those colonies composed of the plump, spindle-shaped cells exhibited fusion and myotube formation. The melanocyte and fibroblastic colonies remained unchanged following the change of medium.

A second subculture was initiated 30 days later. In addition to passage cultures, 20 dishes were plated in GM-I at a density of 4×10^3 cells per petri dish. By 21 days these subcultures had macroscopic colonies (30 to 40 per plate) which were either fibroblastic or "myoblastic" in morphology. At this point ten of the cultures were switched to GM-II. Within 7 days the "myoblastic" colonies exhibited myotube formation, while all cultures remaining in GM-I failed to form myotubes. When the latter cultures were terminated (58 days after subculture), those colonies, originally designated as being "myoblastic," were composed of large, elongate mononucleate cells. Thus these cells which had been carried in culture for a period of 104 days without any overt expression of muscle, could form muscle when plated in a permissive medium.

Other components of the regenerate and mature tail have also been cultured. The ependymal tube, for example, has been cultured in both media as: (i) dissociated cells; (ii) partially dissociated explants of the tube and its associated connective tissue; and (iii) organ cultures on Millipore filters. When grown as dissociated cells, the ependyma forms large plaques of monolayered cells. Intact ependymal tube growing in association with its connective tissues retained its tubular morphology and underwent branching (Fig. 1E).

In a number of the pooled cultures of early regenerates, some wound epithelial cells were inadvertently included. These gave rise to colonies which expanded over the underlying multilayer of mesenchymal cells and produced sheets of keratinized cells for a period of 60 days. Cultures of dissociated mature cartilage tube have been carried in GM-I for a total of 4 months, during which they have been subcultured four times. During this period they have shown no decline in growth. Attempts to obtain overt differentiation of these cells has so far been unsuccessful as judged by studies of S^{35} incorporation and metachromasia after Azure-A staining.

These results, although preliminary, demonstrate the feasibility of conducting both short- and long-term studies on the components of the lizard-tail regenerate in vitro. With these techniques one may be able to approach many of the problems of regeneration that have thus far eluded analysis.

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Reserpine: Effect on Structure of Heart Muscle

Abstract. *We examined the ultrastructure of hearts from dogs given reserpine intramuscularly for 4 days, and from untreated dogs. Sections of the myocardium from treated dogs invariably revealed mitochondrial abnormalities at the 5th and 14th days. These included fragmentation and loss of structure of the cristae, and cyst formation. The appearance at 25 days in the treated as well as in all the untreated dogs was normal. We concluded that reserpine in the dose used produces marked structural changes in the mitochondria of heart muscle, and that these changes are reversible. These changes may account for the myocardial depression sometimes seen after administration of reserpine.*

We have found evidence of impaired myocardial function in dogs given a total of 100 μg of reserpine per kilogram of body weight in divided doses over 4 days (*1*). Uncoupling of oxidative phosphorylation in heart mitochondria has been reported to occur after administration of large doses of reserpine (5 mg/kg) (*2*). In this study we examined the ultrastructure of the heart to see if there were changes in the mitochondria which might explain our own findings.

Twelve dogs weighing 7 to 29 kg were given intramuscular injections of 25 μg of reserpine (Serpasil, Ciba) per kilogram of body weight per day for 4 days (days 1 to 4). Specimens of myocardium were obtained on either the 5th day (six dogs), 14th day (three dogs), or 25th day (three dogs). There were six untreated dogs.

The dogs were anesthetized with intravenous injections of sodium thiopentone and a mixture of nitrous oxide and oxygen (2 : 1). A midline thoracotomy was performed. In nine dogs a fine nylon catheter was introduced into the anterior descending branch of the left coronary artery near the apex, pushed in a retrograde direction for about 1.5 cm, and tied in place. Of these, four were untreated and five had been given reserpine (three at 5 days, one at 14 days, and one at 25 days after treatment). Perfusion was begun immediately with a freshly prepared solution of 0.1 percent osmium tetroxide in 0.1*M* phosphate buffer (*p*H 7.45) and 0.02 percent calcium chloride, at a pressure of 150 to 180 mm-Hg. As the perfusion was begun the artery was clamped just above a bifurcation about 2.5 cm proximal to the end of the catheter, and in this way only a small area of the myocardium was perfused; this area received a normal blood supply until the instant the perfusion was begun.

