

THE MORPHOLOGICAL EFFECT OF THE HETEROCHROMATIC ULTRAVIOLET MICROBEAM IRRADIATION ON THE GILL CILIA OF *MYTILUS EDULIS*

Dr. Çetin ALGÜNES(*)
Edip TUNCER(**)

ÖZET:

Midye (*Mytilus edulis*) solungaç frontal bölge silleri üzerine heterokromatik Ultraviolet microbeam radyasyonunun morfolojik etkisi. Midye (*Mytilus edulis*) nin solungaç sillerinin A, B ve C ile gösterilen muhtelif bölgeleri Ultraviyole microbeam (nokta ışınlandırma) metodu ile ışınlandırılmıştır (Şekil 1).

A bölgesinin (basal corpuscles) ışınlandırılmasında, önce sillerin hareketinde bir hızlanma görülmüş ve 45 saniye ($2 \cdot 10^{-4}$ erg / m² / san) süren ışınlandırma esnasında ışınlandırılan sillerin % 80 in hareketlerini durdurdukları ve ışınlandırmadan sonra da geri kalanlarının % 10 unun hareketsizleştiği tesbit edilmiştir.

B bölgesinin (rootlets) 45 saniye ışınlandırılması halinde ışınlandırma esnasında ve daha sonra ancak sillerin % 40 inin hareketlerini durdurdukları görülmüştür. Bu bölgenin ışınlandırılması 2 dakikayı bulduğu vakit ışınlandırılan silin dibinde önce küçük bir kabarcığın teşekkül ettiği ve sonra bunun dokudan ayrıldığı tesbit edilmiştir. Bunun dışında, bu bölgede ışınlandırma sonucu husule gelmiş morfolojik bir değişiklik görülmemiştir.

C bölgesinin (nuclei) 45 saniye ışınlandırılması, sil hareketi üzerine tesir etmemektedir. Ancak 2 dakikalık bir ışınlandırmadan sonra ışınlandırılan sil ve bunun sağındaki ve solundaki sillerin hareketi bir süre durmakta ve sonra yan siller düzensiz bir şekilde harekete başlamaktadır.

SUMMARY:

The three different regions of the frontal area cilia of the mussel's (*Mytilus edulis*) gills (GIBBONS 1961) were irradiated with ultraviolet microbeam for 45 sec. and for 2 minutes. ($2 \cdot 10^{-4}$ erg / μm^2 / sec.)

Irradiation of the region A for 45 sec. stopped the movement of the 90 % of cilia during the irradiation or shortly after the irradiation.

Only 40 % of the cilia stopped their movement when region B was irradiated for 45 sec. Irradiation of this region for 2 min. caused first a bubble-like formation among the epithelial cells at the irradiated region. This small vesicle departed from the tissue during the next few minutes. It was not possible to observe any morphological alteration in the cells at this region.

The irradiation of region C did not cause a sudden stopping of the ciliary movement. Even two min. after irradiation, the movement of the irradiated cilium and the neighboring cilia stopped only for a few minutes and only the neighboring cilia started to move again, but this motion was irregular.

(*) Trakya Üniversitesi Tıp Fakültesi Tıbbi Biyoloji Anabilim Dalı - EDİRNE

(**) İstanbul Üniversitesi Fen Fakültesi - İSTANBUL

INTRODUCTION

The Ultraviolet (U.V.) microbeam system is frequently used to investigate the behaviour of the specific small organelles or segments of larger functional units such as the chromosomes or areas of unknown function of the cells (SMITH 1964). The U.V. is a radiation type which causes a damage or an alteration only at the irradiated region and has almost no effect beyond this particular area. It has been possible to irradiate a very small area 3-4 microns diameter of mammalian cells grown in tissue culture, U.V. microbeam system has also been used successfully to irradiate the various parts of the microorganisms, such as the cytopharynx, the nucleus and the very small portion of the cytoplasmic membrane of Paramecium (HANSON 1962, and ÖZALPAN et. al. 1970).

