Polyploidy and Aneuploidy in Au 198 Irradiated Rat Liver

The appearance of giant cells in irradiated somatic animal tissue and tissue culture of varied sources is a fact that has been systematically observed and reported[1–9]. Some experimental evidence brought to light in recent years seems to support the general view that this phenomenon is due to an irreparable damage to the cell mitotic mechanism without a concomitant alteration in the synthesis of DNA and protein[6,10].

Here we report and comment on some quantitative data obtained by measurements and countings in livers of Au198 treated rats. Inbred male rats of the August strain were injected intravenously with 15 μc/g of a colloidal solution of Au198. The animals were killed or died after various times following the injection and the livers were removed and processed for examination. In sections 5 micra thick and stained by the Feulgen method, the nuclear diameters were measured directly under oil immersion lens, with a micrometer eyepiece. Only spherical or near spherical nuclei were measured in microscopic fields selected as a row of one field width from each other. 100 nuclei were selected for measurements. 500 nuclei were counted for calculating the % of binucleates, micronuclei and mitosis. The histogram and Tables I and II represent countings and measurements in 5 animals.

The analysis of the histogram shown in the Figure reveals some interesting facts occurring in the irradiated livers. We observe (1) a prolonged latent period between injections and the appearance of parenchymal changes, (2) a striking lateral spread of the histogram towards higher classes of ploidy that continue to take place even at a time when the radioactivity present should be negligible, (3) a complete disappearance of $2n$ nuclei in the irradiated livers examined 2 months following the injection and a progressive reduction in the frequency of $4n$ nuclei, (4) a remarkable degree of aneuploidy.

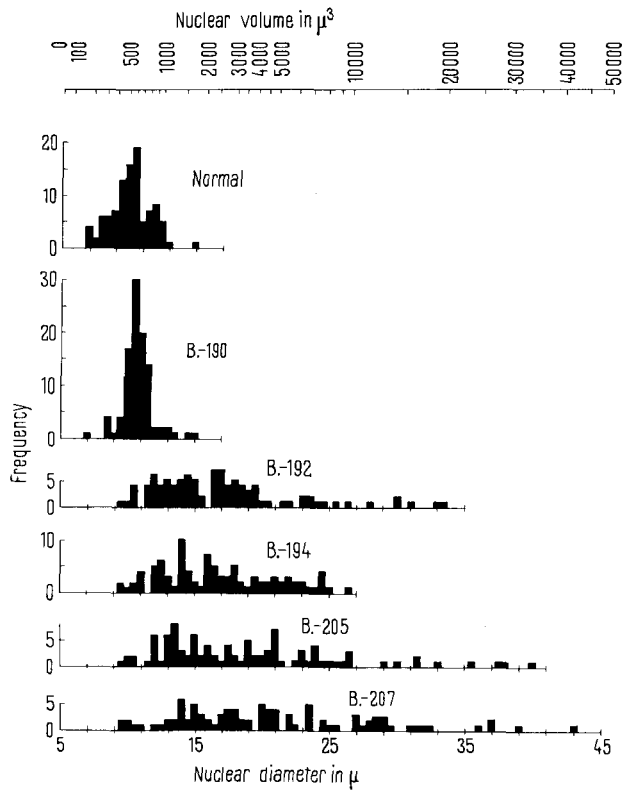
A measurable reduction in DNA synthesis is detected only after high doses and is a transient phenomenon[11–13]. Blockage of mitosis can be achieved with smaller doses and resumes much later than DNA synthesis. The first month after injection corresponds to the period when practically the whole of the administered gold decayed to stable form. Throughout this period of time, both the DNA synthesis and mitosis were blocked and conse-

Table I. % of polyploids and aneuploids taken together, binucleates and mitosis in Au198 irradiated livers (see references in the text)

Rat	Killed (days after injection)	Nuclear volumes $> 8n$ (%)	Bi-nucleates (%)	Micro-nuclei (%)	Mitosis (%)
Normal	–	1	8	0	0
B.190	30	3	10.6	0	0
B.192	64	84.2	12	3.2	0
B.194	74	85.7	8.2	3.4	0
B.205	84	87	10.2	2	0
B.207	93	87	8.2	3.8	0

Table II. Volumetric series observed in livers examined from the second month on after the administration of Au198

Rat B.192 (μ^3)		Rat. B.205 (μ^3)	
Euploid series	550, 1100, 2250, 4000, 8500, 16,000	Euploid series	550, 1200, 2000
Aneuploid series	800, 1550, 3000, 5500, 12,000 6900, 14,500 9500, 19,000	Aneuploid series	850, 1700, 3600, 7200, 14,500, 28,000 2700, 5000, 9500, 19,000 6700, 13,000 17,000, 34,500 23,500
Rat B.194 (μ^3)		**Rat B.207 (μ^3)**	
Euploid series	490, 950, 2000, 4000, 7900	Euploid series	500, 4200, 7900, 27,000
Aneuploid series	700, 1490, 3000, 5900 5000, 9500	Aneuploid series	1490, 3000 1700 5600, 10,500 6500, 13,000, 25,000 42,000



Histogram (see description in the text).

