The cytoplasm of the plasmolyzed cells was reduced to approximately $^2/_3$ its original volume by 12 h and to approximately $^1/_3$ its original volume by 24 h. At 48 h, plasmolysis was complete, the cytoplasm of the cells having been reduced to relatively small masses (Figure 3). Though the shape of the nuclei in the plasmolyzed cells was distorted at times, their spindle shape was still discernible.

Nucleolar enlargement under the abnormal conditions of cell plasmolysis described here lends support to the suggestions of STICH[7,11], that nucleolar growth may be dependent upon the metabolic activity of the cytoplasm, and that under abnormal conditions an enlarged nucleolus is not necessarily a sign of increased protein synthesis. Nucleolar enlargement in lemon fruit cells incubated on distilled water and on a hypertonic mannitol solution indicate that this phenomenon is not a result of changes in the water content of the nucleoli. This is in agreement with the finding[7] that the increase and decrease of nucleolar volume in *Acetabularia mediterranea* was not due to changes in water content.

Note added in proof. The following observation was made after this article was sent to press. Marked nucleolar enlargement was evident in many cells of lemon explants that were placed on a dry glass surface immediately upon removal from the fruit and immediately covered with liquid paraffin (Light Grade) and placed in the dark for 48 h at 25°C.

Riassunto. L'ingrossamento nucleare è stato osservato in cellule plasmolizzate e non plasmolizzate di tessuto di frutto di limone. Queste osservazioni confermano le precedenti investigazioni secondo le quali l'ingrossamento nucleare può dipendere da una attività citoplasmatica e che sotto condizioni non normali un nucleo ingrossato non rappresenta necessariamente un incremento di sintesi proteica.

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Rotation and First Reversion in the *Octopus* Embryo - A Case of Gradual Reversal of Ciliary Beat

Coleoid Cephalopoda show two types of egg deposit. Decapoda enclose their eggs in jelly cases. The egg chorion (a product of the ovary) strongly increases in size during embryonic development, giving the embryo ample space in the perivitellin fluid which it circulates by means of ciliary motion[1]. The Octopoda, on the other hand, lay eggs that are surrounded but by an elongate, stalked chorion, which swells only slightly during development; the embryo remains tightly enclosed.

In 1875, LANKESTER[2] noted that the *Octopus* embryo at an early stage reverses its position in the chorion, turning around from the micropyle to the stalk side. This important phenomenon sunk into oblivion, however, for more than half a century[3]. PORTMANN[4] rediscovered it in 1933, and – referring to it as the first reversion – he described a second reversion performed by animals at a late embryonic stage, compensating for the first reversion in order to bring about a position better suited – but not necessary[5] – for hatching. A double reversion is also known in *Eledone* and *Tremoctopus*[6,7]; most observations were made on *Octopus*, however[8,9]. A time-lapse film study by PAINLEVÉ and ORELLI[10] illustrates all movements of the *Octopus* embryo. Lately, SACARRÃO[11] critically revised the entire problem of the first reversion or 'blastokinesis'[6].

Inspite of this large body of information, the mechanism achieving the first reversion remained unknown, mainly because Octopod embryos were assumed to be entirely devoid of cilia[12]. This is true for the actual embryo, but I observed that the ectodermic surface of the outer yolk sac is covered with ciliary cells. The cilia are very delicate and cannot be seen through the chorion which is transparent, but on bare embryos, they can easily be observed in vivo under the microscope; examination of histological sections also reveals their presence.

Earlier observations. In order to understand the function of the cilia during the first reversion, we have to recall the observations described by the afore-mentioned authors.

The yolk mass is penetrated with a network of plasmic processes of the yolk syncytium that are likely to bring about the contraction along the longitudinal axis of the egg preceding the first reversion[7,11]. The yolk mass forms together with the overlaying embryo and yolk envelope a unity; a gliding of the blastodisc on the yolk[9] is inconceivable[11].

