tolytic enzymes. This viewpoint has been restated more recently (*2*). As far as can be ascertained from the literature, there appears to be no direct experimental evidence that loss of ability by a soft rot bacterium to produce pectolytic enzymes has resulted in loss of pathogenicity. The purpose of the present communication is to report evidence bearing on this aspect of the host-pathogen relationship.

Pseudomonas marginalis (Brown) Stevens, strain P1 pathogenic for lettuce, was selected for study. The bacterium was isolated originally from witloof chicory (*Cichorium intybus* L.) in 1949 (*3*). Cell-free culture filtrates of the pathogen are now known to cause soft rot, russet spotting, and vascular browning of lettuce and to contain protopectinase (or macerating enzyme complex), pectin depolymerase, and pectinmethylesterase (*4*).

Bacterial suspensions of *P. marginalis* in water were irradiated with ultraviolet light (15-watt General Electric germicidal lamp) at a distance of 22.5 cm for varying periods of time up to 10 minutes. Cells surviving exposure were plated out on yeast extract-nutrient agar, and 17 single-colony isolates were tested for pathogenicity. Ten of these showed complete inability, in repeated tests, to infect leaves of witloof chicory and head lettuce (*Lactuca sativa* L.) following needle inoculations with cells grown on yeast extract-nutrient agar.

Five isolates chosen at random from the ten avirulent isolates failed in repeated tests to produce pectolytic enzymes in cultures of lettuce broth and Uschinsky broth, even though they formed heavy, fluorescent growth in the broths. Under similar conditions the pathogenic P1 strain exhibited protopectinase, depolymerase, and pectinmethylesterase activity. Furthermore, the addition of 0.15-percent Seitz-sterilized pectin to an Uschinsky broth culture of an avirulent isolate (strain M4) failed to induce the formation of pectolytic enzymes. Although the mutants have been in culture over 6 months and have been transferred at approximately biweekly intervals, they have not reverted to a virulent state, and they have not produced pectolytic enzymes in culture.

To ascertain whether the avirulent M4 and the virulent P1 strains differed in other respects, the two were compared morphologically, culturally, and physiologically by means of standard methods (*5*). The strains were found to be similar with respect to characteristics previously reported for P1 (*3*), except for their reactions in synthetic broths containing 0.15- to 0.5-percent sodium pectate or sodium polygalacturonate or 1-percent sucrose as the sole carbon source. The avirulent strain M4 failed to ferment these substances, whereas the virulent P1 strain produced alkali from the pectic substrates and acid from sucrose.

Recently it was reported that ultraviolet irradiation of the soft rot bacterium *Erwinia aroideae* produced some specific nutritional mutants which showed a loss of pathogenicity resulting from the inability of the mutants to find on the host tissue the required nutrilites for growth (*6*). Five avirulent strains and strain P1 of *P. marginalis* were grown in minimal broth and minimal agar to determine whether a nutritional mutation was involved in these cultures also. For this purpose eight different, simple synthetic broths [including Davis and Mingioli broth and Entner and Stanier broth (*7*)] and four agar media were used. Inorganic salts or asparagine were used as nitrogen sources, while sodium acetate, glycerol, dextrose, or asparagine were used as carbon sources. Noble (Difco) or washed agar were used for the solid media. Cells washed with 0.8-percent sodium chloride were used to inoculate the media. In all cases, the growth of the five avirulent strains on the minimal media was comparable to that of the virulent parent strain, indicating that a nutritional mutation was not involved.

It is known that the presence of inhibitory substances in plant tissue may affect the host-pathogen relationship (*8*), but in the present study unheated, blended lettuce extracts did not inhibit the growth of the avirulent M4 strain in culture. In addition, it is recognized that there are saprophytic species of *Pseudomonas* and other microorganisms which are able to form pectolytic enzymes in culture but are unable to invade plant tissue (*9*). In the case of these saprophytes, failure to cause disease must be due to some mechanism other than the inability to produce pectolytic enzymes. Although pectolytic enzymes play a decisive role in soft rots of plants, it is quite possible that other enzymes play some role in pathogenesis by *P. marginalis*. In the present study, however, assays for other enzymes were not made.

