niques. Excised mycetomes were either smeared with a needle on a microscope slide and stained with Giemsa fluid, or fixed in Bouin fluid, microtomed into 7 µm sections and stained with Mallory stain for microscopic examination. Mycetomes, dissected under sterile 0.5% NaCl-solution, were incubated in cultures of *Lactobacillus plantarum* and *Streptococcus faecalis*. The test bacteria were inoculated in vitamin assay media containing all necessary growth factors exept patothenic acid and folic acid for *L. plantarum* and *S. faecalis* respectively. By microbiological assay, it was found that the symbionts produce these and other vitamins of the B-group[5].

At the International Atomic Energy Authority, Vienna, tsetse flies were fed on difibrinated horse blood mixed with 25 ppm, 250 ppm and 2500 ppm of oxytetracycline through a silicone-membrane and were sent for further investigation. While there was no difference in longevity after treatment with 25 ppm dosage as compared to the normal flies, higher concentrations always caused increased mortality. LD_{50} for 250 ppm was 18 days, while that for 2500 ppm was 3 days. No offspring were produced in all 3 groups of flies. Microscopical examination proved that the symbionts were severely damaged in all the cases.

A second group of tsetse flies was fed daily on rabbits that were treated with the coccidiostat Sulka (Pharmazeutisches Werk Cuxhaven), containing sulphaquinoxaline in a final concentration of 75 ppm. After 1 and 2 week periods, symbionts in the flies were examined and found to show signs of destruction. Microbiological assay of the mycetomes revealed drastically low amounts of pantothenic acid and folic acid.

In a present research-program on the significance of symbiosis in tsetse flies, lysozyme was applied orally to eliminate the symbionts. It was possible to reduce the dosage of lysozyme so that longevity of the flies was not affected, yet fertility was completely eliminated. Destruction of the symbionts could be proved both by microscopical and microbiological tests.

From these results, it can be concluded that symbionts play an important rôle in tsetse fly reproduction. It is interesting that oxytetracycline does not effect any follicle larger than about 1/4 of its mature size[2]. Recently described rickettsia-like symbionts located in the ovaries of tsetse flies[6], that may also be effected by bactericide drugs, have not been examined in this investigation. Preliminary studies on the significance of endosymbiosis in tsetse flies show that the symbionts provide their hosts with certain vitamins of the B-group[5]. In mosquitoes, where endosymbionts are absent, the reaction of sulphaquinoxaline was antagonized by simultaneous administration of *p*-aminobenzoic acid[7]. In the current experiments with aposymbiotic tsetse flies, it was possible partly to compensate the loss of symbionts by diets supplemented with different B-vitamins.

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Early Development of Gap Junctions Between the Mouse Embryonic Myocardial Cells. A Freeze-Etching Study[1]

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Summary. Using the freeze-etch technique, nexuses have been shown to exist at a very early stage in developing mouse hearts (10 dpc). At this time they are rare, but become more progressively frequent and extensive at 12 and 14 dpc. Special arrangements of particles progressively observed on the fracture faces PF (linear arrays, small associated groups of linear arrays, then hexagonal arrays with 'arms' formed by linear clusters) suggest that in ontogenesis the gap junctions may be built up by successive aggregation of the linear arrays of particles.

Nexus-type junctions or gap junctions, believed to be the sites of low intercellular electrical resistance[3], are common and sometimes extensive in mature mammalian and avian cardiac musculature, particularly in Purkinje fibres. The structure has been described in detail by MCNUTT[4] and MCNUTT and WEINSTEIN[5,6] using both thin sections and freeze-cleave preparations. However, these junctions are rare in early embryonic cardiac muscle, although MUIR[7] observed a few nexuses in the 14-day post coitum (dpc) rat embryonic myocardium. PAGER's[8,9] observations confirm the studies of MUIR[7], and in addition she found 'contact points' (contacts ponctuels) between cardiocyte membranes at the 11th day of the embryonic life in the rat. In embryonic mouse heart, the earliest stage at which gap junctions have thus far been reported is 13-days post fertilization[10,11]. In chick embryonic heart, SPIRA[12] found close oppositions (a 4 nm gap) between cells and freeze-etch studies[13] have also demonstrated small nexuses in chicken myocytes. In addition, gap junctions have been observed in the 9-week human fetal ventricular cardiac muscle[4].