The morphology and physiology of the moluscan cilia have been the subject for several investigations (see for literature GRAY 1930; ATKINS 1938; KINOSITO 1952; YONEDA 1960; TSUCHIYA 1971).

The aim of this study was to irradiate different regions of the cell which are inclose relation to the cilia and so to find out the organel which control the ciliary movement.

MATERIAL AND METHODS

In this work, mussels (*Mytilus edulis*) of 1,5-2 cm average size, collected from Bosphorus shores were used. Pieces of 0,25-0,5 cm² were taken from the gills of the mussels, by the use of a sharp pointed glass rod these pieces were divided into smaller ones on a slide containing a drop of sea water. The slide was then covered with a quartz coverslip.

For U.V. microbeam irradiation, Uretz type microbeam system was used. The U.V. source was Phillips 500 Watt water cooled high pressure mercury lamp. The heterochromatic U.V. beam emerging through a liquid filter having a transmission band of approximately 2100-3200 Å° wavelengths was focused in an area of 3,5 µm diameter on the desired targed using a reflection objective of 74 X magnification. The energy of the radiation on the spot was approximately 2×10^{-4} erg /µm² /sec. (ALGÜNES, 1974).

As can be seen in Fig 1, the three different regions of the epithelial cells of the gills were irradiated with U.V. for two different exposure times, 45 seconds and 2 minutes respectively.

To localize the irradiated area on histological preparations some pieces of gills were fixed with Carnoy's fixative and then paraffin sections were

stained with iron hematoxylineeosin according to the standart methods. Some other pieces of gills were also examined under the phase-contrast microscope.

OBSERVATIONS

The structure of the cilia and of the irradiated area are shown in Fig. 1 schematically. It represents a combined figure drawn from different samples.

