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Intercalated discs, nexuses, sarcoplasmic reticulum and transitional cells in the heart of the adult domestic fowl (*Gallus gallus domesticus*)

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INTRODUCTION

The avian heart enables an Arctic tern to fly 11 000 miles from the Arctic to the Antarctic. It permits migrating geese to fly over the Himalayas at a height of 9000 metres (Dorst, 1974) and a sparrow to remain active and alert at a simulated altitude of 6100 metres whilst its mammalian companion of comparable size was lying moribund (Tucker, 1968). Such a heart deserves both respect and comprehensive study, for its own sake and also as an exercise in comparison with the much better understood heart of the mammal. Whilst it may have the former it does not yet have the latter, although a growing number of papers is accumulating on the subject.

In general terms the avian heart is very like that of the mammal. The most obvious macroscopic difference is the powerful, muscular, right atrioventricular valve in the low pressure circuit to the lungs, the significance of which has never been explained. The most obvious ultrastructural difference is probably the complete absence of T-tubules in any of the myocardial cells. This means that there can be no internal couplings, although peripheral couplings between the sarcolemma and the sarcoplasmic reticulum are frequent and may be relatively more numerous than in the mammal.

Probably no more than about half the total mass of the heart consists of contractile muscle cells. They are relatively small, roughly cylindrical and are connected together to form a functional syncytium so that a stimulus originating in one part of the heart will quickly spread to all other parts. Isolated cardiac muscle cells, grown in tissue culture, have an inherent ability to initiate and to maintain a rhythmic contraction. When associated together in the normal heart they form an electrochemical syncytium, the continuity of which is dependent on a number of cell membrane specialisations which develop between contiguous parts of adjacent cells. The macula adherens (desmosome), fascia adherens (myofibrillar insertion plaque) and nexus (gap junction) are the most important of these specialisations. Whilst the macula adherens is found on the cell membrane, both at the intercalated disc and away from it, the fascia adherens and nexus are restricted to that part of the cell membrane which forms the intercalated disc at each end of the cell.

An intercalated disc refers to the interlocking membranes at the ends of adjacent cells, and consists of a series of transverse folded zones, joined together by longitudinal straight parts (Fig. 1). At the folded zones short finger-like processes from each cell interdigitate so that the terminal sarcomeres attach to them instead of to a Z-line. In this way each contractile muscle cell terminates in a series of transverse steps which can be clearly seen in myocytes disaggregated by treatment with collagenase (Powell, Steen, Twist & Woolf, 1978). For the discrete muscle cells to act as a

functional syncytium a low resistance linkage must exist between each cell and its immediate neighbours. It is now widely, but not universally, accepted that this low resistance link is provided by the nexus.

The present contribution describes the intercalated disc and nexus, as well as the sarcoplasmic reticulum and its peripheral couplings with the sarcolemma, in the adult fowl heart. There is also comment on a spectrum of transitional cells which lie between the contractile and conducting cells.

MATERIALS AND METHODS

Six mature domestic fowl (Rhode Island Red × Light Sussex hybrids) were anaesthetised by intravenous injection of a 50:50 mixture of pentobarbitone sodium and 50% urethane. After exsanguination the animals were perfuse-fixed, using 2% paraformaldehyde and 2% glutaraldehyde in 0·1 M-sodium cacodylate buffer containing 3 mM calcium chloride.

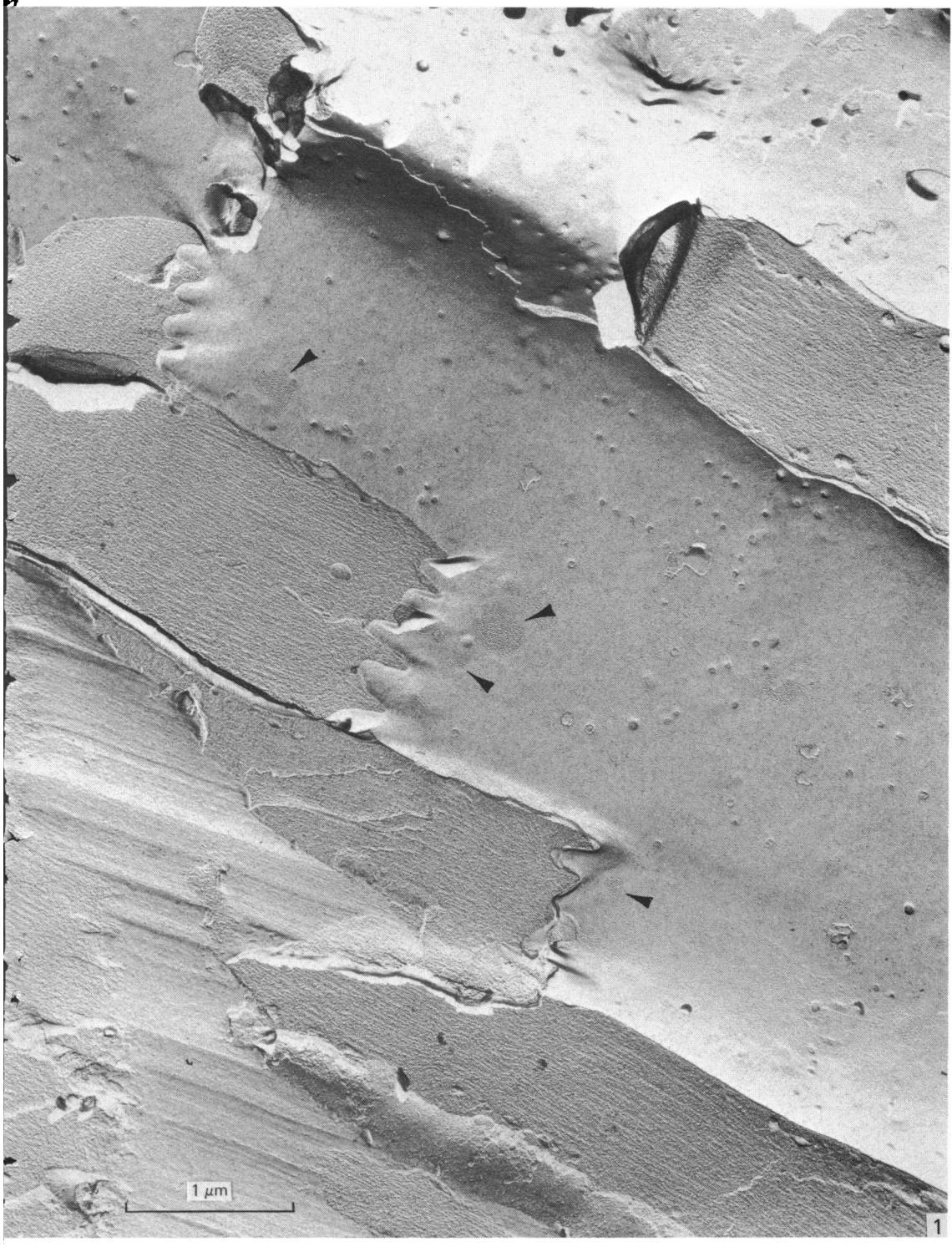
Small pieces of tissue were then taken from various parts of the heart and either cut and stained, or prepared for freeze-fracture replication. Thin sections were stained with 50% methanolic uranyl acetate followed by lead citrate. For freeze-fracture replication the tissue was glycerinated, using 10% followed by 25% glycerol in 0·1 M-sodium cacodylate buffer, quick frozen in liquid nitrogen slush and fractured in a Balzers BAF 300 freeze-fracture plant. Shadowing was by platinum-carbon at 45° and the specimens were examined in a Philips 300 or AEI 6B electron microscope.

RESULTS

The myocytes were about 60 μm long and 6–12 μm wide. A typical intercalated disc consisted of three or four transverse folded zones across the cell at Z-line level, which were connected together by longitudinal stretches of straight sarcolemma (Fig. 1). Each folded zone contained 12 to 18 individual folds (or processes), which protruded from the ends of adjacent cells like fingers. Within each group (or zone) there was usually a staggered row of 8 to 10 dominant processes, with smaller ones at the sides (Figs. 1–5). However, a few folded zones have been seen which were very much smaller than this (Fig. 4*a*) and some that were considerably larger (Fig. 5). The principal processes were fairly uniform in shape and size. They were about 0·5 μm long, with a diameter at the base of about 0·25 μm. On their inner (Protoplasmic-P) surface they were specialised to form the fasciae adherentes, where the actin filaments inserted, and the last sarcomeres of the cell terminated. Presumably by inserting at an angle to the fascia adherens a stronger union was made than if the line of contractile pull had been at right angles.