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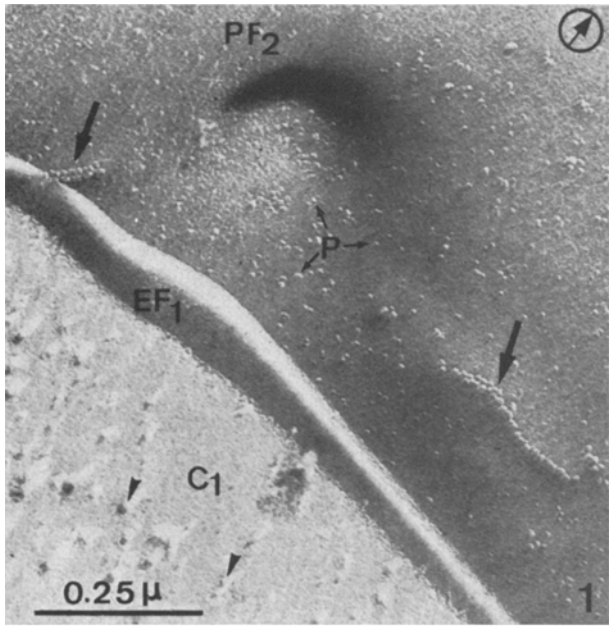
As part of a more extensive study of the ontogenesis of the cardiac musculature, freeze-cleave specimens have been obtained from hearts of embryonic mice with a view to finding the earliest stage at which nexus junctions can be seen, and also to obtain information on how they are formed.

Material and methods. 10, 12 and 14 days pc embryonic hearts were obtained from pregnant mice (ALAS strain). The ventricles were dissected in a physiological salt solution (130 m*M* NaCl, 2.7 m*M* KCl, 2.2 m*M* $CaCl_2$, 1m*M* NaH_2PO_4, 11.9 m*M* $NaHCO_3$, 0.25 m*M* $MgCl_2$, 2 g/l glucose), then immersed successively, for 20 min, with 10, 20, and 30% glycerol in physiological salt solution. The specimens were then immersed for 8–10 sec in liquid Freon 22 cooled with liquid nitrogen. Freeze-etching was carried out using a Balzer BA 360 M freeze-etch device. Following cleavage, samples were sublimated at −100 °C for 1 or 2 min. Replicas were carbon-platinum shadowed and examined using a Siemens Elmiskop I a or Hitachi HU 11 Cs electron microscope. Magnifications were calibrated using a germanium-shadowed carbon replica (54,864 lines per inch).



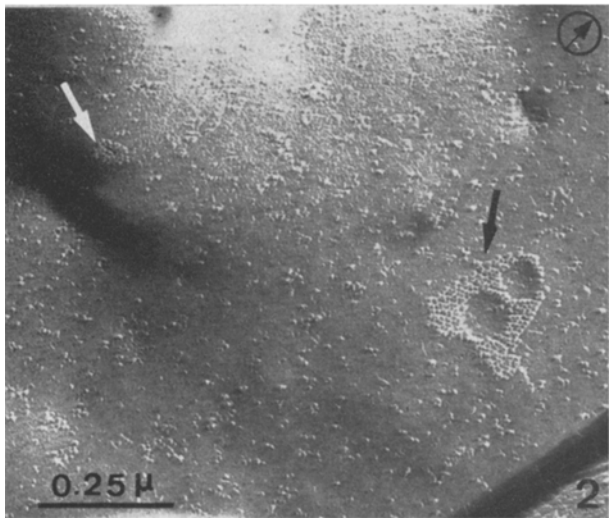
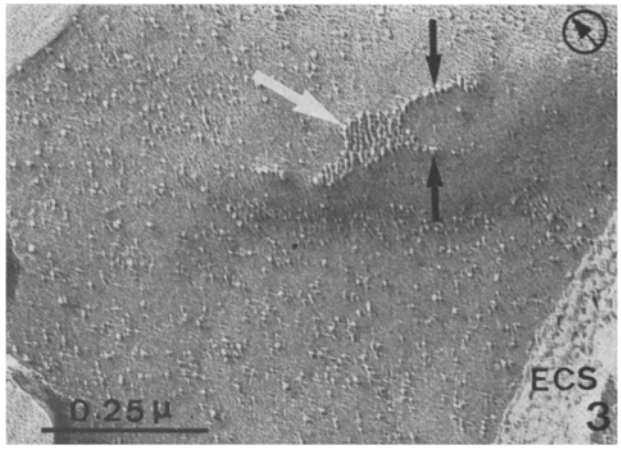