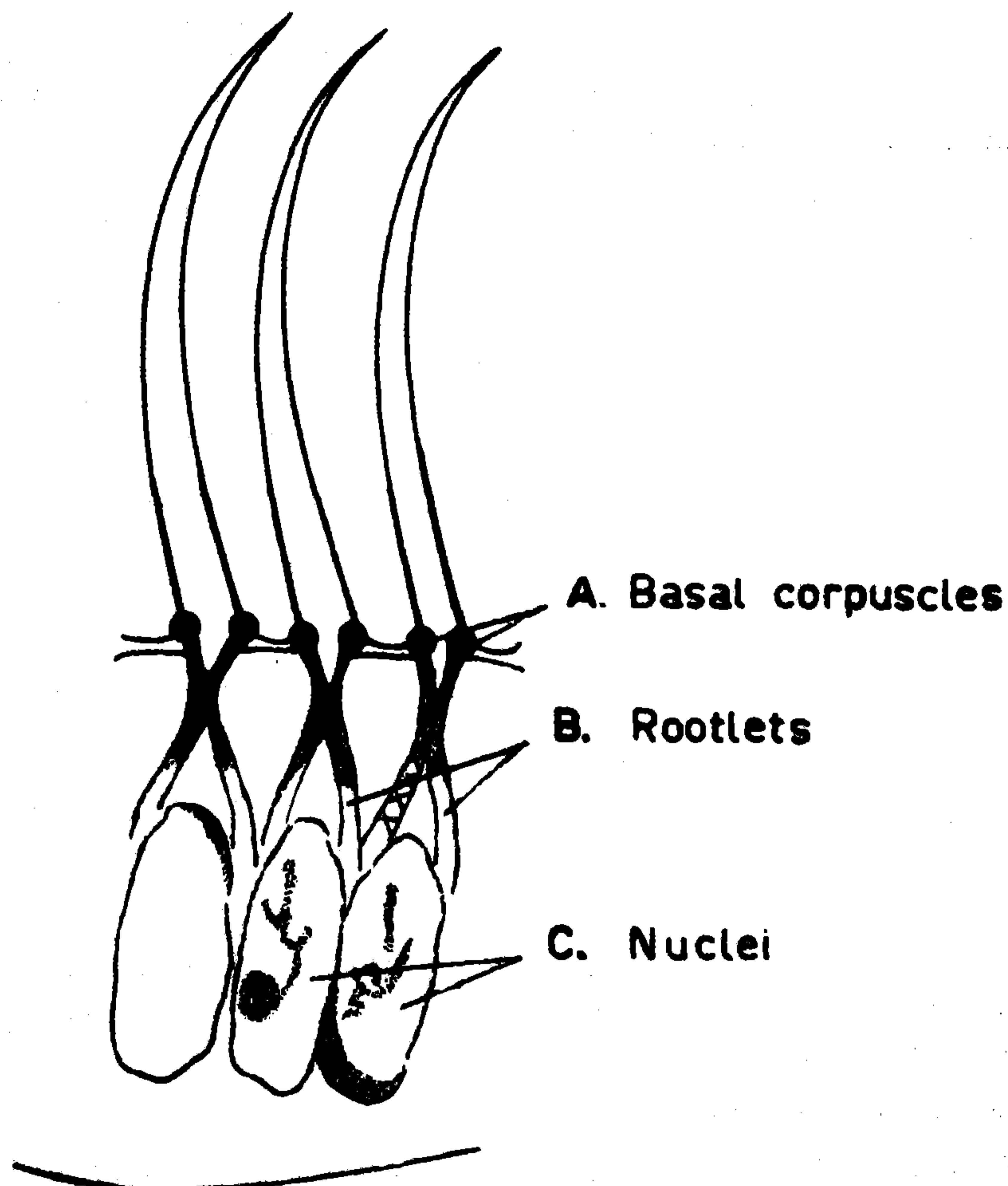


Fig. 1. Diagram of the ciliary apparatus of the gills of the *Mytilus edulis* showing the irradiated regions of it.

I. The effect of U.V. microbeam irradiation on region A:

As in Fig 1 at the border line of the epithelium a small region, approximately $3.5 \mu\text{m}$ diameter, of the cilia's distal part in the epithelial cells is irradiated, where the cilium's basal corpuscle is located.

a- The effect of 45 sec. irradiation.

When the basal corpuscle (region A) is irradiated, an increase in the cilium's frequency of the kinetic cycle is observed during the first 10 sec. of irradiation period. During the next 30 sec. almost 60 % and the next 15 sec. i.e. until the completion of irradiation, about 20 % of the cilia stopped their movement (Table I). A few seconds after the irradiation period 10 % of the cilia, which were moving, stopped this motion. During the 15 min. post-irradiation observation period, the unirradiated cilia did not exhibit any abnormalities in their movements.

Table 1.

Number of experiment	Number of irradiated cilia	Number of Stopped cilia			Number of non-stopped cilia	
		During irradiation		After irradiation		
		First 30' between 30'-40'				
1	14	8	3	2	1	
2	8	7	1	-	-	
3	8	5	2	1	-	
4	12	6	3	1	2	
5	10	7	-	1	2	
6	8	6	1	-	1	
7	12	7	3	1	1	
8	12	8	5	2	3	
		% 59,3	% 19,8	% 9,9	% 11	

b- The effect of 2 min. irradiation.

When the basal corpuscle (region A) is irradiated for 2 min. the same effect with 45 sec. irradiation of the same region, was observed.

II. The effect of U.V. microbeam irradiation on region B

This region is located between the free surface and the nucleus of the cell where the rootlets crossed each other or are divided into two smaller rootlets downward to the nucleus.

a- The effects of 45 sec. irradiation:

45 sec. of irradiation caused only 30 % of the cilia stopped to move whereas 10 % of the moving cilia became motionless during the next 15 min. period following the irradiation and 60 % of them continued to move.

b- The effect of 2 min. irradiation:

During the 2 min. irradiation, the nearest cilium to the irradiated region stopped to move and the other cilia seemed to be uneffected.

c- The morphologic appearance of the cilia were not altered during the first 45 sec. of irradiation of the regions A and B; but the ciliary movement stopped. With the extending irradiation period of region B, the length of the nearest cilium to the irradiation site gradually shortened (fig: 2) and the distal end of the same cilium started to swell to a buble-like body. Almost at the same time, a variation in the refraction index of the irradiated area is observed which begins in the first 1,5 min of irradiation exposure and which continues on, even at the termination of irradiation. Then the irradiated region starts to swell and bubble-like body is extruded between the two cilia from the tissue in about 5–10 minutes and then, the tissue returns to its normal appearance (fig. 3 a and b). It is observed that the round body