In the present study small, localised, discrete patches of particles associated with the 'P' face of the split sarcolemma (Branton *et al.* 1975) have been demonstrated, which it is suggested were avian nexuses. They varied somewhat in shape but were roughly, and frequently almost perfectly, circular (Figs. 6, 7). They occurred on the

Fig. 1. Freeze-cleaved replica of intercalated disc from left ventricular wall (domestic fowl). Three transverse steps of folded sarcolemma are shown connected together by longitudinal regions. Each step consists of four or five processes, although there will be others above and below the plane of cleavage. The upper two folded zones are associated with one cell, whilst the lowest step is linked to a different cell. There is a nexus, on the longitudinal part of the sarcolemma, a short distance to the right of each step (arrowheads). The sarcolemma has been split so as to show the inner face of the outer leaflet (E-face).


1 μm
1

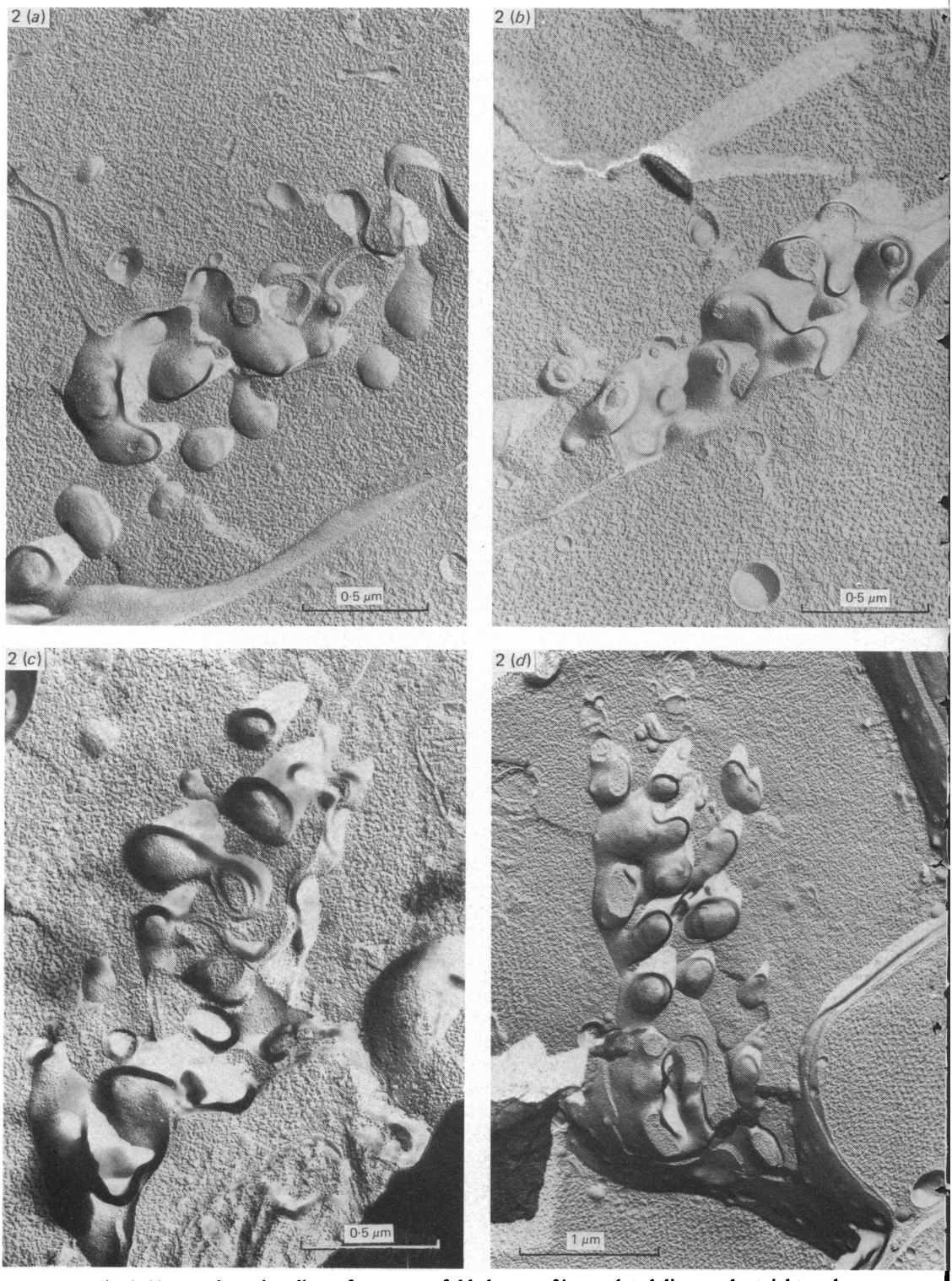


Fig. 2. Freeze-cleaved replicas of transverse folded parts of intercalated discs made at right angles to the long axis of the cells. Each folded zone (step) contains between 15 and 20 processes (left ventricular wall, domestic fowl).

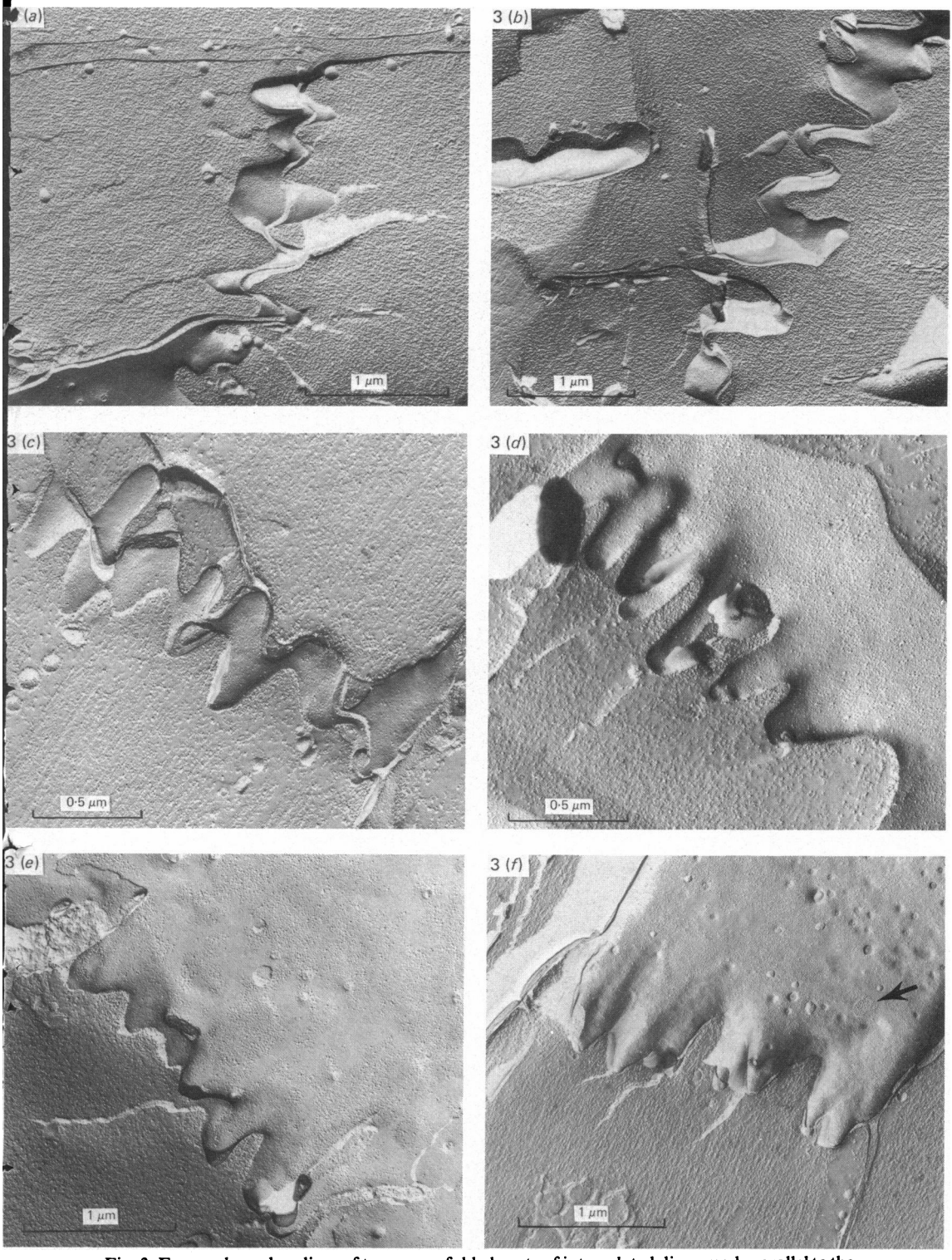


Fig 3. Freeze-cleaved replicas of transverse folded parts of intercalated discs, made parallel to the long axis of the cells (left ventricular wall, domestic fowl). These steps are typical of chicken myocytes and show four to eight processes in the plane of cleavage. The processes are about 0·5 μm long and are commonly subdivided towards their tips (*f*). Only one nexus is shown (arrow).

