intermediates; and deoxygenation reactions (reaction (4), Figure) cannot be a good model for catalase activity (as opposed to the catalatic activity of free iron salts[24]) because catalase compound I can react with numerous other hydrogen donors[14,25] whose oxidation products do not form complexes with ferrous iron.

Résumé. L'auteur critique l'idée que la polarographie démontra l'existence de complexes d'hématine ferreuse avec le peroxide, actifs dans la catalyse. D'autres expériences montrent que les dérivés de l'hématine et du peroxyde sont instables et provoquent la dégradation de la porphyrine. Les complexes de la catalase et du peroxyde ne sont pas actifs polarographiquement et contiennent probablement le fer à l'état ferrique. La théorie de WESTHEIMER sur l'action de la catalase ne s'accorde pas avec les faits démontrant que les dérivés ferreux n'apparaîssent pas dans la réaction et que les complexes avec le peroxyde, autre que le premier composé, sont inactifs. On ne peut pas établir d'analogie bien fondée entre la catalase proprement dite et l'hématine libre.

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An Enzyme(s) from a *Streptomyces sp.* to Prepare Mould 'Protoplasts'

During recent years it has been shown that enzymes from *Streptomyces sp.* are a useful tool for the study of bacterial structures. WELSCH[1] has recently reviewed the field and described the different organisms and the techniques being used by the different workers.

Several instances of 'protoplast' formation from hyphae of a number of mould species were recently reported as resulting from a destruction of mould cell-wall, in particular in the case of gut juice of the snail *Helixpomatia* inducing protoplast formation[2,3]. Reports from our laboratory show the formation of protoplasts like-structures from a large number of moulds prepared by the use of the gut juice of the snail named before[4] as well as the obtention of protoplasts from various yeast species employing an enzyme preparation (strepzyme) from *Streptomyces GM*[5]. Our aims are to test their potential activities upon the various mould components that are being isolated in this and other laboratories, since the results of such work might well enlighten our knowledge of fungal structures.

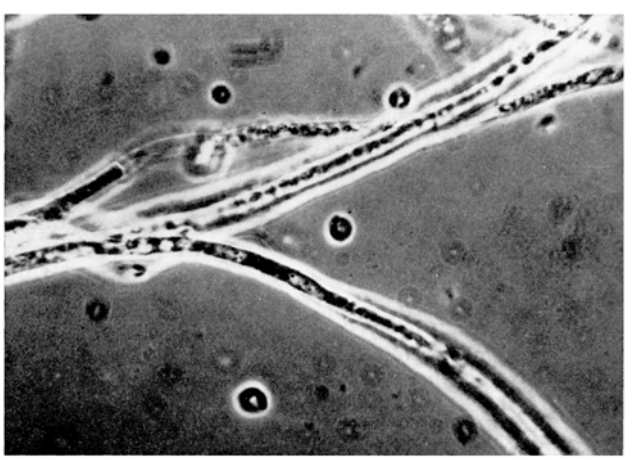
At the moment research is in progress devoted to a comparative study of the action of snail and Streptomyces enzymes upon several yeasts and moulds with the aim of finding out whether transformation into 'protoplasts' by these preparations occurs through similar mechanisms and whether differences are to be found in the response of various species and strains. In the following, we shall only show very briefly that the strepzyme, that is a suitable culture filtrate from our *Streptomyces sp.* strain GM, contains (one or various) principles acting specifically upon given species of fungi. The properties of this agent clearly show that it is a protein and an enzyme[6,7]. Studies are now in progress on identification of the enzyme activities to be found in the culture filtrate preparation of *Streptomyces GM* and will be reported elsewhere.

The study was carried out with the fungi *Mucor sphaerosporum, Penicillium italicum, Aspergillus nidulans, Fusarium culmorum, Verticillium hemileiae, Helmintosporium gramineum* and *Alternaria citri* which were submitted to the action of the *Streptomyces* enzyme. Transformation into 'protoplasts' under the influence of a suitable concentration of strepzyme was obtained with nearly all of them, *Alternaria citri* and *Helmintosporium gramineum* being the only exceptions. It is necessary to state that the rate, completeness and extent of the transformation, vary widely according to the organism under consideration.

Young hyphae of each of the above-named mould species were obtained by inoculating a small volume of a spore suspension of slant cultures into liquid Czapek medium. After a growth period of 18–24 h at 28°C, under vigorous aeration, the mycelium was harvested by centrifugation and thoroughly washed with distilled water. Then the mycelium was resuspended at a low density in 0.1 *M* phosphate buffer pH 6.8 containing 0.8 *M* mannitol. It should be noted that, in our experiments, no Mg^{++} or Ca^{++} were added to the medium.

For conversion of the mould hyphae into 'protoplasts', 0.2 ml of the enzyme preparation was added per ml of incubation mixture. After 3 h incubation at 30° with gentle shaking, the protoplasts began to emerge from the more susceptible mould species, v.g. *F. culmorum.*

When mycelial suspensions, submitted to the action of a suitable concentration of strepzyme in the presence of



The Figure shows 'protoplasts' of *Fusarium culmorum* of various sizes and some empty cell walls after $4^1/_2$ h of incubation under the conditions described in the text.

