Institute of Experimental Medicine of the Hungarian Academy of Sciences, Department of Morphology, Budapest. *(Head: Prof. I. Törő)*

PLASMATIC BUDDING IN ASCITIC TUMOUR CELLS OF MICE UPON THE ACTION OF JANUS GREEN B

Gy. Rappay and *T. Barka*

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Plasmatic buds are known to arise in Ehrlich's ascitic tumour cells suspended in a dilute solution of Janus green B. This phenomenon was also observed by *Lettré* [1, 2, 3, 4] in cells from connective-tissue, epithelium, and carcinoma cultures. His early experiments were carried out with Victoria blue, but later he observed that this action of inducing plasmatic buds was inherent in numerous other substances (cytotoxins, dyes, antibiotics). Although the most diverse chemicals were found to display this effect, in *Lettré*'s view it was specific, and could be traced back to an inhibition of the respiratory and the mitochondrial activitiy, respectively. *Lettré* also established that this process was pervented by ATP and it was upon this that he built up his theory assigning to ATP an important role in the maintenance of the cellular surface structure.

Cellular changes of a similar character, apparently analogous in regard to their mechanism, were observed also in the abdominal cavity of the mouse *(Homann,* [5]) and in tissue cultures. Besides, in the literature there are references to a direct or indirect part being attributed to snapped off portions of cell or cytoplasm in the proliferation of tumour cells *(Revutskaja,* [6]).

Experimental observations and discussion

In tumour cells suspended in Ringer's or in physiological saline Janus green B solution diluted 1 to 10 000 (1 ml of Janus green B solution + 0,05 ml of ascites fluid), plasmatic buds in varying numbers and of different size became visible at room temperature within from 15 to 20 minutes (Fig. 1). Plasmatic buds of a similar character were observable in ascites fluid as well, but they were far less in number. On studying the suspension under the phase-contrast microscope, the buds were found to be forming gradually (Fig. 2). The series of pictures in Fig. 2 was taken of one single cell. In this, budding attained its maximum in 23 minutes. The phase-contrast microscope also showed that the budding portion of the cytoplasm did not contain the mitochondria, and that it appeared optically homogeneous. The budding portion might detach

itself from the cell, and appear in the suspending fluid as a spherical homogeneous structure. Exposed to methyl green pyronine, the buds stained with pyronine, indicating thereby the presence of RNA (basic cytoplasm and possibly micro-

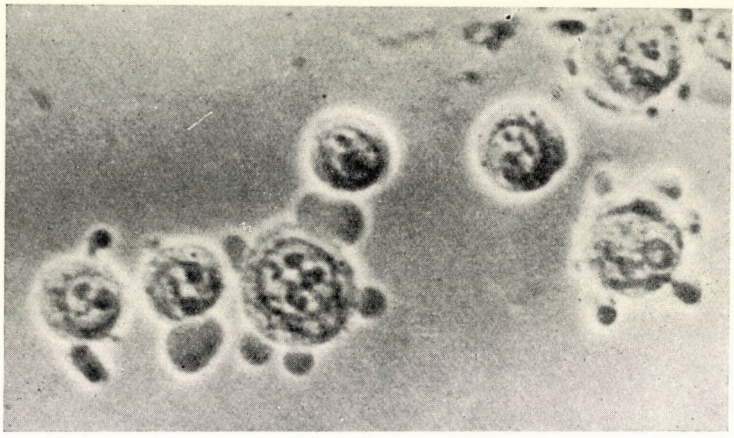

Fig. 1

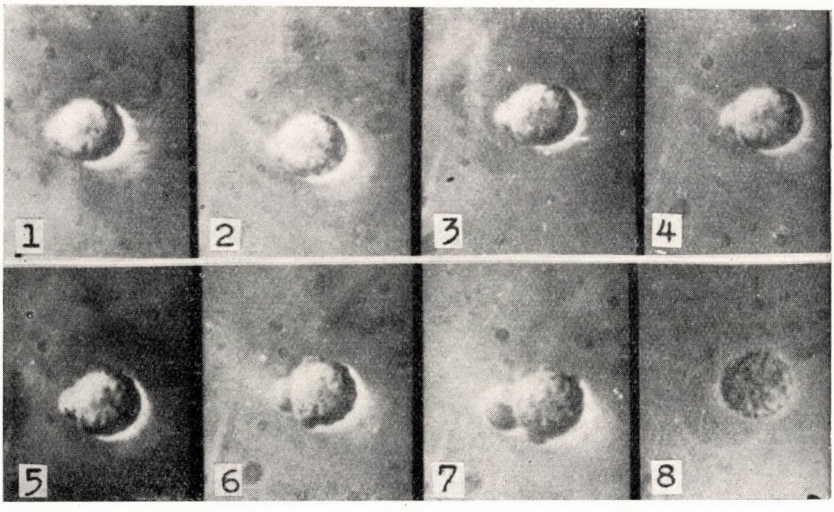

Fig. 2

somes). At 0° C about 10 per cent, and at 37° C from 90 to 98 per cent of the cells displayed budding.