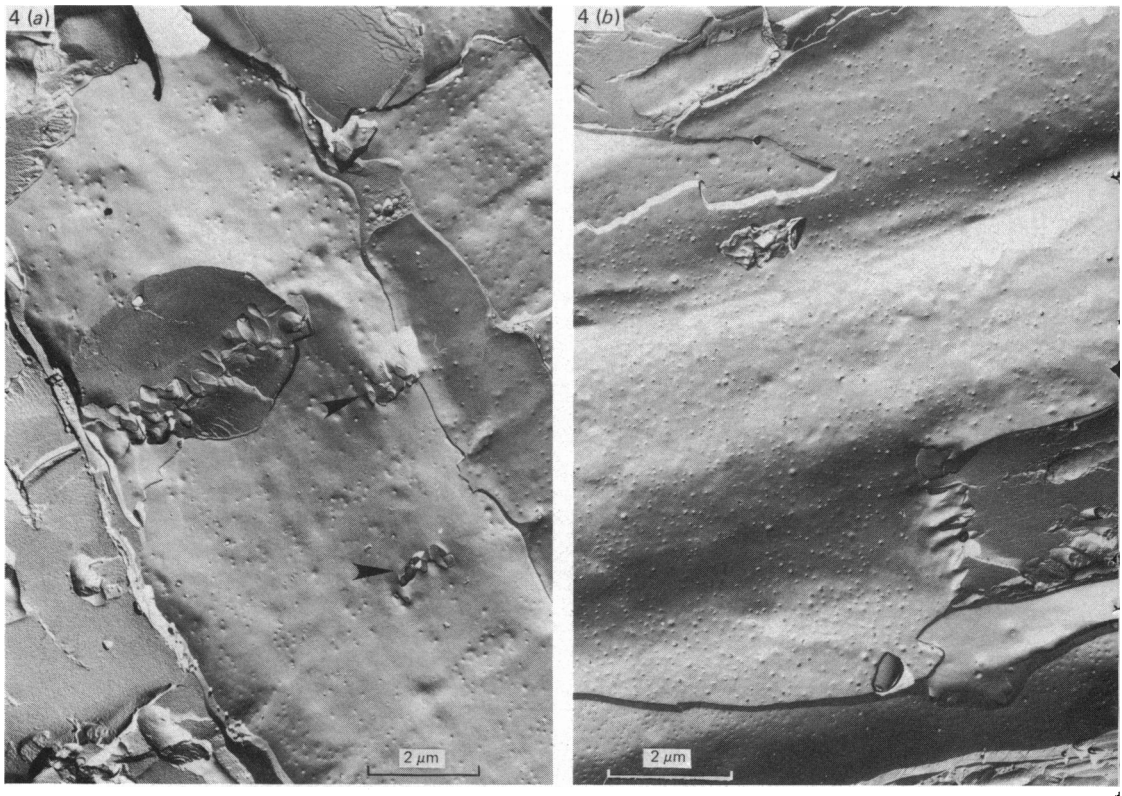


Fig. 4. (*a*) Freeze-cleaved replica made parallel to the long axis of the cell, with a transverse cleavage through one step of an intercalated disc (interventricular septum, domestic fowl). There are three transverse steps, each showing a group of processes; one step (upper left) is very much larger than the other two (centre and lower right, arrowheads).
(*b*) Freeze-cleaved replica made parallel to the long axis of the cell (interventricular septum, domestic fowl) One transverse step of an intercalated disc is shown and there is an extensive area of sarcolemma (E-face) carrying numerous surface vesicles. Many of these have been broken and show only the fractured neck.

straight (longitudinal) part of the intercalated disc, about 200–300 nm away from the bases of the terminal processes. Although many intercalated discs have been studied, only a few extremely small nexuses have been recognised on the folded parts. Since all the nexuses were relatively small the number of particles in them could easily be counted. The largest observed so far on the straight part of the intercalated disc was almost perfectly circular, had a diameter of about 0·33 μm, an area of 0·0872 μm^2 and contained 530 particles (Fig. 7*a*).

The pattern of packing of particles in the nexuses observed during the present study did not show a regular hexagonal array. Instead, the particles seemed to be more randomly packed with occasional lines or strings of them leading towards the nexus, and sometimes continuing within it (Fig. 7*c*). The surface area of four nexuses has been calculated and with a count of their particles a rough idea can be obtained of their packing density. This is shown in Table 1. It would appear that nexuses vary, not only in size and shape but also in the packing density of their membrane particles.

The sarcoplasmic reticulum in the myocytes formed a remarkably regular mesh of

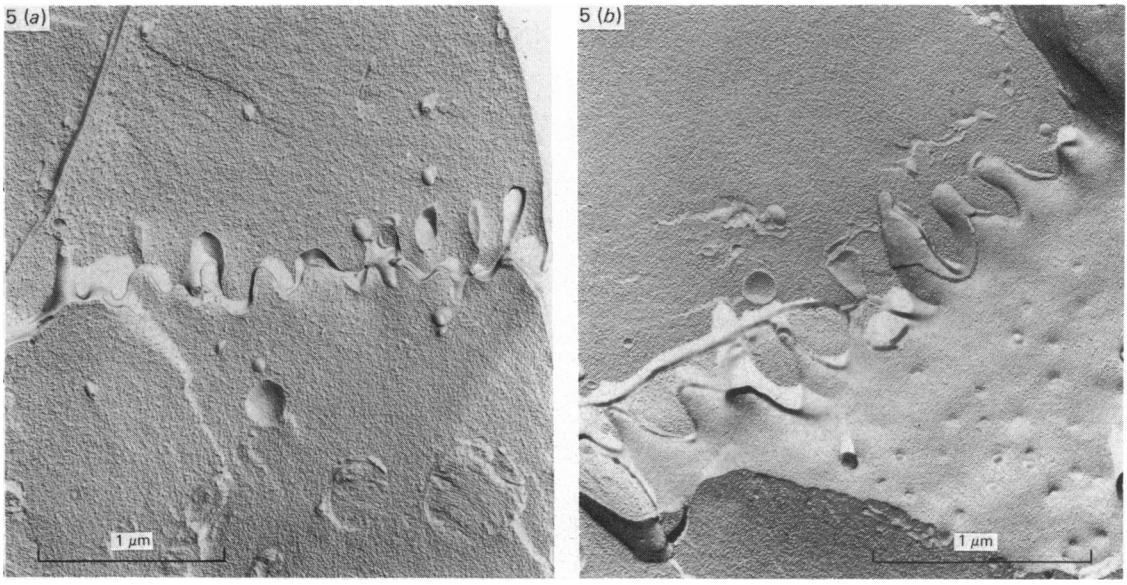


Fig. 5. Longitudinal freeze-cleaved replicas showing the folded zones of transverse steps of intercalated discs (interventricular septum, domestic fowl). These steps are about twice as long as those shown in Fig. 3.

Table 1.

Nexus	Area (μm^2)	No. of particles	Packing density (particles/μm^2)
Fig. 7*a* (small nexus)	0·0069	45	6522
Fig. 7*a* (large nexus)	0·0872	530	6078
Fig. 7*b*	0·0321 (Excluding clear area)	238	7414
Fig. 7*c*	0·0214	195	9112

fine tubes (tube diameter about 70 nm, inside mesh diameter about 200 nm) which lay as a peripheral layer close under the sarcolemma, and which was extended in the form of longitudinal sheets amongst the mass of myofilaments (Fig. 8*b*, *d*, *f*). In this way the myofilament mass was subdivided by sheets of sarcoplasmic reticulum into bundles which in some places were similar in size and shape although in other places were very different. Further extensions of these sheets of sarcoplasmic reticulum surrounded longitudinally aligned groups of mitochondria.

When demonstrated by freeze-cleaved replication the sarcoplasmic reticulum appeared to be restricted to a single layer (Fig. 8*d*). That is, it showed a two dimensional but not a three dimensional pattern of branching. If there had been any three dimensional branching one would have expected to see the broken stumps of those branches which were at right angles to the cleavage plane. In fact, one saw a perfectly smooth, unbroken tubular mesh restricted to the plane of cleavage. Obviously there would be a branch in the mesh when a sheet of it left the subsarcolemmal layer to penetrate the myofilament mass, or to surround a group of mitochondria. Generally, however, the sarcoplasmic reticulum formed a single layered mesh, between longitudinal bundles of myofilaments and mitochondria and spread in a continuous fashion from one end of the cell to the other.

