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REJUVENATION OF CELL-GENERATIONS IN TISSUE CULTURE

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The development of higher organisms starts with the mitosis of the fertilized ovulum ; the final form is attained after a long series of cell divisions. The developmental process consists, however, not only of cell divisions, but of cell degeneration and decay too as of regularly occurring phenomena, all these acting harmoniously with other factors upon the developing form of the organism. In consecutive stages of the fully developed organisms we see in the process of ageing a predominance of degeneration over the division of cells, resulting finally in decay and death of the total organism. The maximum of age, characteristic and well-known for the different species, has no validity as regards the single cells of the organisms. Cells separated from the whole und cultivated under propitious conditions will multiply as cell cultures, not being compulsorily subjected to decay. It is one of the unsolved problems of biology what are the causes of the ageing and decay of the higher organisms while their single cells are capable of infinite — or at least of a considerably longer — life. Several research projects tried to throw some light upon these problems ; cell research proper is linked herewith, examining causes, conditions and course of cell-degeneration and regeneration from the point of view of the individual and separate single cell. Some mitoses promote cell multiplication, and thus induce growth. This is the most common type while sometimes another type is observed with asymmetrical cell division where one of the derivatives is of shorter life and decays speedily ; this is mostly *a priori* smaller. In the present report a symmetrical type of cell division is described resulting in two different daughter cells, the one endowed with less vitality, the other having gained vitality through division in this case inducing growth and storage of protoplasm.

While in cell degenerations connected with morphogenesis and ageing several organising and inducing factors of the whole organism are to be taken into consideration, in pure cell cultures, containing but one type of cells, they can be examined free of these factors so difficult to control. My observation were made on pure tissue cultures of myoblasts and fibroblasts, prepared by Törő's method. In cultures from the heart of young (5 days old) chicken embryos one obtains, by 10 passages at least, a pure culture of myoblasts as proved by Törő's reimplantation experiments, while with the similar method from the heart of older embryos pure fibroblast cultures are obtained.

In a former series of experiments, examining the effects of irradiation upon tissue cultures, I found a great number of radiant granula in the cellular protoplasm in all those cases in which irradiation had an unadvantageous effect upon the cells, similar to granula present in cells not transplanted at the proper time. In the latter this phenomenon is regarded as being due to lack of proper nutrients and oxygenium, and to the increased amount of deleterious metabolites in the culture-media respectively. The protoplasm of such cells appears — if stained with hematoxyline — to be vacuolous ; using lipid stains — as e. g. Janus green — we see instead of vacuoles well stained granula (Fig. 1). This may serve as a proof for the lipid nature of the aforementioned granula. In these former experiments it was conspicuous that a few hours after the

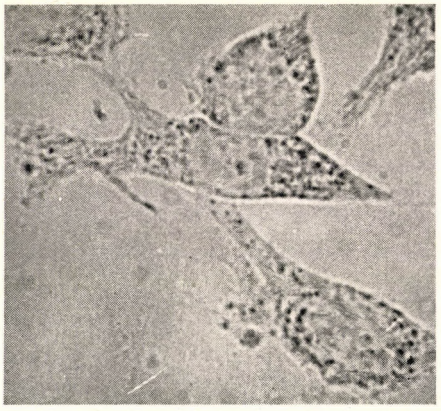

Fig. 1.

irradiation the cells displayed a heterogeneous appearance (although pure cultures were used) in so far that some cells were granulated while others (these were the minority part) offered no degenerative signs whatever. In this connection the first thought was that through irradiation some kind of selection was caused i. e. only the more sensitive cells were damaged since all cells of the cultures, irradiated seriatim through longer periods, received an equal dosage of irradiation.

More recently, when analysing microcinematographs of cytogenetical investigations I found the real cause of the described phenomenon. Following the division of a well granulated connective tissue cell cinematographically I observed a collecting of the granula in the initial, rounded off stage of mitosis in the one pole of the cell (Fig. 2). The division occured in such a manner that most of the granula, characteristic for degeneration, remained in the one daughter cell (Fig. 3, identical with the cell shown in Fig. 2 after the lapse of 6 min.), while the other contained a few granula only and displayed higher motility

and gained supposedly a higher division capacity. Following the later development of the more granulated cell the one (that appeared to be degenerated) I observed a marked diminution of its locomotory speed (up to the half of the

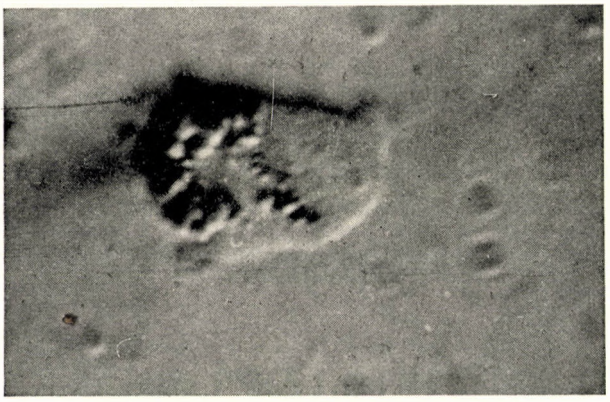

Fig. 2.