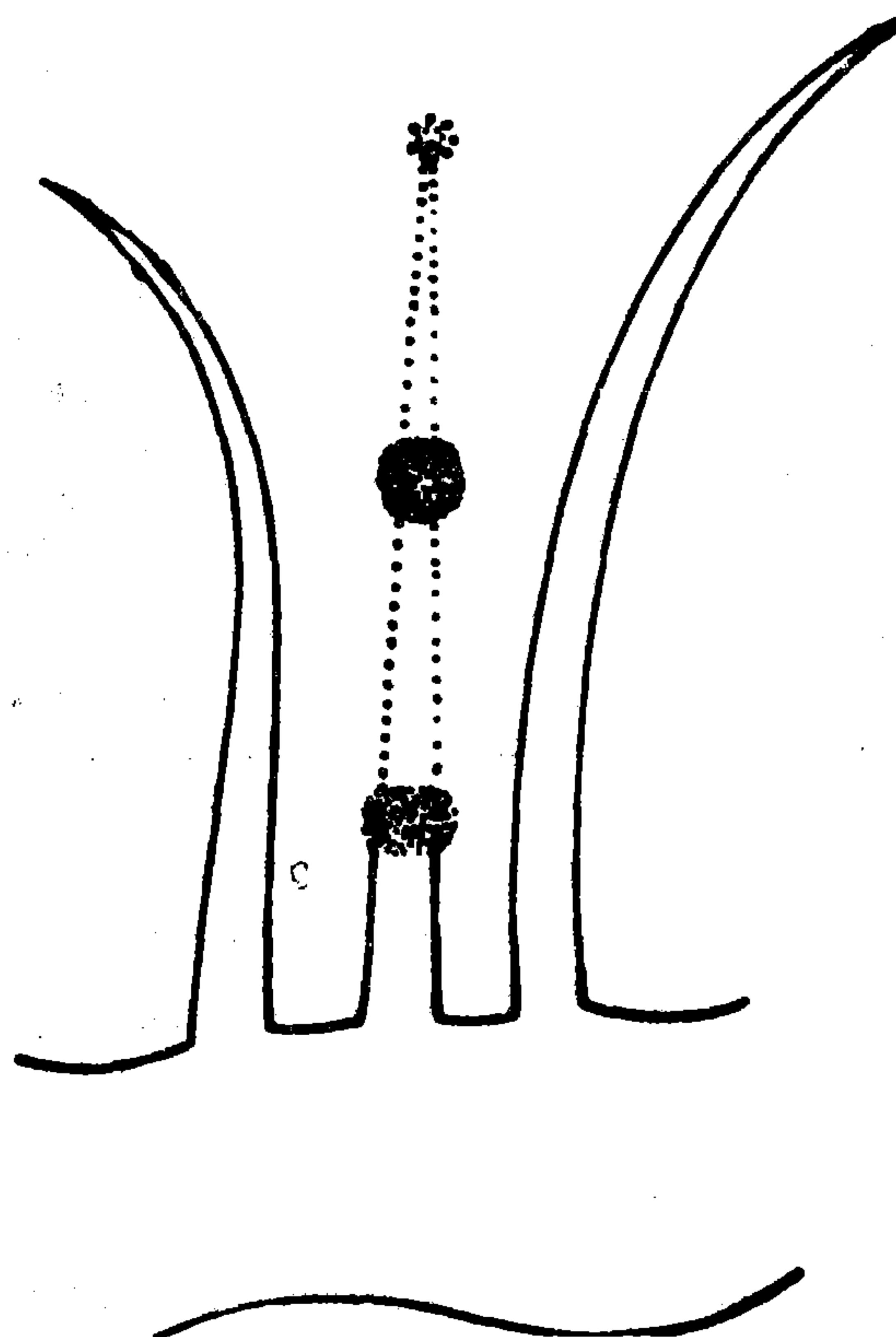


Fig. 2. The changes in the morphological structures of the cilia during the irradiation of the B region

which is discharged does not mix with the water in the medium. It retains its shape in water and flows with the water currents caused by the movements of the other cilia.