From stage VI (staging according to NAEF[3]) on, the embryo slowly rotates around its longitudinal axis[9]. This rotation is directed clockwise when seen from the micropyle end of the chorion and remains so before, during and after the first reversion[10].

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At stage VII or at stage VIII, before or just after closure of the ectodermic yolk sac at the vegetative pole, the embryo (while rotating) starts changing its position in relation to the chorion, turning laterally or ventrolaterally (as a general rule to the right, but sometimes to the left) towards the opposite stalk side of the chorion where it arrives after 7 to 36 h, according to temperature (14–24 °C)[9].

Action of cilia. On the basis of these observations we note that contraction of the yolk mass along its longitudinal axis brings the embryo and yolk sac surface into close contact with the inner surface of the chorion. This leads to the assumption that 1. the cilia of the yolk envelope practise a locomotory action and, with their effective beat directed counterclockwise, give the egg a clockwise rotating movement; 2. the cilia – by gradually and coordinately changing the direction of their effective stroke – turn the whole embryo by 180°, any point on the egg surface describing a spiral movement and the soft yolk mass with the cap of the embryo undergoing a continuous deformation.

The crucial assumption that the direction of the ciliary beat changes was verified by means of small charcoal particles or carmine granules placed on the ciliary surface of the yolk sac of eggs taken from their chorion at different stages before, during and after reversion. Such particles are transported along a straight line that indicates the direction of the effective ciliary beat (Figure 1).

Before the beginning of the reversion, particles are transported in a direction counterclockwise in relation to the longitudinal axis of the embryo and the chorion when looked at from the animal pole; during reversion, in directions counterclockwise in relation to the chorion axis, hence oblique in relation to the longitudinal axis

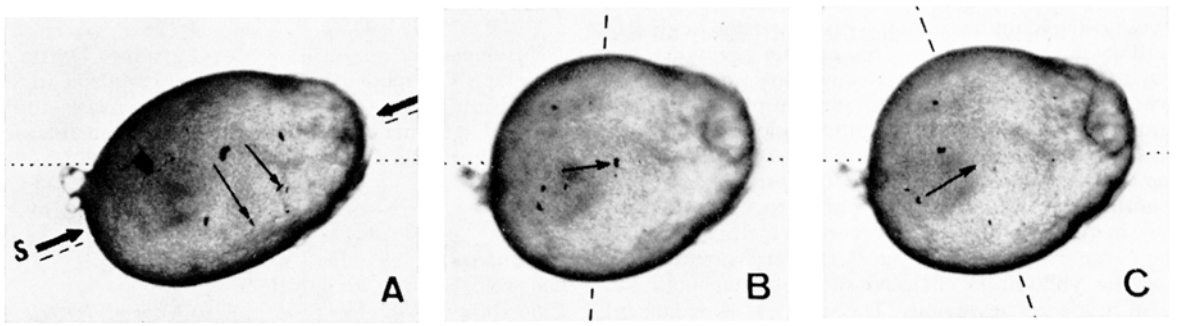

Fig. 1. Embryo shortly after start of first reversion, A) just after being taken from the chorion; broad arrows indicate longitudinal axis of the chorion; s, side of chorion stalk. Broken line indicates axis around which the embryo rotated in the chorion (= chorion axis). Longitudinal axis of the embryo is indicated by dotted line. Arrows on the yolk sac (based on combination of 2 photographs) show track of 2 charcoal particles after a travel of about 40 sec (other particles were stuck on the surface and did not move). Note that track of particles is not at a right angle to axis of rotation, but slightly 'preceding' it. B) Same embryo 3 h later and C) $4^1/_2$ h later. Broken line in B) and C) indicates axis around which the embryo would rotate in the chorion.

