provide a practcal mieans of reducing the severity of the potentially lethal secondary effects of ionizing radiation in the human GI tract.

Zusammenfassung. Nachweis, dass die Gallensalze an der Entstehung des gastrointestinalen Strahlensyndroms mitverantwortlich sind und dass DEAE-Sephadex die Gallensalze bindet und die Überlebenszeit letal behandelter Ratten verlängert.

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26 October 1971.

Characteristics of the Nucleolini Observed Under the Photon Microscope

The term 'nucleolini' is an old one[1] and serves to designate tiny bodies, more or less spherical, that appear in the nucleolus, expecially in relation to a particular functional stage of it[2,3]. While some authors maintain that in the nucleolus there exists a skein-like filament that is to be termed 'nucleolonema'[4,5], other authors consider that the structures actually present in the nucleolus are the nucleolini[6,7] or, at any rate, nucleolar granules[8]. Unfortunately, up to a certain time ago, the use of silver nitrate as impregnation substance[9] often gave rise to super-impregnations that gave the mistaken idea that there actually existed in the nucleolus a filament wound like a skein: namely, the nucleolonema. However, when initially the electron microscope[10,11] showed the existence of a sort of reticulum, this was mistakenly identified with the nucleolonema[12]. And even if one of the authors – namely BERNHARD[13,14] – that contributed in creating this mistaken idea later stated how this reticulum should be considered – that is, not ascribable to the nucleolonema (though he proposed keeping this term) – many subsequent authors have continued to assign it, in fact, to the nucleolonema[15]. Fortunately, at present, in many works on the ultrastructure of the nucleolus, there is mention of its occasional reticulum-like appearance but no longer of 'nucleolonema'[16].

The object of this note is to make a further contribution to the knowledge of the nucleolini, as regards both certain typical aspects of them and their number in relation to dimensions of the nucleoli.

Materials and methods. The material chosen was the following: oocytes of molluscs, echinoderms, anuran and urodelan amphibians; cells of various tissues of vertebrates, particularly those of the nervous tissue; cells of Walker's tumour in the Rat and of human mammary carcinoma; and cells of the root apexes of the plants *Allium cepa* and *Vicia faba*. For the nucleolini, the method of impregnation especially used was that employing platinum chloride[9]. For the nucleolus, material fixed in

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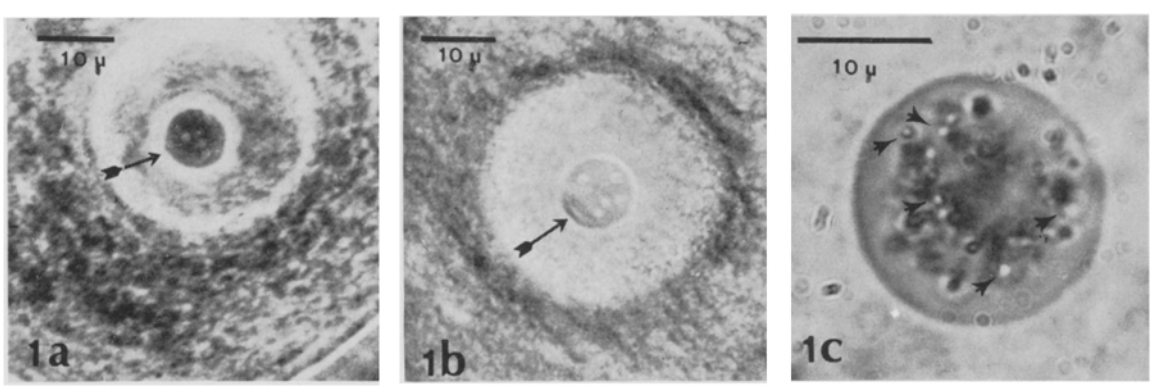

Fig. 1. Oocytes of *Paracentrotus lividus* observed in vivo under the phase-contrast microscope (a) and under the Nomarski's interference phase-contrast microscope (b): the arrows indicate the nucleoli with nucleolini. c) Nucleolus of *Echinus melo* oocyte in a preparation using platinum chloride; there are numerous nucleolini with a high refraction (arrows).

Correlation table of *Echinus melo* oocytes nucleoli

		Nucleolini number													
	Y/X	6	12	18	24	30	36	42	48	54	60	*fy*	Y-B	*fy*(Y-B)	*fy*(Y-B)²
Nucleoli	10					1		1	1			3	5	15	75
diameter (in μm)	9			1	1	3	6	2	1		1	15	4	60	240
	8		1	1	3	2	1		1	1		10	3	30	90
	7	1	3	5	3	1	2	1				16	2	32	64
	6	3	3	5	2							13	1	13	13
	5	3	3									6	0	0	0
	4	16	1									17	−1	−17	17
	3	23	1									24	−2	−48	96
	2	7										7	−3	−21	63
	1	4										4	−4	−16	64
fx		57	12	12	9	7	9	4	3	1	1	115	5	48	722
X-A		−24	−18	−12	−6	0	6	12	18	24	30	30			
fx(X-A)		−1368	−216	−144	−54	0	54	48	54	24	30	−1572			
fx(X-A)²		32832	3888	1728	324	0	324	576	972	576	900	42120			
Σ *fxy*(X-A) (Y-B)		2256	−162	−264	−126	0	186	180	216	72	120	2478			