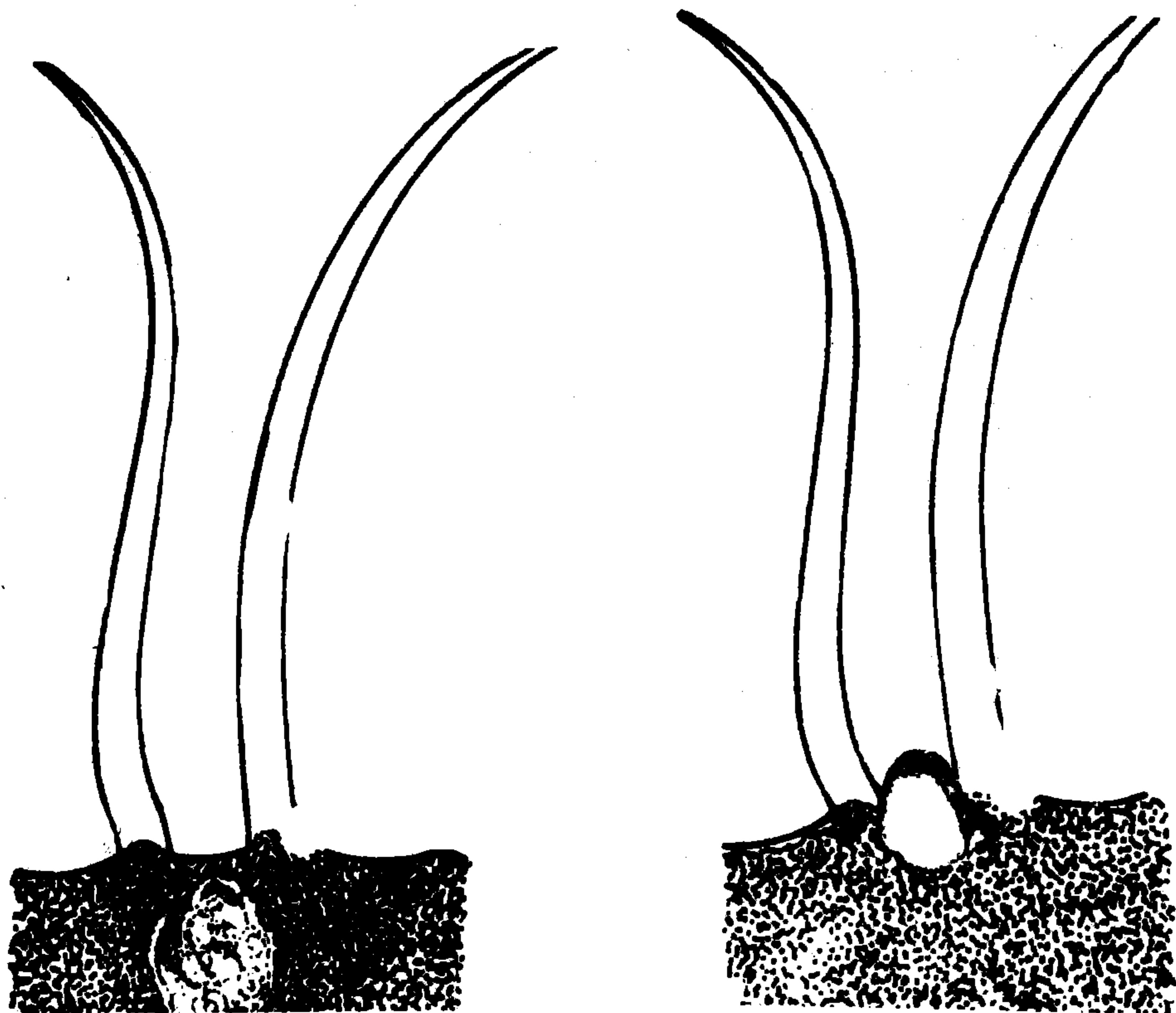


Fig. 3. a) The structure which forms after the irradiation of the region R, and which moves by growing from inside to outside b) The last shape of growth.