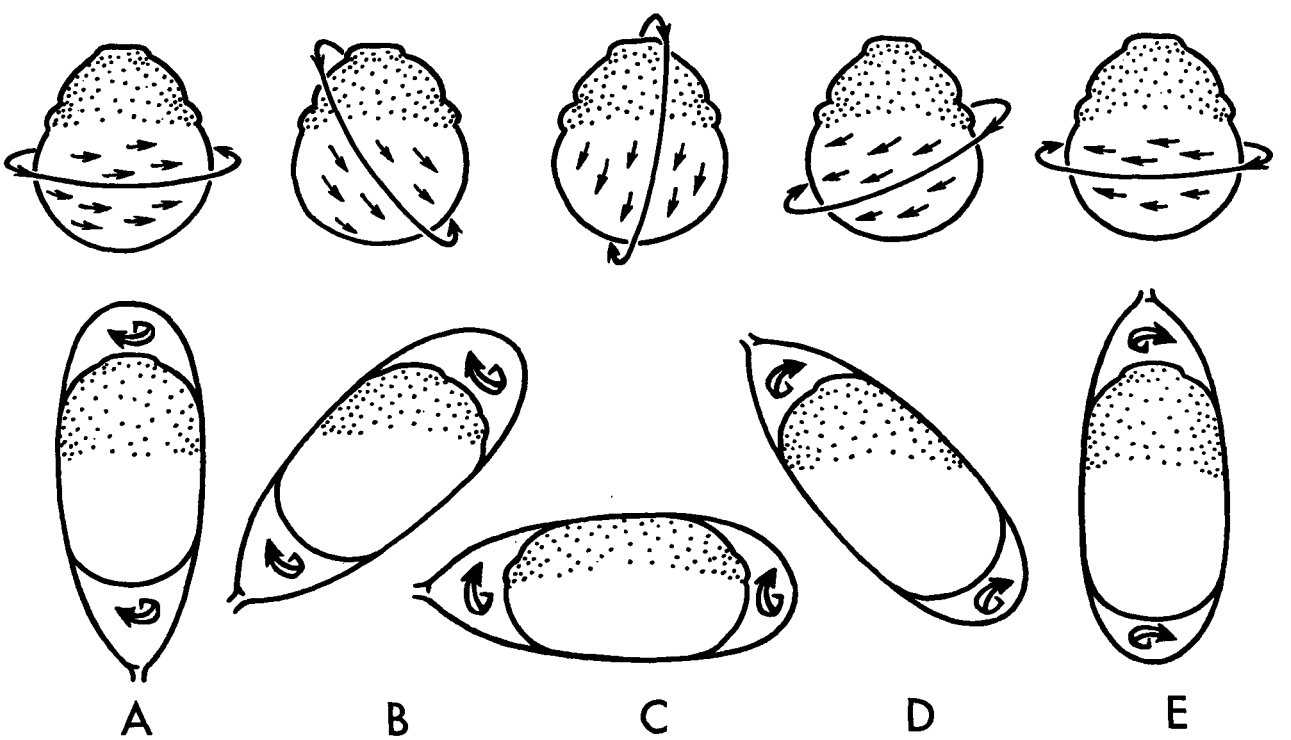

Fig. 2. Diagrammatic presentation of an embryo before (A), during (B–D) and after (E) the first reversion. Broad arrows in the lower row indicate the direction of rotation in the chorion. The upper row shows the embryo when taken from the chorion; arrows indicate direction of effective ciliary beat, circles symbolize plane of ciliary movement. Dotted part marks embryo 'cap'.

of the embryo; and at the end of and after reversion still counterclockwise in relation to the original chorion axis, but clockwise in relation to the axis of the embryo (Figure 2). During reversion, the plane of ciliary movement in fact is not exactly perpendicular to the axis of the chorion (i.e. the axis of actual rotation), but slightly 'preceding' the plane of rotation, thus demonstrating a certain timelag due to the resistance of the bulky eggmass. The change of direction of the ciliary beat can also be followed directly on one and the same embryo by tracking particles at different times (Figure 1).

The pulsation of the outer yolk sac starting at stage IX does not interfere with the rotation, which works through all the stages of early and later organogenesis and ceases at about stage XVI when the ciliary surface in contact with the chorion becomes too small to move the then large embryo.

