Experiments on the Tumour Cells Using Time-Lapse Microcinematography

In cells of rat Walker's tumour[1], human uterine and mammary carcinoma[2,3], and of other tumours[4], it has been ascertained by means of deoxyribonuclease that particular protein globules derived from aggregation of ribosomes could be formed. It had been inferred that ribosomes were concerned, not only from cytochemical examination[4], which had revealed the existence of RNA in the globules, but also from observations under the electron microscope[4], which had shown how these globules derived, in fact, from the union of granulations that corresponded entirely to ribosomes. On the basis of many observations[5–7] it had been found, however, that in the oocytes of certain species of molluscs and echinoderms the yolk globules formed through the aggregation of granulations similar to ribosomes. Moreover, in the same yolk globules the presence of RNA had been demonstrated[8–10] at the beginning of their formation.

Further experiments have now been performed in order to ascertain the possibility of using time-lapse microcinematography to observe the formation of protein globules in the cells of Walker's tumour. For this purpose, fragments of tumour were placed at room temperature (20–22 °C) in an aqueous solution of deoxyribonuclease (crystalline of Light and Co. or of Sigma Chem. Co.: 0,5 mg/ml) and then enclosed between a slide and a coverslip slightly separated. They were also stained directly with a 1% solution of toluidine blue in ordinary physiological solution. For the film sequences, usually taken at a speed of 1–2 frames per sec, a Zeiss plankton microscope was used, fitted in some cases with a phase-contrast system and in other cases with a Normarski's interference contrast system. The objectives usually employed had magnifications of 40 × and 100 × i.o.; when the 40 × objective was used, it was also possible to follow the manifestations of the cells by means of a telecamera connected to a monitor.

The results of the tests carried out were mostly positive; in fact, in the cytoplasm of cells located on the periphery of the fragments, it was normally possible to observe the gradual formation of globules after 8–10 min. By interference contrast these appeared fairly considerable, measuring between 0.5 μm and 1.5 μm, with a tendency to form more or less numerous groups. Usually, after 30 min. at least in the peripheral cells, no further globules were formed. The use of toluidine blue enabled the globules formed to be stained in progressive degrees up to the maximum intensity of orthochromatic colouring; the same colouring was reached, almost simultaneously, by the nucleolus.

The positive result of these other experiments would appear to allow it to be stated that, in the cells of Walker's tumour, it is possible, by artificial means, to cause globules to form through aggregation of ribosomes. Their ribosomal nature is proved by their affinity for toluidine blue, which disappears as a result of digestion with ribonuclease; the globules observed by microcinematography correspond entirely to those of the preparations obtained equally with deoxyribonuclease and then fixed[3]. The present result confirms once more what one of us maintained[1]: namely that the tumour cell is incapable of organizing the ribosomes that reach the cytoplasm in large quantities from the nucleus, or, more precisely, from the nucleolus, the probable site of the tumoural alteration. In this respect we mention that, in normal cells, there always exists a parallelism between RNA basophilia of the nucleolus and the cytoplasm and a capacity for synthesis; this can readily be shown in the oocytes for the purposes of the yolk globules production: in these, the more marked is the degree of nucleolar and cytoplasmatic basophilia attained in the previtellogenesis, the more conspicuous is the synthesis product elaborated in the vitellogenesis[6]. A similar correspondence exists in somatic cells; a scale of decreasing quantities of RNA – and hence of decreasing capacity of synthesis – has been compiled[11]: the first place is occupied by the pancreas, the last by the heart. The tumour cell, on the other hand, though showing a high basophilia due to RNA, does not reveal any concrete synthesis product. The fact of having succeeded, in various types of tumour cells, by means of a contrivance, in provoking the constitution of a product in the form of globules should leave no further doubt regarding an aspect of its alteration: namely, that there is a system for which an abnormal production of ribonucleoproteins is not followed by the product of the synthesis activity. This idea is also supported by the fact, already recorded[6], that, whereas is any normal cellular system there is always – relative to the syntheses – an adaptation of the cytoplasm volume to the nuclear and nucleolar volume, in the tumour cell system this adaptation does not exist: the volume of the cytoplasm does not increase in relation to that of the nucleus and the nucleolus.

Riassunto. Viene descritta mediante riprese microcinematografiche a tempo in cellule del tumore di Walker trattate con desossiribonucleasi la formazione graduale di globuli conseguenti alla aggregazione di ribosomi. Si Ritiene pertanto che venga confermata l'ipotesi che nella cellula tumorale esista una condizione particolare per cui, ad una anormale produzione di ribonucleoproteine, non segua la produzione di concreti prodotti di sintesi.

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[1] A. Bolognari, Nature, Lond. *190*, 358 (1961).
[2] A. Bolognari, M. P. Albanese and A. Donato, Nature, Lond. *200*, 809 (1963).
[3] A. Bolognari, M. P. Albanese and A. Donato, Antol. Med. Igiene *1*, 5 (1963).
[4] M. P. Albanese, A. Bolognari and A. Donato, Atti Soc. Peloritana Sc. F. M. N. Messina, *10*, 269 (1964).
[5] A. Bolognari, Atti Soc. Peloritana Sc. F. M. N. Messina, *7*, 1 (1961).
[6] A. Bolognari, Boll. Zool., *28*, 597 (1961).
[7] A. Bolognari and U. Faraone, Atti Accad. pelorit. Messina *50*, 51 (1970).
[8] A. Bolognari and A. Donato, Nature, Lond. *199*, 697 (1963).
[9] A. Bolognari and A. Donato, Acta Histochem. *19*, 285 (1964).
[10] A. Donato, L. D'Este and D. Teti, Experientia *23*, 433 (1967).
[11] A. Ficq, Inst. Int. Sci. nucl. Monographie 9 (Bruxelles 1961).