In summary, the results in the present study of the soft rot pathogen *P. marginalis* indicate that loss of pathogenicity by the radiation-induced mutants is genetic in nature and is linked with their inability to form pectolytic enzymes and with their consequent inability to attack the pectic substrates present in inoculated host tissues.

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Intracellular Impulse Conduction in Muscle Cells

Abstract. A hypothesis, suggested previously by morphological studies, for impulse conduction from the sarcolemma to the contractile material via the sarcoplasmic reticulum is discussed. The relation of reticulum morphology and cell size to speed of contraction in smooth and striated muscle agrees with the hypothesis and thus supports it. Additional support comes from evidence concerning an unusual morphological relationship between the sarcolemma and contractile fibrils in striated muscle of amphioxus.

Studies of the fine structure of cells by recently developed physical means, such as electron microscopy, have stimulated the formulation and study of hypotheses of the mechanisms of various cellular functions. The worth and durability of these hypotheses will depend ultimately, of course, on sound physiological demonstration that the mechanisms are in reality those described by the hypotheses. It is valuable in the meantime, however, to formulate hypotheses, provided that they suggest new lines of experimental investigation. Such investigation, when carried out, will lead to validation or rejection of the hypothesis.

It is the purpose of this report to discuss a hypothesis of intracellular impulse conduction in muscle cells that has been suggested by cytological observations recently made with the electron microscope. As is shown, the hypothesis is justified in that it brings the previously paradoxical time-distance relationships in muscle into proper order and suggests investigation directed toward the demonstration of the presence of intracellular membrane potentials and depolarization phenomena.

The paradox referred to is as follows: Striated muscle cells, which average 50 to 100 μ in diameter, contract very quickly and across the entire diameter of the cell very soon after the action potential has passed over the cell membrane. Smooth muscle cells, on the other hand, which average 6 to 10 μ in diameter, contract slowly, and parts of the cell come into play only at relatively long

times after the cell membrane action potential has passed. It is immediately obvious that the relationship between cell size and delay time is reversed from what one would expect if the contraction-inducing impulse were to pass from the cell surface to the contractile elements by the same mechanism in each case, and if, of course, the other events, such as the activation of the contractile material, required the same time in both striated muscle cells and smooth muscle cells. This forms the paradox referred to above.

The possibility of the diffusion of a substance from the cell surface toward the central axis of the cell has been considered by Hill as a possible mechanism of impulse transfer (*1*). Hill shows, however, that diffusion would be too slow to account for the rapid sequence of events in striated muscle cells (*2*). This does not, however, rule out the possibility that this mechanism could act in smooth muscle cells. Indeed, the paradox suggests that the over-all mechanism is not the same in striated and smooth muscle cells, and our calculations show that diffusion times over distance of the order of the radii of smooth muscle cells (3 to 5 μ) fall well within the limit of observed delay times for smooth muscle. The problem then becomes one of finding an alternate mechanism which is coupled to both the cell surface and the contractile elements and which is fast enough to account for the short delay time in striated muscle.

The mechanism discussed here is based on electron-microscope studies of striated muscles of several types (*3–5*) that rediscovered a system of the sarcoplasm which presumably corresponds to a system described in light microscope images by Thin in 1874 (*6*).

The major characteristics of this system, called the "sarcoplasmic reticulum," are that it is a continuous, membrane-limited, reticular system whose structural organization repeats longitudinally and synchronously in parallel with the bands which form the sarcomeres, and which is continuous laterally between the fibrils across the whole diameter of the cell. It is closely associated with the external membrane of the cell on the one hand, and with the myofibrils on the other. Indeed, the latter association is characterized in vertebrate skeletal muscle by a special differentiation opposite the I-band level of each sarcomere, where contraction presumably is initiated (*7*), and points to a probable role in the contractile function of the cell.