Perfusion was maintained for 3 minutes; while the heart was beating strongly an area of muscle changed color as blood was replaced by the fixative. A small strip of muscle was excised from this area and immediately placed in 1 percent osmium tetroxide in Palade's buffer (*3*), cut into cubes of approximately 1 mm^3, and rapidly transferred to fresh osmium tetroxide (1 percent) in Palade's buffer. After 2 hours at 5°C we removed the fixative by washing the muscle in tap water.

In the remaining nine dogs (two untreated and seven treated) fixation by

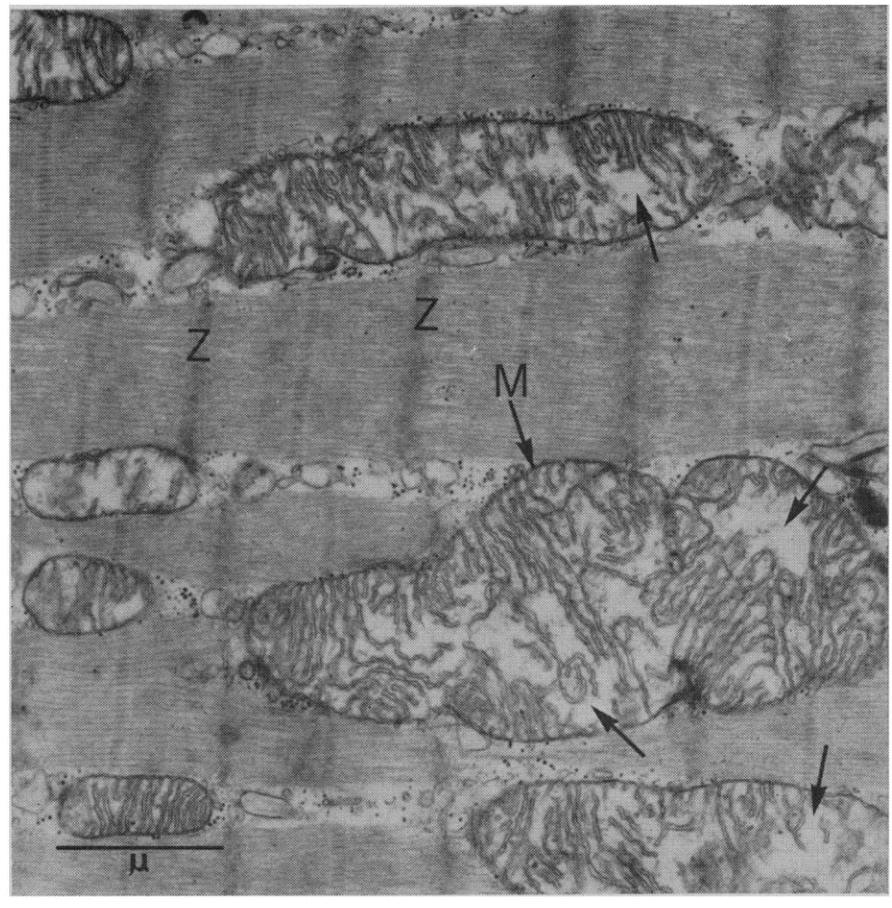

Fig. 1 (right). Changes in the mitochondria of the left ventricle on the 5th day after treatment with reserpine. There is extensive fragmentation of cristae in each of the mitochondria (*M*). Structureless areas and cyst-like spaces can be seen. Arrows indicate cystic areas. The sarcomeres appear normal. Two adjacent Z-lines are shown.

perfusion was not used. For these the fixative was ice-cold glutaraldehyde (6.25 percent) and osmium tetroxide (1 percent) in 0.1*M* collidine buffer (*p*H 7.3) as described by Trump (*4*). Specimens were dropped into fixative within 10 seconds of removal, immediately cut into cubes of 1 mm^3 and transferred to fresh fixative, which we removed after 1 hour by washing the tissue in 0.1*M* collidine buffer. The samples were from identical sites in all dogs.