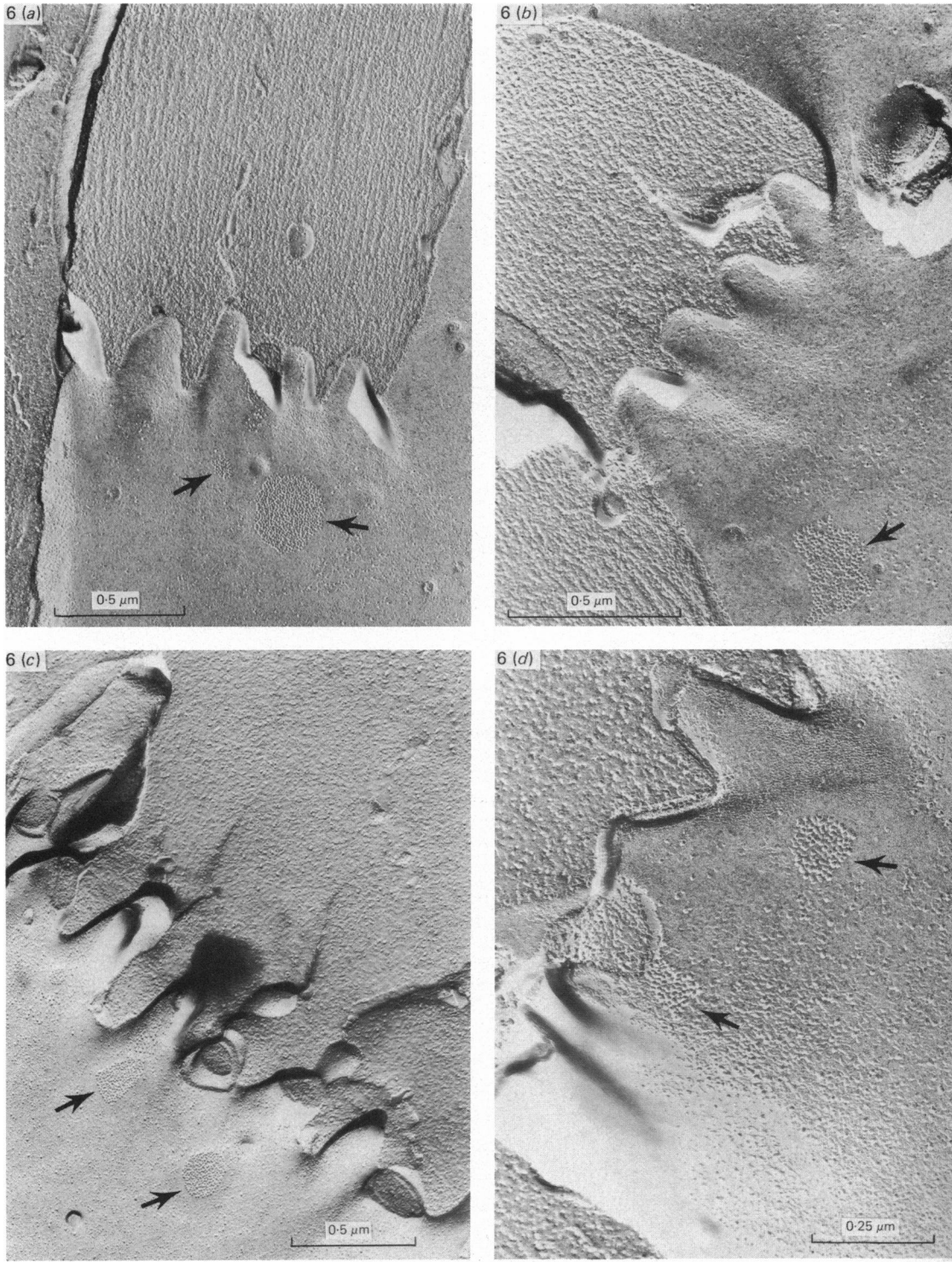


Fig. 6. Freeze-cleaved replicas of transverse folded zones of intercalated discs on the longitudinal parts of the sarcolemma (left ventricular wall, domestic fowl). There are one or two nexuses just before each step of the folded sarcolemma (arrows).

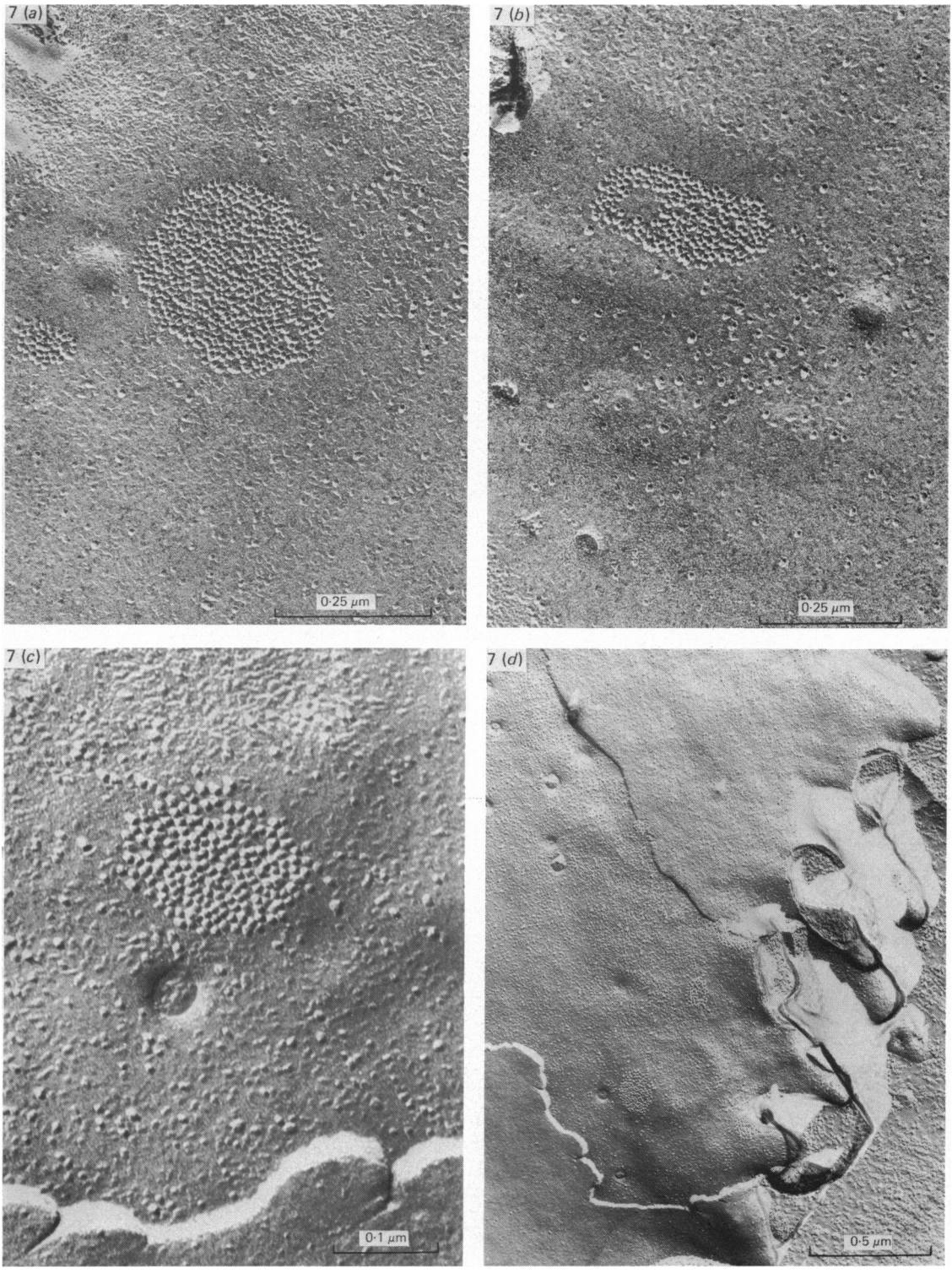
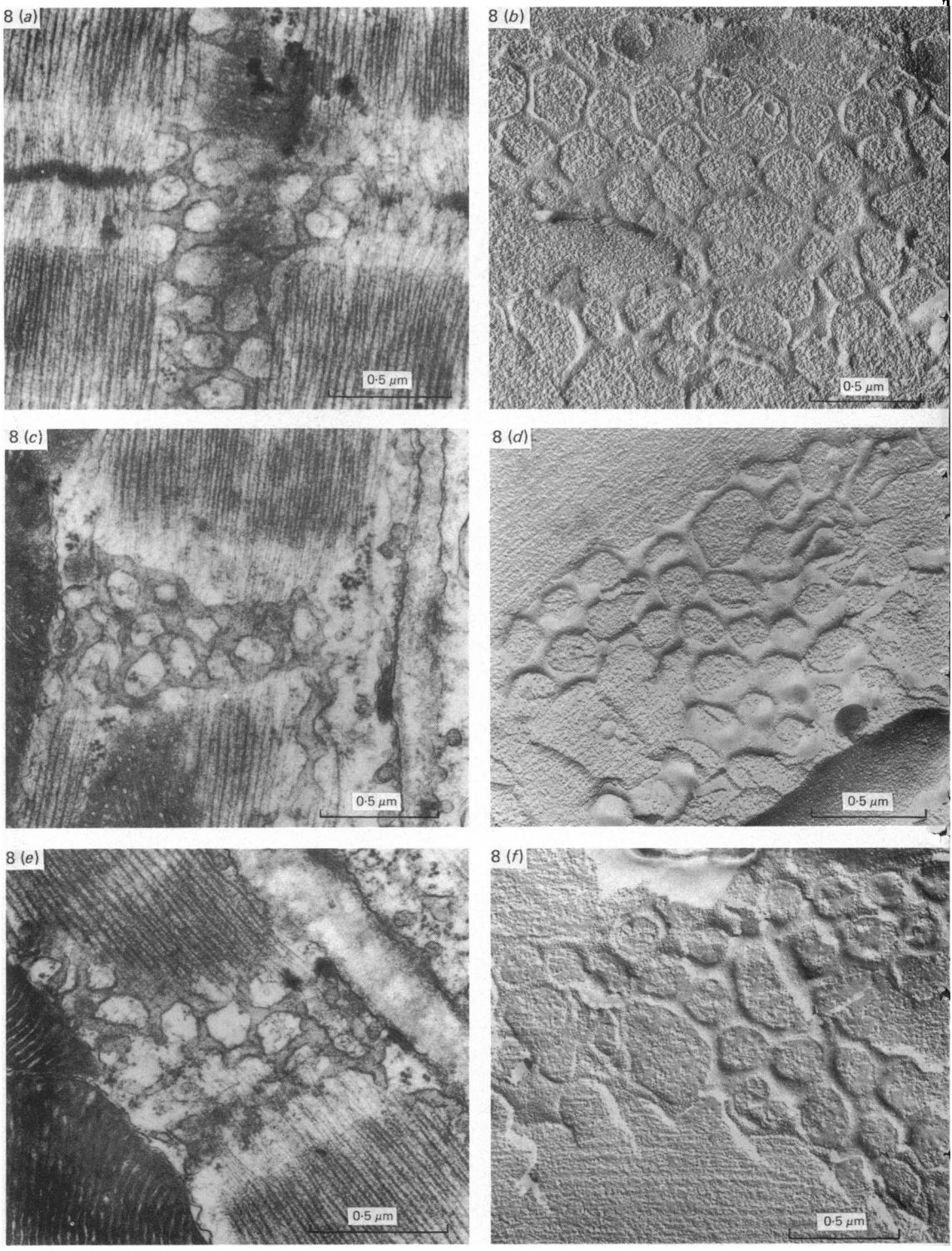