III. The effect of U.V. microbeam irradiation on region C

According to the histological preparations this region corresponds to the place where the nuclei of the cells are located.

a) *The effect of 45 sec. irradiation:*

When the C region is irradiated to 45 sec. a variation was not observed in the movements of cilia, both during the irradiation and post-irradiation periods.

b) The effect of 2 min. irradiation:

During the 2 min. irradiation of region C the irradiated cilium was stopped. On the other hand the two neighbouring cilia (just at the right and left) of region C stopped their movements following irradiation. However, in the next few minutes the two cilia at both sides started to move disorderly and their kinetic cycle became normal gradually during the next 15 min. But no movement of the cilium in the middle was seen during the observation time.

DISCUSSION

The gill cells of mussels have prismatic cilia. Their nuclei are localized in the basal region of the cell. The space between the free surface of the cell and the nucleus is occupied by the basal corpuscles of cilia and by the rootlets. The ends of rootlets towards the free surface of cell are tightly connected with basal corpuscles whereas those towards the nucleus become invisible in the vicinity of nuclear membrane in the cytological preparations. But it seems so as if the ends of rootlets and the nuclear membrane contact each other. At least they are very close to each other. These brief description of the gill cell will help to understand the results obtained.

Region (A) includes the cell surface and the basal corpuscles of the cilium. Irradiation of this region for 45 sec. stops the movement of 90 % of the cilia. This shows that this part of cell, and possibly the basal corpuscles, is more sensitive to U.V. microbeam irradiation and that it controls directly the ciliary kinetic cycle. SASAKURA's (1966) results are in agreement with these results. On the other hand KINOSITO (1952) who has investigated the effect of isotonic KCl had come to the conclusion that the effect of this substance on the ciliary movement is caused by the alteration of the activity of this region, i.e. of the basal corpuscles.

Irradiation of region (B), even for 2 min. stops the movement of only 40 % of cilia indicating that this region controls the ciliary movement indirectly.

The rootles of cilium are streched in this region between the basal corpuscles of the cilium and the nucleus of the gill cell. BOLETZKY (1973) could show that there is a close relation between the cilia's rootles and the giant mitochondria. So it may be assumed that U.V. microbeam irradiation disturbs the energy formation in mitochondria or disturbs the transfer of energy through the rootlets to the basal corpuscles. GÖKDOĞAN (1972) could show that hydrazine hydrate cause the various granules, lysosomes and

mitochondria in the cytoplasm to move. We could prove by a series of experiments that the granules in the cells which are caused to move by hydrazine hydrate, stop their movements in a great percentage when these cells are irradiated with U.V.

The results obtained from this experiments are show that the movement of the cilia is controlled by each of the three irradiated regions of the gill cells of mussels (Fig. 1 A, B and C). But the effect of each region on the ciliary movement is different from the effect of the other regions. The existence of center (A) which control the movement of cilia could be shown first by Paramecium (HANSON 1962, ÖZALPAN et. al 1970). However, certain structure (A) responsible for the kinetic cycle of cilia could not be demonstrated in these investigations. The presented results make it possible to develop a theory how the cilia move. The nucleus stimulates the energy formation in mitochondria. The transfer of energy to basal corpuscles is realised through the rootlets. Basal corpuscles turn the chemical energy to kinetic energ which cause the movement of cilia.

The morphological alteration which occurs when the B region is irradiated for 2 min. is the most interesting part of the work, for the irradiation of this region:

- a- stops the ciliary movement and beside this effect causes,
- b- a reduction of the size of the cilium nearest to the irradiated region and,
- c- an extrusion of a substance from the irradiated region,

A similiar process as a results of U.V irradiation is not known with other types of cells. This phenomenon is being investigated in detail and the results will be published elsewhere.

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