After dehydration, the muscle bundles were infiltrated for 24 hours with araldite and added accelerator. Final embedding was carried out in fresh araldite which was polymerized at 60°C for 36 to 60 hours. The resulting blocks were sectioned on an LKB ultratome. Sections were picked up on grids coated with nitrocellulose and carbon and stained with lead hydroxide solution or lead citrate. They were examined on a Philip's EM 200 electron microscope or a Siemens Elmiskop I at 60 kv.

The mitochondria were abnormal in all sections taken from dogs examined at 5 days and at 14 days after treatment with reserpine. The mitochondria were normal in appearance in the dogs examined 25 days after treatment and in all the untreated dogs. The nuclei, myofibrils, and other intracellular structures were normal in both treated and untreated dogs.

A representative example of the changes seen in the mitochondria at 5 and 14 days is shown in Fig. 1. This is contrasted with Fig. 2 which shows the appearance of the mitochondria found in the controls and in the dogs examined at 25 days. On the 5th and 14th day nearly all the mitochondria showed changes in the cristae, which were fragmented and frequently destroyed leaving structureless areas containing the remains of cristae. In some mitochondria there was a patchy distribution, with normal looking cristae adjacent to abnormal areas. In others the whole mitochondrion appeared abnormal, containing cyst-like spaces and fragmented cristae. The changes on the 14th day appeared quite as severe as those found on the 5th day. By light microscopy no abnormalities could be detected at 5 and 14 days.

The dogs themselves were not greatly affected by this dose of reserpine, though all dogs were less active for a few days and mild looseness of stools occurred. Serum electrolytes measured on the 5th and 14th days were normal. The dogs killed on the 14th and 25th days appeared normal by the 9th day;

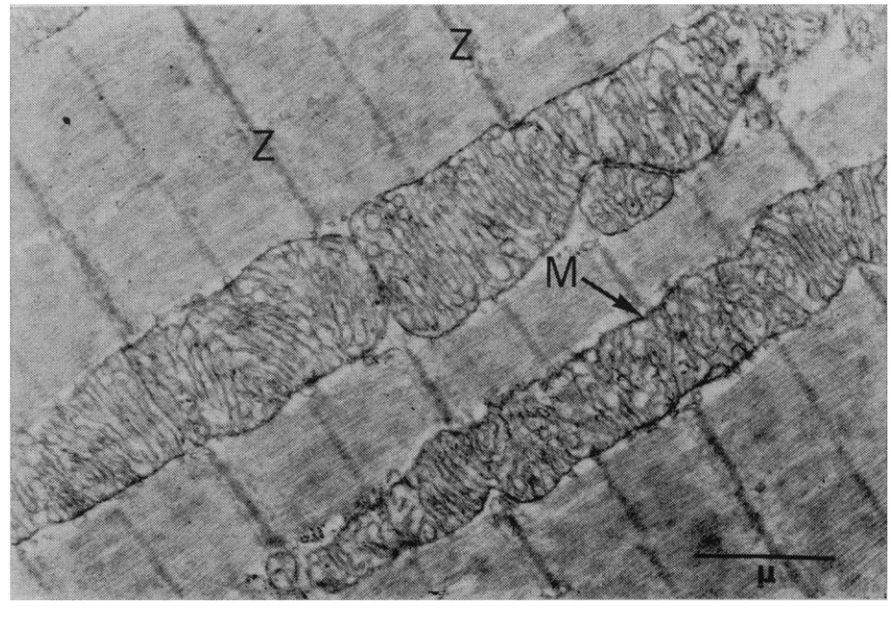

Fig. 2. (right). Normal mitochondria (*M*) from the left ventricle of an untreated dog. There is no evidence of disruption of the cristae. *Z*, Z-line.

their appearance was in no way different from that before treatment.

The changes we found in the mitochondria of the treated animals were gross and, as far as we are aware, have not been described previously. Zaimis (*5*) described chronic inflammatory cell infiltration and focal necrosis in the heart after long-term administration of reserpine; these changes were acute after large doses (1 mg/kg). These seem to be additional and possibly unconnected changes probably caused by the larger amounts of reserpine used. In our study changes were confined to the mitochondria, and the muscle appeared normal by light microscopy.