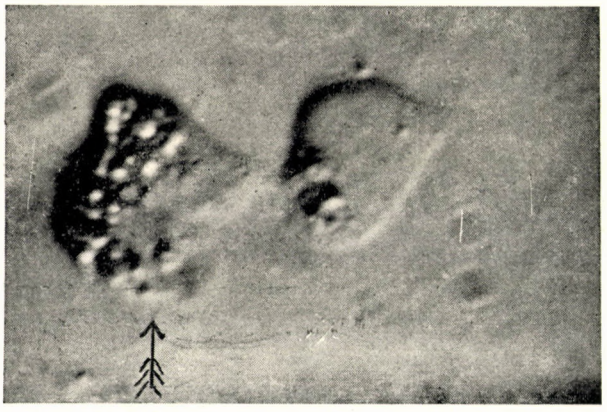

Fig. 3.

normal, to approximately 35 micra/hour) ; in the next division of this cell — that occurred after some delay in comparison with cells free of such granula — the phenomenon was repeated accurately (Fig. 4). The granulated derivative of this second division, being to some degree increased in volume, displays hardly any sign of locomotion, it becomes rounded off (Fig. 5) ; finally all signs of movement disappear, as prodromum of the final decay (Fig. 6, identical with the cell shown in Fig. 5 after the lapse of 60 minutes). To sum up, three healthy and one unprospering cell stem from one cell displaying signs of degeneration. In which cellgeneration a cell incapable of further life will arise

depends surely upon the quality of the deleterious environmental and circumstancial influences ; under unpropitious conditions this may occur in the first generation ; if the mother cell is seriously damaged, it decays without being able to divide.

Fig. 4.

This type of cell division — the formation from a degenerated cell of a cell with increased vitality and of another cell with decreased vitality, condemned to decay, — where the capacity of life was unequally distributed, may be regarded as cell rejuvenation. This process is not identical with dis-

Fig. 5.

charging of a part of the cellular protoplasm in eliminating inclusions since the part condemned to decay is of an equal size with the surviving one ; furthermore the decaying granulated derivative has a nucleus with normal tinction,

mostly somewhat swollen. Further observations are required to clarify the problem whether a mitosis or an amitosis occurs, though the present observations point with marked probability to mitotic cell division.

I observed repeatedly the appearance of such granulated cells ; as regards the distribution of the granula in cell division I have seen always an identical behaviour. Thus, I have to suppose that this process regularly occurs in regene-

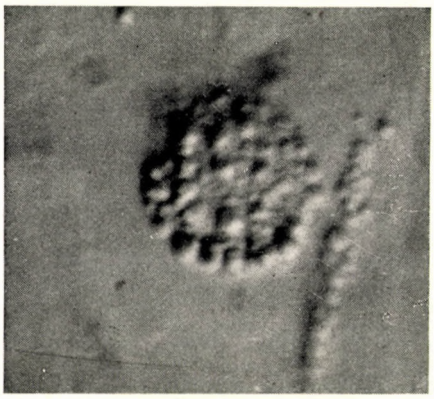

Fig. 6.

ration following degenerations. Hereby the genesis of rounded off cells, in the liquified zone of cell cultures, full of granula, void of motility, seen often in cell cultivation, is clarified too.

In this connection it seems necessary to compare this phenomenon with the wellknown fact that by X-ray or radium irradiations in special dosage cell proliferation, formation of malignant neoplasms may be induced. One has to suppose that contemporaneously with the series of cell divisions as described above the transformation of normal cells to neoplastic ones occurs, a process of which no particulars are yet known.

Summary

By observing microcinematographs of single cells in cell cultures over a longer period (48—72 hours) the hitherto unknown fact was detected that the derivates of cells displaying externally and in the motility sign of degeneration, receive these signs unequally transmitted; in cell division only one of the daughter cells receives all of these degenerative signs. Of all cells of one division period only one comes into the decaying final stage of degeneration while the others — becoming rejuvenated as it were — obtain higher vitality.

REFERENCES

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ОБМОЛОЖЕНИЕ ПОКОЛЕНИЙ КЛЕТОК В ТКАНИЕВЫХ КУЛЬТУРАХ

А. Вадас

Р е з ю м е

Наблюдая долгое время (48—72 ч.) путем микрокинематографических снимков, отдельные клетки тканиевых культур, автору удалось установить, что потомки клетки потерпевшей по какой либо причине дегенерацию, и показавшей все внешние и функциональные признаки перерождения, не наследуют это свойство в равной степени. При делении клетки всегда только одна из дочерных клеток наследует это свойство, так что после нескольких делений среди возникших многочисленных клеток только одна достигает конечную фазу перерождения: гибель, а все остальные клетки, как-будто обмоложены, проявлаяют признаки повышенной витальности.