Soc. London, Series B*, 1960, **152**, 353.

BINGGELI, M. F., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 143.

CHAMBERS, V. C., and WEISER, R. S., *Cancer Research*, 1964 *a*, **24**, 693.

CHAMBERS, V. C., and WEISER, R. S., *J. Cell Biol.*, 1964 *b*, **21**, 133.

EPSTEIN, M. A., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 567.

EPSTEIN, M. A., *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 153.

GAY, H., *Proc. Nat. Acad. Sc.*, 1955, **41**, 370.

GROSS, P. R., PHILPOTT, D. E., and NASS, S., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 135.

HARRISON, G., American Society for Cell Biology, Second Annual Meeting, Abstract, 1962.

HOSHINO, M., *Cancer Research*, 1963, **23**, 209.

HRUBAN, Z., SWIFT, H., DUNN, F. W., and LEWIS, D. E., *Lab. Invest.*, 1965 *a*, **14**, 70.

HRUBAN, Z., SWIFT, H., and SLESERS, A., *Cancer Research*, 1965 *b*, **25**, 708.

HSU, W. S., *Z. Zellforsch.*, 1963, **58**, 660.

KANE, R. E., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 21.

KARNOVSKY, M. J., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 729.

KAYE, G. I., PAPPAS, G. D., YASUZUMI, G., and YAMAMOTO, H., *Z. Zellforsch.*, 1961, **53**, 159.

KESSEL, R. G., *Anat. Rec.*, 1963 *a*, **145**, 363.

KESSEL, R. G., *J. Cell Biol.*, 1963 *b*, **19**, 391.

KESSEL, R. G., *Z. Zellforsch.*, 1964 *a*, **63**, 37.

KESSEL, R. G., *J. Ultrastruct. Research*, 1964 *b*, **10**, 498.

KESSEL, R. G., *J. Cell Biol.*, 1965, **24**, 471.

KING, R. C., and DEVINE, R. L., *Growth*, 1958, **22**, 299.

LANSING, A. I., HILLIER, J., and ROSENTHAL, T. B., *Biol. Bull.*, 1952, **103**, 294.

LEIGHTON, J., MERKOW, L., and LOCKER, M., *Nature*, 1964, **201**, 198.

MAHOWALD, A. P., *J. Exp. Zool.*, 1962, **151**, 201.

MAHOWALD, A. P., *Dev. Biol.*, 1963, **8**, 186.

MCCULLOCH, D., *J. Exp. Zool.*, 1952, **119**, 47.

MERRIAM, R. W., *Biol. Bull.*, 1958, **115**, 329.

MERRIAM, R. W., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 117.

OKADA, E., and WADDINGTON, C. H., *J. Embryol. et Exp. Morphol.*, 1959, **7**, 583.

PALADE, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 567.

PALADE, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.

PASTEELS, J. J., CASTIAUX, P., and VANDERMEERSSCHE, G., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 575.

PORTER, K. R., *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1961, **2**, 621.

REBHUN, L. I., *J. Biophysic. and Biochem. Cytol.*, 1956 *a*, **2**, 93.

REBHUN, L. I., *J. Biophysic. and Biochem. Cytol.*, 1956 *b*, **2**, 159.

REBHUN, L. I., *Ann. New York Acad. Sc.*, 1960, **90**, 357.

REBHUN, L. I., *J. Ultrastruct. Research*, 1961, **5**, 208.

REYNOLDS, E. S., *J. Cell Biol.*, 1963, **17**, 208.

ROSS, M. H., *J. Ultrastruct. Research*, 1962, **7**, 373.

RUTHMANN, A., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 267.

SCHULZ, H., *Oncologia*, 1957, **10**, 307.

STAY, B., *J. Cell Biol.*, 1965, **26**, 49.

SWIFT, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 415.

WADDINGTON, C. H., and OKADA, E., *J. Embryol. et Exp. Morphol.*, 1960, **8**, 341.

WESSEL, W., and BERNHARD, W., *Z. Krebsforschung.*, 1957, **62**, 140.

WISCHNITZER, S., *J. Ultrastruct. Research*, 1958, **1**, 201.

WISCHNITZER, S., *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 558.