Schwartz and Lee (*2*) found approximately 25 percent uncoupling of oxidative phosphorylation in heart mitochondria of cats and guinea pigs 24 hours after treatment with 5 mg of reserpine per kilogram body weight. In our study the dose of reserpine used per unit weight was not greatly in excess of that which may be used clinically.

It seems likely that depression of myocardial function would accompany the changes we have described. Such depression has been found by some investigators (*6*) but not by others (*7*). It also seems possible that the depletion of catecholamines caused by reserpine might be due, at least in part, to an action of the drug on the mitochondria of sympathetic nerves. It is known that noradrenaline is formed in the nerve cell body and then transported down to the nerve terminals (*8*). However, we were unable to identify any nerve tissue in our sections, and it is therefore not possible to say whether the mitochondria of the adrenergic nerves supplying the myocardium were similarly affected.

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Role Differentiation in Copulating Cicada Killer Wasps

Abstract. *Copulating male and female cicada killer wasps have distinct behavioral roles independent of reproduction. Males terminate copulation, and females initiate the copulatory flight in which the pair in copulation escape potential danger. Separation and escape behavior are mutually exclusive. Separation occurs because the female clings to the substrate and fails to join the male in his frequent attempts at flight; thus he eventually pulls free. Escape occurs when the female begins flight, which the male readily joins. Differences in thresholds for flight probably largely determine both roles. There appears to be an evolutionary balance in escape and separation behavior determined by the behavior of the female, and illustrative of behavioral homeostasis. The female remains still in the presence of mild stimuli, such as attempted male flights, and thereby aids in separation; she initiates escape in the presence of strong stimuli such as potential enemies.*

A 10-year field study (1956 to 1966) of cicada killer wasps *Sphecius speciosus* was conducted in New York. Seventy-nine copulating pairs were observed (*1*). The male mounts the female in a manner similar to that described for the copulation of other sphecid wasps such as *Bembix* (*2*), *Ammophila* (*3*), *Mellinus* (*4*), *Oxybelus* (*5*), *Stictia* (*6*), or *Tachyspex* (*7*), but upon insertion of the genitalia he immediately dismounts and faces in the opposite direction, thereby assuming a linear position (*8*, *9*). Copulation is lengthy; six copulations seen in entirety ranged from 29 to 51 minutes, with a mean of 37 minutes. Separation of the pairs was observed 13 times, and the male broke the connection by attempted flight with one possible exception (Fig. 1). The possible exception involved attempted flights by the male, but in the actual separation the male may have withdrawn without flight. Such "spontaneously" occurring attempts at flight by the male (separation behavior) begin early in copulation, even seconds after insertion of the genitalia. Consequently most of the copulation period involves or is primarily devoted to disengaging behavior.

In the cicada killer, genitalia that are difficult to disconnect are hypothesized to have evolved as a consequence of selection for genitalia that are never dislodged by the frequent pouncings of conspecific males on couples (*9*). Frequent interference occurs because cicada killers are highly gregarious; colonies may exceed 900 individuals (*9*, *10*). Lengthy copulation, however, probably occurs because the couple has difficulty in disconnecting, an apparently "undesirable" consequence of conflicting selection pressures in the aforementioned evolution. Lengthy copulation and difficulty in separation probably influenced the evolution of special roles of copulating males and females. Males, being smaller and weaker, were probably preadapted to the separator role, using the heavier counterweight of the females to pull against. The larger females were probably preadapted to the escaper role because of their greater strength which enables them to pull males behind them in the copulatory flight in the event of danger. The actively flying male supports its own weight in flight but does not appear to fly backward. The copulatory flight is usually elicited by movement or tactile interference of conspecific males or humans. It probably evolved as an escape mechanism used during the long and vulnerable copulation period (*9*).

The copulatory flight occurred in all 24 cases in which the female was observed to attempt flight, but there were no separations. Flight attempts by males were considerably more common; however, in 69 recorded attempts, females never joined the males but clung to the substrate, apparently resisting their pull. There were 13 separations. When females began flight, males appeared to "cooperate" fully. They did not cling to the substrate or