Fig. 7. Detail of nexuses by freeze-cleaved replication (domestic fowl). The size and particle packing density of these nexuses is given in Table 1.


8 (a)
0·5 μm
8 (b)
0·5 μm
8 (c)
0·5 μm
8 (d)
0·5 μm
8 (e)
0·5 μm
8 (f)
0·5 μm

Replicas of quite large sheets of sarcoplasmic reticulum have been obtained. The one in Figure 8*d* is about 2·3 μm long and it was presumed that they were extensive enough to be regarded as representative of the sarcoplasmic reticulum as a whole. They appeared to be completely without fenestrations. The same replicas, in which the sarcoplasmic reticulum spread across more than a complete sarcomere, showed no sign of dilated cisternae.

T-tubules were completely absent from all the cardiac muscle cells, so there could be no internal coupling with the sarcoplasmic reticulum. However, peripheral couplings were numerous in which an extension of the subsarcolemmal layer of sarcoplasmic reticulum approached the sarcolemma more closely, and over a distance of about 0·2 μm showed the modifications that have come to be regarded as a junctional complex (Fig. 9*a*, *b*, *c*). These peripheral couplings were widely scattered over the sarcolemma and were not restricted to a position either side of a Z-line.

Peripheral couplings were not made by terminal branches of the sarcoplasmic reticulum associating with the sarcolemma as blind-ending extensions. Instead, loops of the continuous mesh approached the cell membrane at intervals, formed a junctional complex and then separated from it in order to approach the membrane again a short distance away to form the next coupling. Pairs of couplings were opposite each other in neighbouring cells, suggesting that this may be the basis of a synchronised Ca^{2+} release and hence contractile response in certain parts of the heart (Fig. 9*c*). The freeze-cleaved replica illustrated in Figure 9*d* shows sarcoplasmic reticulum from adjacent cells close to the sarcolemma but there is no sign of any junctional processes so these may not be junctional sarcoplasmic reticulum.

Freeze-cleaved replicas of myocyte cell membrane faces showed with remarkable clarity the large numbers of small surface vesicles that penetrated the outermost zone of the underlying cytoplasm. They had a narrow neck (diameter about 50 nm) and just below the cell surface they dilated to a diameter of about 90 nm. The total depth from the outer surface of the sarcolemma was about 100 nm. Their distribution was not uniform but a typical area of cell membrane might carry 20 vesicles/sq. μm. These vesicles were the only structures that broke the surface of the myocyte. There were no T-tubules nor was any evidence seen for the speculative suggestion by Sommer & Johnson (1970) that tubules of the sarcoplasmic reticulum may, intermittently, unite with the sarcolemma to void their contents by some kind of tubular exocytosis. On the other hand these surface vesicles were frequently to be seen protruding in between the mesh of subsarcolemmal sarcoplasmic reticulum (Fig. 9*e*, *f*) and it is tempting to wonder whether the vesicles may unite with the tubes under certain circumstances, but there is no evidence for this at present.

Conducting cells have been found throughout both atria and ventricles, and even outside the heart in the posterior vena cava which contains cardiac muscle in its wall (Akester, 1971). These cells are very obvious (Akester & Akester, 1971; Scott, 1971) and are mainly characterised by a very small amount of contractile material organised into sarcomeres. However, there would appear to be a complete spectrum of muscle

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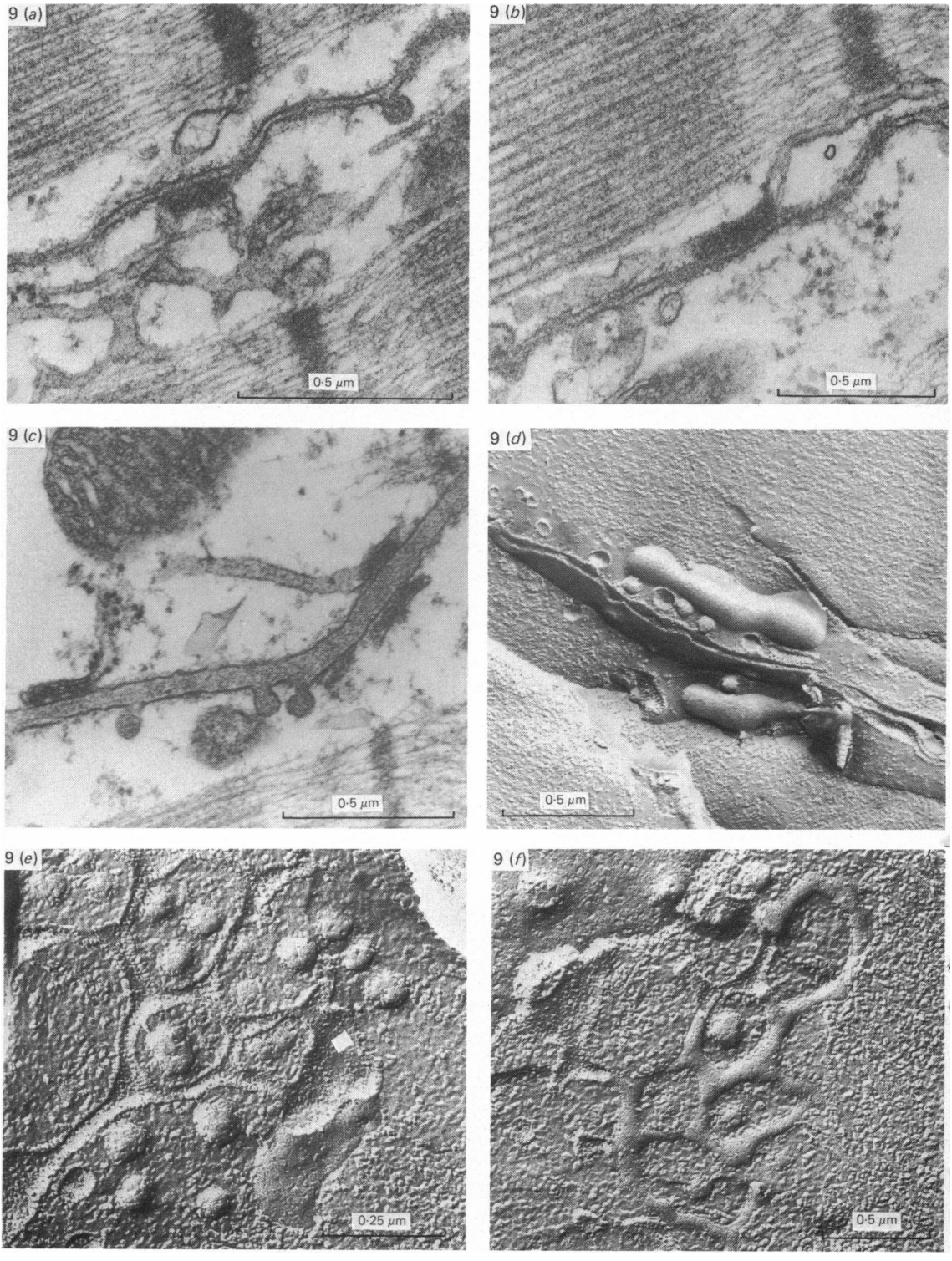
Fig. 8. (*a*, *c*, *e*) Stained section transmission electron micrographs showing sarcoplasmic reticulum passing the Z-line region of myofilament bundles without dilating to form cisternae (left ventricular wall, domestic fowl). It is possible that on the left hand side of (*c*) two coated vesicles are attached to the sarcoplasmic reticulum mesh. There is no sign of fenestration in the sarcoplasmic reticulum. (*b*, *d*, *f*) Regions of sarcoplasmic reticulum demonstrated by freeze-cleaved replication. Each shows part of a sheet lying between bundles of myofilaments. There is no sign of any extended junctional sarcoplasmic reticulum nor are there any fenestrations (interventricular septum, domestic fowl).