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the stabilizer, are frequently examined, one can generally observe several successive stages occurring between the initial normal mycelium and the final typical sphere. This was particularly well seen in the case of *Fusarium culmorum*. The first effect to be observed after 3 h incubation is the concentration of a region of the cytoplasm in one point of the filament. In a few cases, a large portion of the filamentous part remained empty. The cytoplasm, enclosed in its semipermeable membrane, bulges through some points of possibly lowered resistance, assuming progressively a spherical shape, finally taking the shape of a typical 'protoplast'. At a given moment the appendages were lost and spherical protoplast-like bodies of various sizes were left. When the concentration of strepzyme is too high, the transformation may occur so quickly that the sequence of events cannot be followed.

'Protoplast' forms appear as highly contrasted structures in phase-contrast microscopy. During the course of a prolongued incubation, and mainly for dilution with water, these structures become thinner and undergo lysis. Under optimal conditions, the osmotic structures were visible for after 26–72 h. With the organisms most sensitive to lysis, ghosts of spherical forms were not seen, sometimes a few debris were observed in the preparations.

Transformation of mould mycelia into 'protoplasts' under the influence of strepzyme appears to be a rather general phenomenon. Among the large number of organisms studied, the *Streptomyces* preparation induced morphological abnormalities of the mycelium of most of the strains tested, which can be considered as intermediary stages. The same type of abnormalities were also observed in germinating conidia of various fungi.

The stability of the strepzyme-induced 'protoplasts' is also quite variable: in some cases, lysis is evident after short incubation, while, in some others, spherical bodies are stable for many hours, suggesting that such bodies are not quite identical with true protoplasts[5]. Studies on the stability, regeneration and other properties of the osmotic structures will be described elsewhere.

Résumé. Les auteurs montrent l'action d'une préparation enzymique du *Streptomyces GM* sur la paroi cellulaire des champignons, donnant lieu à la formation de «protoplastes». De tous les champignons traités avec l'enzyme (strepzime), *Fusarium culmorum* semble s'être montré le plus sensible à cette digestion enzymatique.

M. J. R. Aguirre, I. Garcia-Acha, and J. R. Villanueva

Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid (Spain), July 30, 1962.

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Aggregation of Cells Isolated from Vegetalized and Animalized Sea Urchin Embryos

In a previous paper it has been shown that cells isolated from sea urchin embryos aggregate, giving rise to almost normal larvae (Giudice[1]). Some of the metabolic properties of the isolated cells have also been described (Giudice[2]).

The problem of whether or not the cells isolated from stages of development from blastula to the early pluteus are able to change their pattern of differentiation, was discussed but no satisfactory answer could be given. In an attempt to contribute to its solution, experiments have been performed using chemically vegetalized or animalized embryos. The results described in this note show that the altered pattern of differentiation is retained in the process of aggregation thus strengthening the suggestion that, at the stages examined, the possibility of changing the pattern of differentiation is very limited, if it exists at all (Giudice[1]).

Vegetalization was achieved by treating the fertilized eggs with 0.066 *M* LiCl in sea water for a period of 18 h (Lindahl[3]).

Animalization was induced by treatment with 0.001 *M* $ZnSO_4$ in sea water for the same length of time (Lallier[4]).

At the time of the disaggregation, the embryos had reached the stage of young blastula. In the case of the vegetalized embryos, some experiments with exo-gastrulae were also performed.

Cell isolation and aggregation were carried out following the procedure previously described.

The cells isolated either from vegetalized blastulae or from exogastrulae (i.e. before or after the appearance of the visible signs of vegetalization), aggregate within a few hours in the same way as the cells of normal embryos. After about 24 h, the aggregates are of the average size of the normal embryos and appear as solid spheres but they fail to exhibit the rotary motion typical of the aggregates from normal embryos. Sometimes they cluster together into larger masses. The histological sections show that the aggregates almost completely lack a continuous outer ectodermal lining. The ectodermal cells in fact are clustered into small groups scattered on the surface of the aggregates. Most of the surface instead is covered by a layer of endothelial-like flattened cells (Figure 1). The aggregates remain as solid masses and neither blastocoelic cavity nor intestine, nor spicules have ever formed. Sometimes a few pigmented cells have been seen. After a few days (about 4) in culture, they usually degenerate. Also the cells isolated from the animalized embryos aggregate in the usual way during the first few hours. However, they give rise to aggregates usually three to four times smaller than a normal embryo. After about 24 h the aggregates appear as hollow spheres surrounded by long cilia, which keep them in motion. Some of the aggregates are very small and in this case it is doubtful whether or not a cavity exists. The histological sections confirm these observations.

No further evolution of these aggregates has been observed, and they degenerate after a few days. Neither pigmented cells nor spicules have been found.

The almost complete lack of external epithelial lining is clearly demonstrative of the vegetalized character of the aggregates from vegetalized embryos. The lack of blastocoelic and intestinal cavities (which on the contrary appear very early in the aggregates from normal embryos), as well as of skeleton, may be due to the absence of the

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