Discussion. All observations thus far reported in the literature perfectly match the 'model' described here. Embryos failing in undergoing the first reversion evidently start ciliary activity at a stage too late for reversion, the embryo having lost its high deformability; at stage X, however, when normal embryos have completed the first reversion, they also rotate counterclockwise thus demonstrating the autonomy of the reversal of ciliary beat. In species with very large eggs (e.g. *Octopus briareus*), the early embryo is very small compared to the egg mass and is – in the case of belated reversion – dragged forth by the large rotating yolk sac[13]. Embryos with only part of the yolk mass enclosed in the outer yolk sac can also undergo reversion. The partial reversion of aberrant, invaginated germs[9] can be explained by contraction only of the yolk mass that forces the uvula of embryonic tissue sooner or later into a curved, oblique position; its gradual deformation is about 10 times slower than normal reversion.

The fact that rotation does not cease after completion of the first reversion endorses the assumption that rotation also fulfills other physiological tasks such as acceleration of oxygen uptake by the yolk sac and maintainance – by revolving the perivitellin fluid – of a balanced microenvironment. This physiological viewpoint is meaningful for any attempt to evaluate the evolutionary pathway leading to the establishment of such a reversion, the significance of which is unknown. From a more biophysical point of view, however, the first reversion stands out against other functions of the ciliary activity. Whereas processes such as revolving the perivitellin fluid can be carried by cilia that never change the direction of their beat[1], the first reversion demands a more sophisticated ciliary apparatus. The present study coping only with the performance of this apparatus, the problems of the structure of the cilia envolved[14] and of the control and coordination system for this well-timed process are still open.

Zusammenfassung. Cilien des äusseren Dottersackes versetzen Octopoden-Embryonen in Rotation innerhalb ihrer Eihülle. Die erste Umdrehung oder «Blastokinese» ist die Folge einer einmaligen, innert Stunden ablaufenden Schwenkung der Cilienschlagrichtung um 180°.

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Influences of Cell Cycle on Uptake of SV40-DNA by Diploid Human Cells

Cells from a variety of animal species can be transformed in vitro by SV40. The transformed cells show loss of certain regulatory properties such as cellular sensitivity to contact inhibition and changes in morphology. Both structural and numerical aberrations of chromosomes occur in transformed cells[1,2]. Cells transformed by SV40 contain virus specific tumor antigens as well as messenger RNA which hybridizes specifically with SV40 DNA. These observations indicate that the viral genome interacts with that of the host cell and that all or part of the genetic information of the virus persists in the transformed cells. It has been demonstrated that cells transformed by DNA tumor viruses contain at least part of the virus genome in stable association with the host cell DNA[3–5]. The present study was undertaken in an attempt to utilize autoradiographic methods to determine the fate of tritium labeled SV40 DNA following infection of synchronized human cells by the virus and thus to get an insight into the influence of the physiological state of the cells on virus infection.

Materials and methods. SV40 clone 307L was grown in monolayers of CV-1 cells. For preparation of tritium labeled SV40, growth medium (Eagle's medium supplemented with 10% calf serum) was removed from 7-day-old CV-1 monolayer cultures and replaced with maintenance medium (Eagle's with 2% calf serum). In addition the maintenance medium contained tritiated-thymidine (TdR-H^3). 24 h later the cultures were infected with SV40 at 10 PFU per cell. After infection the culture were incubated at 37 °C in maintenance medium containing TdR-H^3 at 0.5 μc/ml. Cultures were harvested on the seventh day after infection when they were extensively cytopathic. Cells and maintenance medium from 10 cultures were pooled and the virus was purified and concentrated by a technique similar to that of LUBORSKY[6]. The infectivity titer of the final material was 6.5×10^6 PFU/ml and the specific activity was 1.5×10^{-3} counts/min per PFU. It is important to point out that the final virus suspension contained no free or DNAase-sensitive counts higher than background. This indicates that there was no free TdR-H^3 or cellular DNA in the preparation.

A human diploid cell line developed from skeletal muscle tissue at our laboratory was used in this study and grown in Ham's F-10 medium supplemented with

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