9 (a)
0·5 μm
9 (b)
0·5 μm
9 (c)
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9 (d)
0·5 μm
9 (e)
0·25 μm
9 (f)
0·5 μm

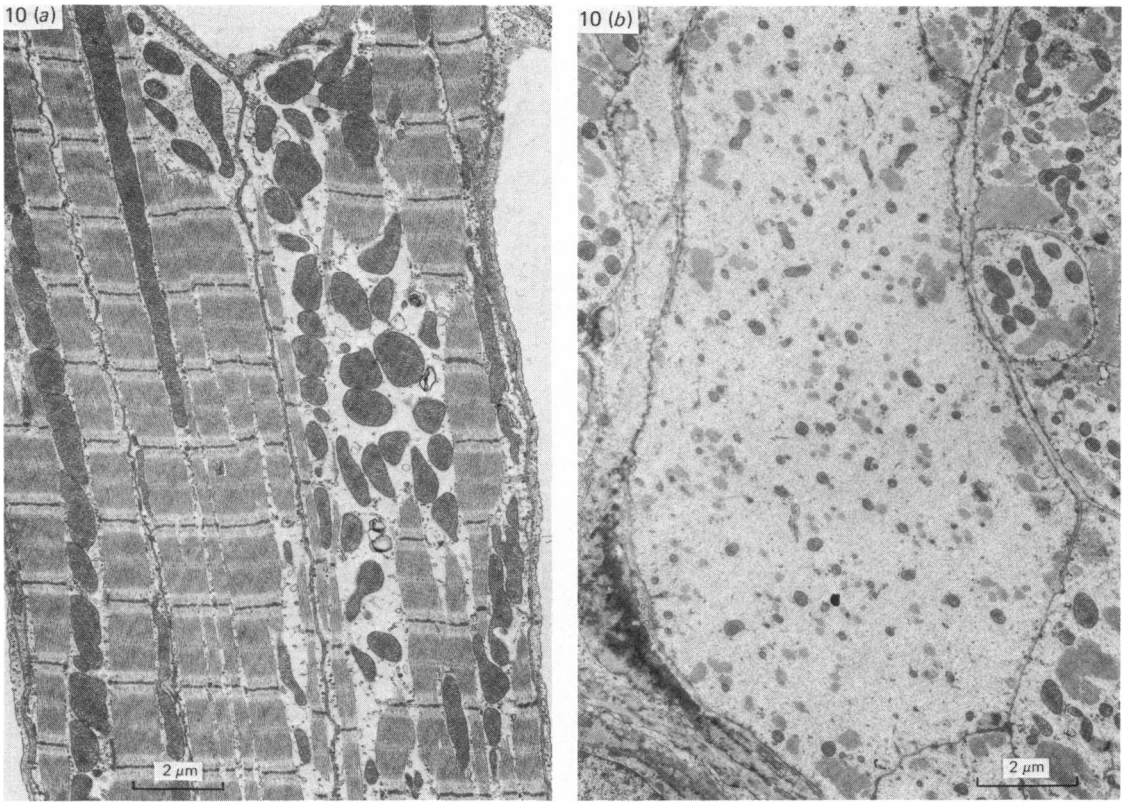

Fig. 10. Stained section transmission electron micrograph to show gradation of cell types (left ventricular wall, domestic fowl). (*a*) shows three transitional cells. They all contain considerable regions of highly organised sarcomeres. At this level of sectioning the left and centre cells contain more than the cell on the right. The cell on the right contains a large area devoid of myofilaments (light area with scattered mitochondria) but the rest of this cell contains much myofilament material organised into sarcomeres. (*b*) Stained section transmission electron micrograph showing a typical conducting cell almost devoid of organised sarcomeres (left ventricular wall, domestic fowl).

cell types in the chicken heart, from the contractile (working) muscle cell packed full of myofilaments highly organised into sarcomeres, at one end of the scale, to the distinctive conducting (Purkinje) cell, at the opposite end, which contains virtually no organised sarcomeres at all (Fig. 10*a*, *b*). It is suggested here that not enough attention has been paid to this population of transitional cells (Akester, 1971). Presumably the relative importance of their dual role, of contraction and conduction, depends mainly on the amount and organisation of the myofilaments they contain.

A transitional cell is one in which some parts of the cell contain highly organised myofilaments, in the form of a continuous series of sarcomeres, with mitochondria longitudinally arranged beside them, while other parts are largely devoid of organised

Fig. 9. (*a*, *b*, *c*) Stained section transmission electron micrographs showing junctional sarcoplasmic reticulum. (*c*) shows junctional sarcoplasmic reticulum forming a pair of couplings opposite one another in adjacent cells. The sarcoplasmic reticulum leading to the coupling at the left side of this illustration carries ribosomes which is unusual (left ventricular wall, domestic fowl). (*d*) Freeze-cleaved replica of sarcoplasmic reticulum in adjacent cells separated by surface vesicles (left ventricular wall, domestic fowl). (*e*, *f*) Freeze-cleaved replicas of subsarcolemmal sarcoplasmic reticulum surrounding surface vesicles (interventricular septum, domestic fowl).

sarcomeres. The latter parts contain fewer mitochondria which are scattered throughout a region containing great numbers of randomly distributed microfilaments. In other words, one part of a transitional cell is indistinguishable from a contractile, working muscle cell whilst the other part is identical with a conducting (Purkinje) cell. At the junctional region between the two parts, the last sarcomeres of the contractile zone peter out amongst the background microfilaments of the conducting zone and do not meet a Z-line. The proportion of these two zones in individual transitional cells varies considerably, hence the suggestion that these cells may form a complete spectrum between the two extremes.

Conducting cells make intercalated discs with other conducting cells, with contractile cells and also with what have been described above as transitional cells. There is a tendency for intercalated discs between conducting cells to be rather more simple and less obviously stepped than is the case with contractile cells. The sarcoplasmic reticulum in conducting cells is much less well developed than in contractile cells, but it still makes peripheral couplings with the sarcolemma.

DISCUSSION

It is now widely accepted that the nexus (gap junction) is the most likely site for a low resistance electrical pathway between cardiac muscle cells and that there may even be protoplasmic continuity (trans-nexus channels) through each of the subunits. Electrical coupling between cardiac muscle cells persists after desmosomes and fasciae adherentes have been separated in a Ca^{2+}-free medium, but it is lost when nexuses are separated in a hypertonic medium (Barr, Dewey & Berger, 1965).

On the other hand, the longitudinal resistivity of cardiac muscle is greatly increased by an increase in the resistance of the extracellular fluid (Ringer solution). If nexuses provide low resistance channels between contiguous cells, one might have expected the longitudinal resistivity of the muscle cells to be largely unaffected by increased resistance in the extracellular fluid (Sperelakis, 1979).

Nexuses have not previously been demonstrated in the chicken's heart by freeze-cleaved replication and it was suggested by Sommer & Johnson (1969) either that they did not exist, or that they were so labile that they dissociated on preparation. According to the same authors (1970), whilst nexuses are frequent in mammalian hearts, "they seem to be rare, small or absent altogether in the chicken". However, Scott (1971), using stained sections, showed that they did exist in the domestic fowl.

