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.

[5] S. Brenner et al., Nature *181*, 1713 (1958).

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

[1] G. Giudice, Dev. Biol., in press.
[2] G. Giudice, Arch. Biochem. Biophys. *99*, 447 (1962).
[3] P. E. Lindahl, Acta zool. Stockh. *17*, 179 (1936).
[4] R. Lallier, Exp. Cell Res. *8*, 230 (1955).

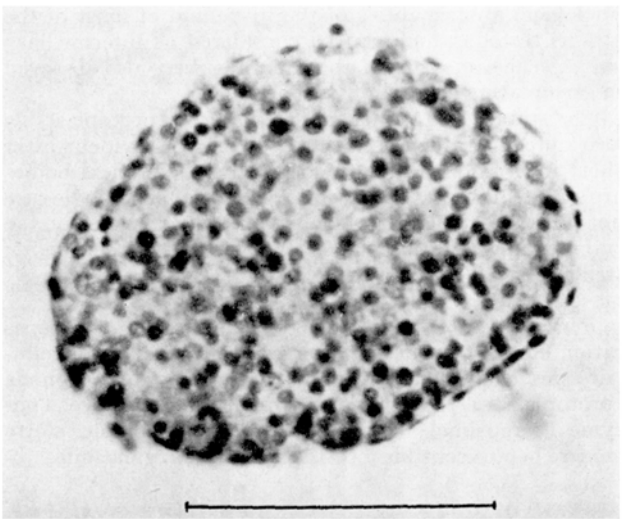

Fig. 1. Solid aggregate derived from cells isolated from blastulae of *Paracentrotus lividus* treated with lithium. Note the flattened cells lining the surface of the mass. Reference line = 50 μ.

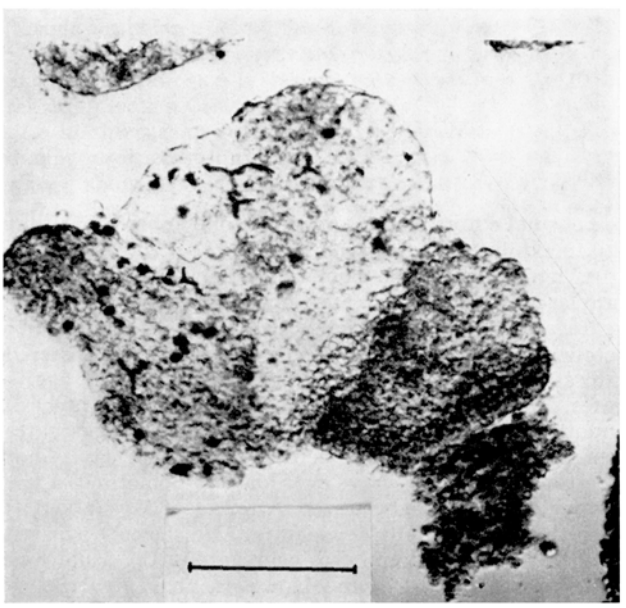

Fig. 2. A mixed aggregate of cells from vegetalized and animalized blastulae of *Paracentrotus lividus* as viewed *in toto*, after flattening between slide and cover-slip. The black spots are pigment cells. Note several triradiate spicules. Reference line = 50 μ.

ectoderm. In fact, the formation of the intestine has been suggested (GUSTAFSON[5]) to depend upon the drawing of the presumptive endodermic cells towards the ectoderm by the mesenchymal cells which in turn need the ectodermal contact to perform their function.

Also the development of the skeleton is considered to be due to the interaction between mesenchymal and ectodermal cells (UBISCH[6]; WOLPERT and GUSTAFSON[7]). It is, on the other hand, a common observation that, in vegetalized embryos, the more reduced the ectodermal covering is, the less developed the skeleton.

A number of experiments have been carried out in which we have tried to mix together cells isolated from animalized and vegetalized embryos in the hope of re-establishing a normal mixture of ectodermal endodermal and mesodermal cells. In only one case did we obtain embryos of vegetalized-like type but which developed many triradiated spiculae (Figure 2). Although this is fairly meagre evidence it may be taken at least as an indication in favour of the above suggestion as to the effect of the ectoderm on the differentiation of the skeleton.

The present results cannot be considered as a final proof that normally the dissociated cells did not dedifferentiate during the formation of the aggregates. They do, however, suggest that the induced changes which bring about vegetalization or animalization (which on the whole, are an exaggeration of the main differentiation in the early development of the sea urchin egg) may be retained by the cells during isolation and aggregation[8].

Riassunto. Blastule di *Paracentrotus lividus* trattate con LiCl (vegetalizzate) o con $ZnSO_4$ (animalizzate) sono state disaggregate con la tecnica precedentemente descritta.

Le cellule da blastule vegetalizzate si riaggregano in masse solide che non differenziano nè scheletro nè intestino. Quelle da blastule animalizzate danno origine a vescicole ciliate. Tentativi di combinazione di cellule da blastule animalizzate e vegetalizzate in un caso hanno dato origine ad aggregati con abbozzi di scheletro.

G. GIUDICE

Istituto di Anatomia Comparata, Università di Palermo (Italy), November 5, 1962.

[5] T. GUSTAFSON and H. KINNANDER, Exp. Cell Res. *10*, 733 (1956).
[6] L. v. UBISCH, Pubbl. Staz. Zool. Napoli *30*, 279 (1957).
[7] L. WOLPERT and T. GUSTAFSON, Exp. Cell Res. *25*, 311 (1961).
[8] This work has been supported by Grants from the Consiglio Nazionale delle Ricerche (Research Group on the Problems of Differentiation) and the National Institutes of Health, U.S. Public Health Service (RG-06211) to the Laboratory of Comparative Anatomy.

Zur Trennung der C_{19}-Steroide des Harns im Dünnschichtchromatogramm

Bei der Anwendung des Dünnschichtverfahrens nach STAHL[1,2] zur Chromatographie der C_{19}-Steroide des Harns gelangten wir zu einer Änderung der ursprünglichen Versuchsanordnung, über die wir hier berichten.

Sowohl das von STAHL angegebene aufsteigende Verfahren auf den 200 × 200 mm-Platten als auch das Durchlaufverfahren nach BRENNER und NIEDERWIESER[3] haben sich zur Trennung der 17-Ketosteroide des Harns nicht bewährt. Auf den immer noch kurzen Trennstrecken liess sich eine genügende Scheidung der einzelnen Substanzen, besonders aber des Androsterons vom Dehydroepiandrosteron, nicht erreichen. Beim Versuch, das Verfahren von

[1] E. STAHL, Chemikerzeitung *82*, 323 (1958).
[2] E. STAHL, Pharmazeutische Rundschau *1*, Nr. 2, 1 (1959).
[3] M. BRENNER und A. NIEDERWIESER, Exper. *17*, 237 (1961).