It has occurred to several authors, in discussing the sarcoplasmic reticulum (*3, 5, 8*), that it is possible that this system could serve to transmit the excitatory impulse intracellularly. One possibility is that the membranes forming the sarcoplasmic reticulum are electrically polarized in a manner similar to the polarization of the muscle and the external membrane of the nerve cell. Since the sarcoplasmic reticulum divides the sarcoplasm into two compartments which communicate only *through* a membrane, it seems likely that the two phases thus delineated differ in type or activity of chemical species, or in both, and therefore the presence of a chemical and electrical potential gradient seems not only possible, but probable.

Assuming this to be true, we can further propose that the polarized membrane is capable of conducting an impulse, and that this conduction is coupled to the depolarization of the cell external membrane by its close proximity to the latter and to the contractile elements by diffusion of an activating substance over relatively short distances. Thus we have postulated the existence of a new *excitable unit* at the level of the fibril and even at the level of certain sensitive sections of the fibril. This changes the diffusion distance in striated muscle, which corresponds to the diffusion distance of 3 to 5 μ in smooth muscle, from 25 to 50 μ, which is the radius of the striated muscle *cell,* to 0.5 to 1.0 μ, which is the radius of the striated muscle *fibril.* The time-distance paradox is thereby resolved, and the possibility that a diffusion mechanism of activation of contraction applicable to both striated and smooth muscle is once more admissible.

The hypothesis has been able to explain previously inexplicable data. It now remains to examine the validity of the hypothesis both by examining its implications in the light of available data, and with experimental tests suggested by the hypothesis.

We can determine the effect the new hypothesis, which was derived from considerations of striated muscle, will have on our picture of time-distance relationships in smooth muscle. Examination of smooth muscle cells in the electron microscope reveals the presence of only a small complement of sarcoplasmic reticulum (*9*). There certainly appears to be no massive, well-developed system such as has been seen in most striated muscle cells thus far examined. Thus, the excitable membrane in smooth muscle would seem to remain the external membrane of the cell. This consideration, therefore, strengthens the hypothesis.

We can deduce from the hypothesis that the speed of contraction should increase as the size of the excitable unit decreases. This has been found to be the case for the limited data available at present, which concern only the difference between the two major classifications of muscle cells, smooth and striated. That is to say, most striated muscle cells examined have been from fast muscles and have had well-developed reticula surrounding small fibrils, while no smooth muscle has been found with such a well-developed reticulum, leaving the relatively large single cell as the excitable unit in this type of muscle. A more careful correlation of size of the excitable unit and the speed of contraction for various muscles within each of the major classifications is planned. This correlation will help to evaluate the validity of the hypothesis. There is supporting evidence at present, however, from some work by one of us (L.D.P.) on the myotomes of the primitive chordate amphioxus (*Branchiostoma carribbaeum*). These muscles are fast, judging from the quick swimming motion of the animal, but do not have a well-developed reticulum. The myofibrils are, however, in the form of flat sheets or lamina about 1 μ thick, and both large faces of each fibril are closely associated with the external membrane of the cell, which completely covers each of the flat fibrils. Thus the action potential is carried directly by the cell membrane to within 0.5 μ of the center of the fibril, and the time-distance relationship becomes approximately the same as that for striated muscle cells with cylindrical fibrils of this size sheathed in sarcoplasmic reticulum.

Other lines of investigation which are presently being carried out involve determination of the effect of changes in intracellular ionic concentrations on the morphology of the reticulum, with correlation of the reticulum morphology with the functional state of the muscle cell, and an investigation of the morphology of muscle cells from a wide variety of species with particular attention to the spatial relationships of the cell membrane, the sarcoplasmic reticulum, and the contractile material. We hope that information will soon be available which will allow a more critical evaluation of the hypothesis discussed here than is presently possible.

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