There is an obvious similarity between the hearts in these two groups of vertebrates. They overlap in overall size and in beat frequency. If anything, the avian heart may beat more rapidly than that of the mammal of comparable size. Certainly the absence of mammalian-type large nexuses is no disadvantage to the avian heart which has smaller ones and fewer of them. It has been suggested that nexuses are not essential for electrical coupling between cardiac muscle cells of non-mammalian species (Sperelakis, Mayer & MacDonald, 1970), but this suggestion was made before avian nexuses had been recognised.

There is some difference of opinion concerning the most common position of nexuses in the mammalian heart. Sommer, Steer, Johnson & Jewett (1972) found them more frequently on the longitudinal parts of the intercalated disc, whilst McNutt (1970) considered them to be more common on the transverse folded parts. The present study suggests that nexuses in the fowl heart, although smaller and less numerous than in the mammal, are usually to be found on the longitudinal parts of

the intercalated discs. Presumably the few extremely small nexuses that were recognised on the folded parts by freeze-cleaved replication represent the same type of membrane specialisation as was described by Scott (1971) in stained sections.

The observations presented here for the domestic fowl resemble those described by Shibata & Yamamoto (1979) for the sparrow. It would be interesting to know how nexuses are formed and what determines their definitive size. There does not seem to be any obvious relationship between size or number of nexuses on the one hand, and speed of conduction or beat frequency on the other. As has been suggested by Shibata & Yamamoto it does not necessarily follow that the larger nexuses are more efficient than the smaller ones in providing a low resistance pathway between cells.

Presumably nexus particles are largely of a protein nature and are synthesised by the cell for incorporation into the membrane. There is a great deal of lateral movement of macromolecules within the cell membrane, so that particles are free to move and to aggregate into clusters (nexuses). It seems likely that there will be a constant turnover of these particles, so that a disposal mechanism would be required. It is at least possible that the very large aggregations of nexus particles found in the mammal represent an accumulation of 'expended' particles which are being collected together for disposal in some way, and that the smaller nexuses are the more electrochemically active. Body temperature may have something to do with it. The body temperature of birds is several degrees (about 6°F) higher than that of mammals. Turnover of membrane particles may be accelerated so that 'expended' particles are disposed of more rapidly and thus do not have time to accumulate beyond a certain stage in birds.

Nexus particles observed during the present study of the chicken heart have not shown the hexagonal packing which has been described in the mammal. This may be due to shearing forces, during the freeze-cleave preparation, disturbing such an arrangement, or it may indicate that this type of packing was not there in the first place.

It is not known if nexuses develop *in situ* or are formed by the aggregation of isolated particles moving at random, or under 'guidance' within the sarcolemma. It is not known if their position is pre-determined and fixed once they have been formed or whether they move about and so behave something like a log-raft. It is not known how long they last or what their disposal mechanism is: whether larger ones are better able to provide a low resistance pathway or whether smaller ones are the more active. In support of the possibility that they may form by movement and aggregation, is the fact that some nexuses show lines of particles which look as if they might be moving towards the nexus. Of course, they might equally well be moving away from it, but one can detect lines or 'strings' of particles, sometimes following a gentle curve, both within the nexus and also leading towards its outer margin. Further support for this suggestion comes from the fact that some nexuses appear to have a different concentration of particles than others. The four nexuses illustrated in Figure 7*a*, *b*, *c* have approximate concentrations of 6078, 6522, 7414 and 9112 particles per μm^2 respectively (Table 1). Although these calculations must be regarded as being very general, they do appear to show a significant difference in particle concentration. They also suggest that particle concentration in neighbouring nexuses, which may form under similar local membrane conditions, resemble each other more closely than do those taken from widely separated parts of the heart.

It would be very useful, in the study of nexus dynamics, if there were some way to label these particles. If this could be done, possibly in tissue culture, then the size and distribution of nexuses could be studied under variable environmental conditions of

temperature, pressure, gravity and electrical or magnetic fields. If nexuses really are low resistance pathways between cardiac muscle cells, the fact that they occur between liver cells and several types of epithelial cell as well, suggests that they have some other function which, as yet, is not understood.

The sarcoplasmic reticulum has been shown to take a variety of forms in the cardiac muscle cells of different species. Saetersdal & Myklebust (1975) have described dilated cisternae in the sarcoplasmic reticulum of the papillary muscle of the pigeon heart, which occur each side of the Z-line and at the level of the H- and I-bands. Sommer & Waugh (1976) and Jewett, Leonard & Sommer (1973) have described fenestrations in chicken myocyte sarcoplasmic reticulum. Neither of these features were found in the present study.

Another puzzling aspect of the avian heart lies in the complete absence of T-tubules. This is usually explained by the fact that mammalian cardiac muscle cells (with T-tubules) have a greater diameter, which makes it necessary for the sarcolemma to penetrate into the depths of each cell to provide sufficient couplings with the sarcoplasmic reticulum. However, many mammalian atrial contractile muscle cells are said to be without T-tubules. They are also said to be smaller than their ventricular counterparts (McNutt & Fawcett, 1974). Certainly a significant body of opinion has been established in favour of the idea that above a certain diameter, which is given as low as 7 μm by Sommer & Waugh (1976), muscle cells require T-tubules.

However, this is unlikely to be the full explanation. The diameter of mammalian cardiac muscle cells probably ranges from about 10 to 30 μm. Some of the reported measurements are as follows: Sommer, Steer, Johnson & Jewett (1972), 10–15 μm; McNutt & Fawcett (1974), 20–30 μm; Shibata (1977), 5·5–19 μm; Powell *et al.* (1978), 17 μm; Carlson *et al.* (1978), 20–25 μm for isolated cells. Avian myocytes, on the other hand, are about half the diameter of their mammalian counterparts. Some of the reported diameters for avian cardiac muscle cells are as follows: Sommer & Johnson (1970), 2–5 μm; Jewett, Sommer & Johnson (1971), 4–10 μm; Sommer *et al.* (1972), 6–8 μm; Sommer & Waugh (1976), 8–10 μm; Shibata (1977), 3·3–6·3 μm. Those measured for the present study in the chicken vary from 5·5–11·5 μm. It therefore seems very probable that the largest avian myocytes (without T-tubules) are larger than the smallest mammalian cells (with T-tubules).

Sommer & Johnson (1970) have already raised this problem and have pointed out that, although mammalian myocytes are larger than their avian counterparts, both are very much smaller than skeletal muscle cells (diameter about 100 μm) and neither should require an infolding of the sarcolemma in the form of T-tubules. They have even suggested what some might regard as a rather unlikely role for T-tubules in mammalian cardiac muscle cells; that is, that T-tubules have developed as a kind of evolutionary insurance policy against the possibility that the cells may be called upon to undergo a compensatory hypertrophy. Certainly such a hypertrophy in the mammal can cause a most impressive increase in diameter of the individual myocytes, up to about 200 μm according to McNutt & Fawcett (1974). It would be interesting to know whether avian cells can respond in the same way and if they do, whether they still manage to function without developing T-tubules.

The only case of any sarcolemmal invagination occurring in bird's myocytes, so far reported, is by Kelly & Chacko (1977). However, although they (perhaps unfortunately) used the term 'T-tubules', these were formed under most unusual conditions in tissue culture. None the less it is interesting that bird cardiac muscle cells have the ability to form tubular invaginations which even develop internal couplings with

the sarcoplasmic reticulum. It is probably true to say that the real role for T-tubules in mammalian cardiac muscle cells has still to be worked out and that the significance of their absence in birds is no better understood.

Conducting cells are said to have a considerably greater diameter than contractile cells (up to 5 times as great, according to Sommer & Johnson (1970)), but as has been emphasised in this paper, there may be a complete spectrum of cell types blending the characteristics of the two extremes in different proportions. In this way a large conducting cell 'half full' of contractile material might contain more of this material than a 'fully loaded' but smaller contractile cell. In the mammal the former would be without T-tubules whereas the latter would have them. There can be no doubt that active contractile material in the centre of a large avian conducting cell is further from its sarcolemma (without T-tubules) than similar material is from its peripheral sarcolemma in a smaller mammalian contractile cell (with T-tubules).

Not only may there be a complete spectrum of transitional cells, blending the characteristics of conducting and contractile cells, but with the development of more myofilaments by a process akin to hypertrophy, a conducting cell might become more and more like a contractile cell. In fact, the heart may be able to convert conducting cells into contractile cells and *vice versa* through the intermediary of the transitional cell. This facility, if it occurs, may carry some evolutionary potential for response to stress or environmental change. The fact that some of these transitional cells are difficult to recognise with the electron microscope means that they would be quite impossible to see with the light microscope. Routes of electrical conductivity in the chicken heart, between the S-A and A-V nodes may therefore be more complicated than the atrial bundles recently suggested by Mathus & Shrivastava (1979).