As has already been mentioned, *Lettre*' found that the presence of ATP prevented the cytoplasm-budding. Yet, on adding to the suspension of the above composition 10 mg of neutralized ATP dissolved in a phosphate buffer

of pH 7,2, we observed that in the ascites cell ATP was unable to prevent plasmatic budding consequent upon the action of Janus green B. No budding occurred, however, if the ATP was added to the suspension without being previously neutralized, supposedly because of the pH effect asserting itself.

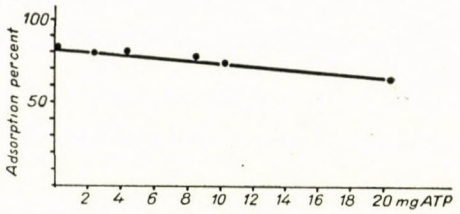

Fig. 3

Though not preventing the cytoplasm-budding, ATP was found to reduce to some small extent penetration of the Janus green B into the cell. Of tumour cells 200 mg (wet weight) were suspended in 3 ml of Ringer's solution containing Janus green B in dilution of 1 to 10 000 with increasing amounts of neutralized ATP (0,5—20 mg). The suspension was left standing for three minutes at room

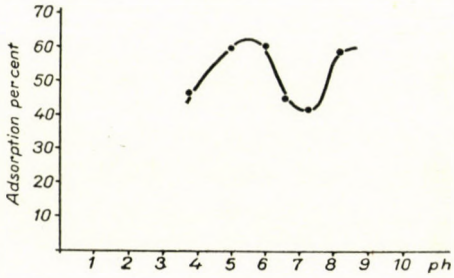

Fig. 4

temperature, then centrifuged for ten minutes at 3500 r.p.m. The light absorption of the supernatant was measured with Lange's photometer. As can be seen from Fig. 3, the decline in the rate at wich the dye had penetrated into the cells or had been adsorbed onto them, was approximately linear with the rise in the concentration of the ATP. On suspending equal amounts of cells (200 mg wet weight) in 1,5 ml of *Mc. Illoain* buffers of different pH (3,8 to 8,0), and mixing them to an equal volume of Ringer's solution containing Janus green B 1 to 5000, the rate at which the dye had adsorbed to and penetrated the cells was found to depend on the pH value as well (Fig. 2).

On the basis of the above experimental results, and duly considering time, temperature, and pH, we arrived at the conclusion that the formation of the cytoplasm-budding and their snapping off were indicative of some process

of enzymatic nature, and that the conditions of diffusion were unlikely to be playing a primary part. It being known from the literature that Janus green exerted an effect on oxidative processes (*Lazarov*, *Cooperstein*, [7]), we deemed it necessary to examine the question as to whether the different enzymatic inhibitors were bringing about cytological changes similar to those induced by Janus green B? To tumour cells suspended in 0,05 ml of Ringer's solution 0,1 ml was added from a solution of potassium cyanide, mono-iodo acetic acid, urethane, sodium arsenate, sodium fluoride, and dinitrophenol, respectively, in a concentration of M/1000. None of these cytotoxins was found to be able to produce an effect similar to that of Janus green B, either in itself or in combination with any of the others. It was, however, established that monoiodo acetic acid and urethane were each capable of blocking plasmatic budding caused by Janus green B.

The mechanism of the phenomenon observed is unknown. It is probable, in our opinion, that the buds and the snappings off are outcomes of complex processes. As no such effect is elicited by individual enzym inhibitors or by combinations of them, we think it cannot be explained solely by an inhibition of mitochondrial respiratory activity, as *Lettré* would have it. It might at any rate be indicative of the mitochondria's being instrumental in the maintenance of the normal structure of the cell wall. Clearance of the mechanism of the phenomenon, and of the question as to whether it is due to purely energetistic factors or to the interference of some other, principally adsorption factors, requires further studies.

It has not been possible for us to follow up the further fate or development of the snapped off parts. We think they are of but secondary importance from the point of view of tumour cell proliferation.

Summary

Plasmatic budding and snapping off have been observed to arise in Ehrlich's ascitic tumour cells upon the action of Janus green B. Neutralised ATP has been found unable to inhibit this process. Monoiodo acetic acid and urethane have each been observed to be able to prevent plasmatic budding caused by Janus green B. The further fate and development of the snapped off parts have not been followed up.

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ДЕЙСТВИЕ ЯНУСОВОЙ ЗЕЛЕНИ "B" ПРИ АСЦИТЕ МЫШЕЙ НА ОБРАЗОВАНИЕ ВЫБУХАНИЙ ПЛАЗМЫ В КЛЕТКАХ ОПУХОЛЕВОЙ ТКАНИ

ДЬ. РАППАИ и Т. БАРКА

Авторы наблюдали в клетках ткани асцитной опухоли Эрлиха вызванные действием янусовой зелени "B" выбухания или же отшнурования плазмы. Нейтрализированным аденозинтрифосфатом (АТФ) нельзя препятствовать этому процессу. Моноиодуксусная кислота и уретан могут препятствовать вызванному янусовой зеленью "B" выбуханию плазмы. Дальнейшую судьбу или развитие отшнуровавшихся частей авторы не наблюдали.

György *Rappay*, Budapest, IX., Tűzoltó u. 58. Hungary.
Tibor *Barka*, Budapest, IX., Tűzoltó u. 58. Hungary.