$\overline{X} = 16.3$ $\overline{Y} = 5.4$ $\sigma^2x = 178.57$ $\sigma^2y = 6.04$ $P = 26.98$ $r = 0.82$ $by/x = 0.15$

various ways (Zenker, Helly, Bouin, Carnoy) was stained by the methods of Mallory, Unna-Pappenheim, Dominici and 1% toluidine blue; the existence of RNA was demonstrated by means of checking with ribonuclease. The Feulgen-Rossenbeck test was carried out on preparations fixed in Carnoy and Helly. Observations were made not only with the ordinary microscope but also, especially in vivo, with the phase-contrast microscope, the Nomarski's interference phase-contrast microscope, and the polarizing microscope.

Results and discussion. The results obtained may be summarized as follows: 1. Nucleolini are present in any type of cell whatsoever: animal or vegetable, somatic or germinal, normal or tumoral. It is not possible to discover them either in the nucleoli, immediately after mitotic division, or in the oocytes or in neoplastic cells at the beginning of their growth. Normally, while they are absent in relatively small nucleoli, they are present in the larger nucleoli in numbers that increase with the volume attained by the latter.

2. The nucleolini mostly appear spheroidal, though it is possible to find more or less elongated examples. In every nucleolus, their distribution, as well as their size (from 0.25 μm to 1.8 μm), is highly varied.

3. As a result of impregnation with platinum chloride (Figure 1c), the nucleolini appear as of an intense garnet red colour, which stands out against the background of the nucleoli, which is emerald green. As a result of varying

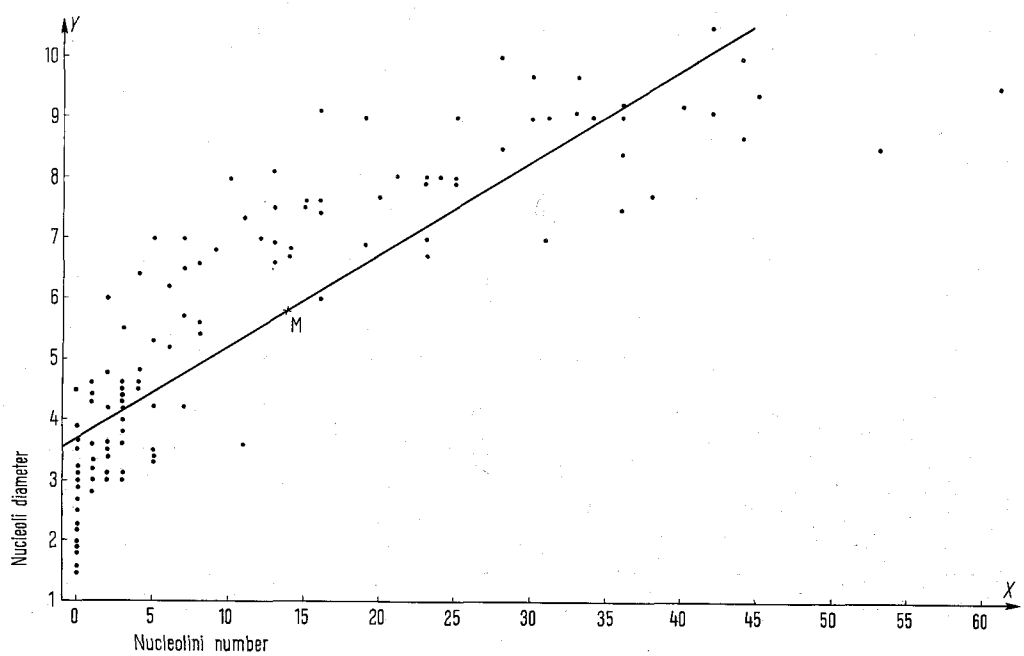

Fig. 2. Dispersion diagram of the nucleoli diameter and nucleolini number of *Echinus melo* oocytes.

the angle of incidence of the light of the microscope, as is normally achieved by turning the micrometer screw, the garnet red colour tends towards a yellow tone. Under the microscope with polarizing filters set at 90°, the nucleolini appear divided into 4 very bright quadrants by a black interference cross. In the present state of our studies, it appears that the nucleolini possess no staining affinity and that even less do they have positive cytochemical reactions. There is not even a Feulgen-positive reaction. One can be deceived into thinking that a reaction of this kind exists by the characteristic garnet red colour of the nucleolini; however, it is easy to check this by varying the angle of incidence of the light of the microscope. Under the polarizing microscope, in the preparations obtained by the ordinary techniques, the nucleolini still show the characteristic black interference cross.