Jewett *et al.* (1971) reported that distinct, cistern-like dilations of the sarcoplasmic reticulum were well developed in birds with rapidly beating hearts, like finch and hummingbird, but these were poorly developed and hard to find in the chicken (Jewett *et al.* 1973). These dilations were full of an electron-dense material and were said to occur at the level of the Z-I region in the sarcomeres. They also carried processes identical to those found at peripheral couplings but without any junctional sarcolemma. These dilations in the sarcoplasmic reticulum have been called extended junctional sarcoplasmic reticulum, and their presence in the finch has been confirmed by Bossen, Sommer & Waugh (1978). Similar structures have been described by Saetersdal, Myklebust, Justeen & Olsen (1974) in the pigeon, but although many regions of sarcoplasmic reticulum have been observed at the Z-line level (Fig. 8*a*, *c*, *e*), extended junctional sarcoplasmic reticulum has not been found in the chicken during the present study.

The complete absence of T-tubules in avian myocytes naturally makes internal coupling with the sarcoplasmic reticulum impossible. However, external couplings with the sarcolemma are frequent. Jewett *et al.* (1971) have suggested that these couplings in both mammal and bird occur most commonly on the sarcolemma each side of a Z-line. The present study has shown them to be more widespread than this, but the exact pattern of distribution and concentration per unit area of cell surface is not known.

Further observations are required on the ultrastructure of avian hearts and should include a greater number of species than has been studied to date. It is again suggested that the part played by transitional cells requires much greater study, not only by electron microscopy to determine their distribution, but also by electrophysiological mapping of their action potentials. It may be possible to choose different

types of myocyte for selective tissue culture and the possibility should be borne in mind that one type of myocyte may be able to transform into another. There is still much to be learned about the avian heart.

SUMMARY

Intercalated discs usually consisted of three or four transverse steps of folded cell membrane connected by straight parts running longitudinally.

The transverse folded parts contained a variable number of interlocking processes from contiguous cells. Large processes were frequently flanked by smaller ones.

Nexuses appeared to be better developed on the straight parts of intercalated discs than on the folded parts. They varied in size, shape and particle-packing density.

The largest nexus observed was almost perfectly circular. It covered an area of 0·087 μm^2, contained 530 particles and had a particle-packing density of 6078 particles/μm^2.

The sarcoplasmic reticulum formed a uniform mesh of fine tubes which was distributed in sheets between bundles of myofilaments and longitudinally aligned mitochondria.

Neither extended junctional sarcoplasmic reticulum nor fenestrations in the sarcoplasmic reticulum were observed.

Peripheral couplings were numerous and widespread. They were not restricted to the sarcolemma in the regions of the Z-line, H- or I-bands.

Transitional cells combine the characteristics of contractile and conducting cells in varying proportions.

There is some speculation about the mechanism of nexus formation and the interconvertibility of contractile and conducting cells via the intermediary of the transitional cell.

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REFERENCES

AKESTER, A. R. (1971). The heart. In *The Physiology and Biochemistry of the Domestic Fowl*, (ed. D. J. Bell & B. M. Freeman), vol. II, pp. 745–781. London: Academic Press.

AKESTER, A. R. & AKESTER, B. V. (1971). Some ultrastructural characteristics of conducting cells in the avian heart. *Journal of Anatomy* **108**, 613–619.

BARR, L., DEWEY, M. M. & BERGER, W. (1965). Propagation of action potentials and the structure of the nexus in cardiac muscle. *Journal of General Physiology* **48**, 797–823.

BOSSEN, E. H., SOMMER, J. R. & WAUGH, R. A. (1978). Comparative stereology of the mouse and finch left ventricle. *Tissue and Cell* **10**, 773–784.

BRANTON, D., BULLIVANT, S., GILULA, N. B., KARNOVSKY, M. A., MOOR, H., MUHLENTHALER, K., NORTHCOTE, D. H., PACKER, L., SATIR, B., SPETH, V., STAEHLIN, L. A., STEERE, R. L. & WEINSTEIN, R. S. (1975). Freeze-etching nomenclature. *Science* **190**, 54–56.

CARLSON, E. C., GROSSO, D. S., ROMERO, S. A., FRANGAKIS, C. J., BYUS, C. V. & BRESSLER, R. (1978). Ultrastructural studies of metabolically active isolated adult rat heart myocytes. *Journal of Molecular and Cellular Cardiology* **10**, 449–459.

DORST, J. (1974). *The Life of Birds*, vol. 1, p. 35. London: Weidenfeld & Nicolson.

JEWETT, P. H., SOMMER, J. R. & JOHNSON, E. A. (1971). Cardiac muscle. Its ultrastructure in the Finch and Hummingbird with special reference to the sarcoplasmic reticulum. *Journal of Cell Biology* **49**, 50–65.

JEWETT, P. H., LEONARD, S. D. & SOMMER, J. R. (1973). Chicken cardiac muscle. Its elusive extended junctional sarcoplasmic reticulum and sarcoplasmic reticulum fenestrations. *Journal of Cell Biology* **56**, 595–600.

KELLY, A. M., & CHACKO, S. (1977). Internal membrane junctions and unusual T-tubules in cultured chick cardiac muscle cells. *Journal of Molecular and Cellular Cardiology* **9**, 419–424.

MCNUTT, N. S. (1970). Ultrastructure of intercellular junctions in adult and developing cardiac muscle. *American Journal of Cardiology* **25**, 169–183.

MCNUTT, N. S. & FAWCETT, D. W. (1974). Myocardial ultrastructure. In *The Mammalian Myocardium*, (ed. C. A. Langer & A. J. Brody). New York: John Wiley & Sons.

MATHUS, R. & SHRIVASTAVA, A. (1979). Cardiac conducting system of *Gallus domesticus* with special reference to the atrial bundles. *Acta morphologica neerlando-scandinavica* **17**, 181–189.

POWELL, T., STEEN, E. M., TWIST, V. W. & WOOLF, N. (1978). Surface characteristics of cells isolated from adult rat myocardium. *Journal of Molecular and Cellular Cardiology* **10**, 287–292.

SAETERSDAL, T. S., MYKLEBUST, R., JUSTEEN, N-P. B. & OLSEN, W. C. (1974). Ultrastructural localization of calcium in the pigeon papillary muscle as demonstrated by cytochemical techniques and X-ray analysis. *Cell and Tissue Research* **155**, 57–74.

SAETERSDAL, T. S. & MYKLEBUST, R. (1975). Ultrastructure of the pigeon papillary muscle with special reference to the sarcoplasmic reticulum. *Journal of Molecular and Cellular Cardiology* **7**, 543–551.

SCOTT, T. M. (1971). The ultrastructure of normal and Purkinje cells of the fowl heart. *Journal of Anatomy* **110**, 259–273.

SHIBATA, Y. (1977). Comparative ultrastructure of cell membrane specializations in vertebrate cardiac muscle. *Archivum histologicum japonicum* **40**, 391–406.

SHIBATA, Y. & YAMAMOTO, T. (1979). Freeze-fractured studies of gap junctions in vertebrate cardiac muscle cells. *Journal of Ultrastructure Research* **67**, 79–88.

SOMMER, J. R. & JOHNSON, E. A. (1969). Cardiac muscle. A comparative ultrastructural study with special reference to frog and chicken hearts. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **98**, 437–468.

SOMMER, J. R. & JOHNSON, E. A. (1970). Comparative ultrastructure of cardiac cell membrane specializations. A review. *American Journal of Cardiology* **25**, 184–194.

SOMMER, J. R., STEER, R. L., JOHNSON, E. A. & JEWETT, P. H. (1972). Ultrastructure of cardiac muscle. In: *Hibernation and Hypothermia, Perspectives and Challenges* (ed. T. E. South, J. P. Hannon, J. R. Willis, E. T. Pengelley & N. R. Albert). Amsterdam–London–New York: Elsevier Publishing Co.

SOMMER, J. R. & WAUGH, R. A. (1976). The ultrastructure of the mammalian cardiac muscle cell – with special emphasis on the tubular membrane systems. A review. *American Journal of Pathology* **82**, 191–232.

SPERELAKIS, N. (1979). Propagation mechanisms in heart. *Annual Review of Physiology* **41**, 441–457.

SPERELAKIS, N., MAYER, G. & MACDONALD, R. (1970). Velocity of propagation in vertebrate cardiac muscles as functions of tonicity and $(K^+)_o$. *American Journal of Physiology* **219**, 952–963.

TUCKER, V. A. (1968). Respiratory physiology of house sparrows in relation to high-altitude flight. *Journal of Experimental Biology* **48**, 55–66.