Fig. 1. 10 dpc embryonic heart. The cleavage plane has passed from the cytoplasm (C_1) of one cell (1) and has followed the plasma membrane, revealing face EF. After passing through the membrane, the cleavage plane penetrates the plasma membrane of a second cell (2) revealing face PF of the membrane. The fracture face PF_2, studded with particles (P) distributed at random, shows linear arrays of particles (right black arrow) and aggregates of linear arrays (left black arrow). Small head arrows indicate myofilaments protruding from the cytoplasm of the cell 1. Encircled arrow indicate the direction of platinum shadowing. ×90,000.

Fig. 2. 10 dpc embryonic heart. Fracture face PF of a myocardial cell displaying 2 gap junctions (arrows). The larger gap junction shows 2 zones containing only a few particles. Close examination of the nexus marked by the black arrow shows the particles to be hexagonally arrayed. ×72,000.



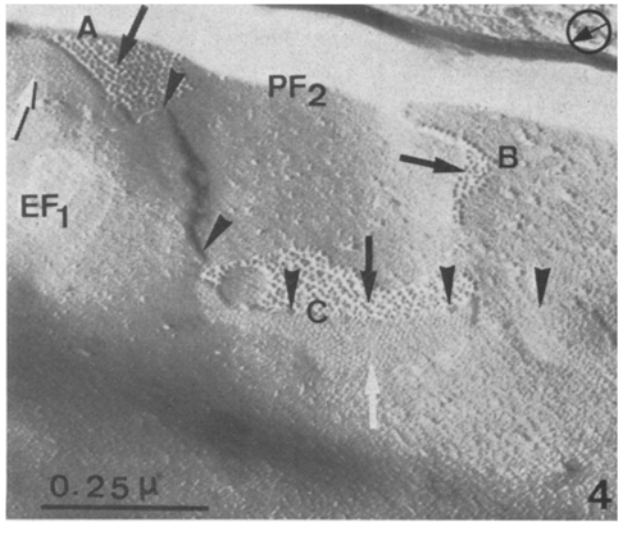


Fig. 3. 12 dpc embryonic heart. Fracture face PF of a myocardial cell. The gap junction (white arrow) presents 2 'arms' formed by linear arrays of particles. ECS: extracellular space. ×90,000.

Fig. 4. 14 dpc embryonic heart. The cleavage plane has broken through the plasma membrane of a first cell (1) revealing face EF, then has passed through the plasma membrane of a second cell (2) revealing face PF. The edge of the fracture face EF is marked with black head arrows. 3 nexuses (A, B and C) are visible in a face view, and for 2 of them (A and C) it is possible to distinguish both faces PF (black arrows) and faces EF (white arrows). Face EF of the gap junction B has been cleaved away. Nexuses faces EF (white arrows) contain closely arrayed pits while faces PF (black arrows) are studded with particles. ×90,000.

Results and discussion. Freeze-cleaving splits plasma membranes producing 2 fracture faces[14,15]: face PF (or A) directed toward the exterior of the cell, and face EF (or B) directed toward the cytoplasm[16]. Fracture faces PF and EF of embryonic myocardial cell plasma membranes are studded with many 6–10 nm particles (as measured in replicas) distributed at random (Figure 1).