4. Nucleolini may coexist, in one and the same nucleolus, with one or more vacuoles. There is a very sharp difference between them, since the former show high refraction, which is not revealed by the content of the latter.

5. Nucleolini are especially abundant in the nucleoli of vitellogenetic oocytes and developed neoplastic cells, and this is found in concomitance with the following conditions presented by these nucleoli: a more or less marked affinity for the aniline blue of Mallory's method, little or no affinity for the pyronine of the Unna-Pappenheim method and the toluidine blue of the Dominici method. A check with ribonuclease shows that the affinity for pyronine and toluidine blue is due to RNA. Thus it is that the nucleolini are found in greater numbers in those nucleoli in which there is a condition of lower density of their constituents[2], as also of a smaller quantity – or possibly the absence – of ribonucleoproteins.

6. In the cells observed in vivo, the nucleolini are evident, especially in nucleoli of oocytes and neoplastic cells. They appear of a faint red colour, which turns to a more yellow tone as the angle of incidence of the light of the microscope is varied; these colours stand out against the background of the nucleoli, which is faintly green. Under the phase-contrast microscope, as also under the Nomarski's interference phase-contrast microscope (Figure 1 a and b), the nucleolini appear more evident than under the ordinary microscope. Under the microscope with polarizing filters set at 90°, it is not possible to identify the black interference cross.

Regarding the earlier statement that the greater the volume of the nucleoli the more numerous were the nucleolini, previous studies[17] had been carried out on the oocytes of *Bufo vulgaris*, which are polynucleolate. Also RAMON CAJAL[18] had pointed out, in nerve cells, a relationship between the number of the nucleolar granules and the dimensions of the nucleoli. We have now turned our attention to the oocytes of the Echinoid *Echinus melo*; during their whole growth these oocytes have only one nucleolus. For the species under examination, the criteria adopted for elaborating the statistics were the same as those employed for the oocytes of *Bufo*; the measurements were taken in 115 nucleoli of different oocytes.

The smallest nucleolus diameter was 1.5 μm, the largest 10.5 μm; the maximum number of nucleolini counted was 61, a number that may be considered as the experimental limit of the count. Up to a nucleolus diameter of 2.7 μm no granules were found. The mean values obtained were: diameter of nucleoli: 5.8 μm; number of nucleolini: 13.7. With the values found, the dispersion diagram (Figure 2) was drawn up relative to nucleoli diameter and number of nucleolini, as well as the correlation Table. From these were obtained a coefficient of regression (by/x) equal to 0.15 and a coefficient of correlation (r) equal to 0.82. From the diagram it is easily deduced that the number of nucleolini increases in relation to the increase in the nucleolar diameters. However, the distribution of the points does not appear uniform along the line of regression, since there are maximum values of one dimension that do not correspond to the maximum values of the other. Since the coefficient of correlation is equal to 0.82, we can equally assert that between the two magnitudes considered in the oocytes of *Echinus melo* there is a significant correlation on the statistical level.

Riassunto. I risultati delle ricerche condotte al microscopio fotonico sul nucleolo di cellule vegetali e animali, somatiche e germinali, normali e tumorali hanno permesso di affermare ancora una volta che le sole strutture presenti in esso sono i nucleolini. L'esistenza dei nucleolini è stata comprovata in vivo, come pure con varie tecniche in seguito ad osservazioni compiute con diversi tipi di microscopio. L'analisi statistica effettuata su ovociti di *Echinus melo* ha permesso di confermare che i nucleolini, che sono assenti nei nucleoli più piccoli, sono poi tanto più numerosi per quanto più elevato è il volume dei nucleoli più grandi.

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Morphological Basis of Follicle Cells - Oocyte Interaction in Normal Pupae and Isolated Pupal Abdomina of *Galleria mellonella* L.

The complex structure of follicular cell/oocyte interface in vitellogenetic insect oocyte was observed by several authors. However, the observations on the changes of its pattern in the course of later development of the egg vesicle in normal and hormonally disturbed conditions have not been signalized. The present study gives some informations on this subject.

Material and methods. The follicular cell/oocyte interface was studied in the egg vesicles of *Galleria mellonella* L. bred on bee's wax under laboratory conditions. The

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Fig. 1. Oocyte (ooc.)/follicular epithelium (f. ep.) interface in terminal egg vesicle of 6th-day pupa. Numerous pinocytotic canaliculi and vesiculi are seen. m, mitochondrium. Fig. 2. Fragment of a similar region as in Figure 1. The oocyte and follicular epithelium plasmalemma are marked. Fig. 3. Oocyte/follicular epithelium interface in egg vesicle at early vitellogenesis. The agglomeration of electron dense material filling the interspace of these cells is visible. Pinocytotic vesicles marked by arrow. vm., vitelline membrane. Fig. 4. Oocyte/follicular epithelium