Thus far the investigation has shown the earliest signs of nexus formation to be at 10 dpc. At that time the faces of PF of the plasma membranes frequently show linear arrays of particles (consisting of 6 to 20 particles) and sometimes aggregates made up of 2 or 3 rows of linear arrays (Figure 1). Much less frequently, one can observe small clusters of particles in hexagonal arrays with a 9–10 nm center-to-center spacing with the corresponding fracture face EF showing corresponding arrays of 35–50 nm depressions (Figure 2). These structures are characteristic of nexuses. Sometimes, nexuses faces PF (averaging 50 to 90 nm in width), show, in the central areas, one or two approximately circular zones containing only a few widely spaced particles (Figure 2). At 12 dpc both linear and hexagonal arrays of particles are found on the faces PF and both are more common than at 10 dpc. The smallest nexuses, usually oval or circular in form, sometimes have 'arms' formed by one or two rows of particles (Figure 3). At 14 dpc, on the fracture faces PF, linear arrays or particles have become rarer, whereas the hexagonal arrays are now relatively numerous. Their size (on average 250 nm in the greater length) is also larger than at 10 and 12 dpc. The nexuses faces EF appear studded with small pits, hexagonally arrayed (Figure 4).

The establishment of nexuses at this very early stage suggests that this type of intimate intercellular contact with low electrical resistance may be an important feature in the development of the heart as a whole, particularly in the establishment of good electrical contact for the passage of the depolarizing impulse.

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Localization of Wheat Germ Agglutinin Receptor Sites on Yeast Cells by Scanning Electron Microscopy

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Summary. WGA receptor sites on the surface of *Saccharomyces cerevisiae* cells pretreated with an α- mannanase were localized by gold granules labelled with WGA. The receptor sites were found on the bud scars, the mother cell-bud junction (chitin) and the bud, but not on the mother cell.

Recently we have developed a new technique for the visualization of cell surface receptor sites by scanning electron microscopy (SEM) using gold granules labelled with antibodies[1] We report here the localization of wheat germ agglutinin (WGA) receptor sites on the cells of *Saccharomyces cerevisiae* by SEM. WGA is a lectin (MW 26 000) whose combining site is complementary to sequences of β-(1→4)-N-acetyl-D-glucosaminyl units[2,3] which in budding yeasts are predominantly found in the chitin of the bud scars[4–7]. However, a significant amount of glucosamine is also found elsewhere in the cell wall[5,6,8] as in the mannan protein. The main constituents of *S. cerevisiae* cell walls are a β-glucan (49%) and the α-mannan-protein (40%)[5] whose polysaccharide moiety is in the outer layer of the wall[9]. The cell wall mannan is attached through N-acetyl-β-D-glucosamine (possibly in the form of di-N-acetylchitobiose) to asparagine units of the protein[10].

WGA receptor sites appeared on the surface of yeast cells only when the cells were first treated with an α-mannanase known to cleave most of the α-(1→2)- and α-(1→3)-linked side chains of *S. cerevisiae* mannan[11].

Experimental. Labelling of gold granules. Gold granules could not be labelled and stabilized against flocculation with WGA[1] due to its too low molecular weight. WGA was therefore cross-linked to bovine serum albumine (BSA). WGA (L'Industrie Biologique Française, 1 mg) and BSA (4 mg) were dissolved in 0.005 *M* NaCl (0.25 ml) and the solution was neutralized to pH 7 with 0.2 *N* K_2CO_3. The proteins were cross-linked with 0.25% glutaraldehyde (0.05 ml)[12]. After 2 h at 25 °C, 0.005 *M* NaCl (12.2 ml) was added.

Colloidal gold granules (Sol. I) of a size suitable for SEM[1] (50 nm) were prepared according to Frens (Sol C of ref.[13]). The optimum amount of protein necessary to label 5 ml of Sol I was determined by adding diluted WGA-BSA solutions (1 ml). After 1 h at 25 °C, too small an amount of protein caused agglutination of the colloid, seen as a decrease of the absorbance at 540 nm.

Gold granules (Sol II) of a size suitable for TEM (5 nm) were prepared according to FAULK and TAYLOR[14]. The optimum amount of protein necessary to label 5 ml

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