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quently no volumetric changes could be detected. From the first to the second month, the liver cells progressively resumed their DNA synthesis activity while the mitotic recovery was still lacking. The outcome of this is the appearance of giant cells as the regenerative process tries to compensate for the cell necrosis induced by the irradiation.

The high frequency of aneuploids deserves special comment (Table II). The lateral spread of the histogram accompanied by a disappearance of $2n$ nuclei and a progressive decrease in the frequency of $4n$ nuclei suggest that the aneuploidy is not due to abnormal mitosis with unequal distribution of chromosomes between the daughter cells, but more likely results from asynchronous endoreduplication of chromosome sets. The constancy in frequency of the binucleates shows that the giant cells are not produced as a consequence of mitosis with a common spindle in these elements. Micronuclei and bizarre nuclei were observed 2 months after injection, when the liver cell population was almost entirely composed of polyploids and aneuploids. The presence of these aberrant elements indicates that few cells resume their mitotic activity although faulty mitosis is the result. Again, the absence of mitotic figures together with the observation that the % of micronuclei is kept constant from the moment of their appearance throughout the whole period of observation, suggests that those cells that regain their divisional capacity died after a few divisions, sometime between 30 and 64 days after the gold administration (Table I).

The occurrence of polyploidy and aneuploidy and the progressive increase in the frequency of chromosome aberrations in aging mammal liver is a fact already established and well documented[14–17]. Irradiated livers look 'older' when compared to the same organ of the intact animals of the same age. Polyploidization and aneuploidization in normal livers can be understood as a phenomenon peculiar to reverting post-mitotic cells in which the usual process of cell turnover by means of mitosis does not take place at a proper rate and where the 'physiological' cell loss is deficiently compensated by increasing the size of the remaining elements. By inducing necrosis and concomitantly blocking mitosis almost indefinitely, what radiation did in our material was to accelerate a physiological process or, to say it in a rhetorical way, to condense the biological time of the system under consideration. It is highly probable that polyploids and aneuploids, due to their unbalanced chromosome sets, are defective elements within the whole system and are unable to cope properly with the metabolic tasks that are the burden of the liver cells. As a matter of fact, KOLETSKI[2] has already demonstrated by means of the bromosulphalein test that these giant cells are functionally incompetent.

If we define aging as a progressive and irreversible disruption of form and impairment of function that leads to gradual decrease in the resistance to environmental stress, we might perhaps say that as the number of these giant elements increases the liver 'ages'. The experimental results discussed above and expressed in the histogram and Tables I and II, confirmed previous observations by others, revealed some new aspects of the irradiation effects, and suggest the following conclusions: (a) At high doses of radiation both the DNA synthesis and mitosis are inhibited. (b) The DNA synthesis resumes much sooner than the mitosis activity after irradiation. A number of cells never recover their divisional capacity, indicating that the DNA synthesis and mitosis although related to each other are controlled by different and independent mechanisms. (c) Regenerative effort in a cellular system where mitosis is inhibited leads to progressive polyploidization and aneuploidization. (d) Polyploidy and aneuploidy in our material seem to be due to synchronous and asynchronous endoreduplication of chromosome sets and not to aberrant mitosis. (e) Cells that recover their divisional capacity following high doses of radiation seem to die after a few divisions. (f) The morphological and quantitative similarities between radiation induced changes and aging changes in rat liver, indicate a common biological mechanism underlying both processes. Polyploids and aneuploids being physiologically incompetent cells, we suggest that their increased frequency with time is the 'cause' of aging in livers of rats.

Résumé. On a etudié l'induction de polyploidie et aneuploidie dans les foies des rats à la suite d'injection intraveineuse d'or colloidal radioactif. On souligne l'analogie entre ce phénomène et l'observation de phénomènes semblables faites sur les foies normaux d'animaux âgés. On suggère une relation 'causale' entre l'augmentation de la fréquence de ces éléments polyploidiques et aneuploidiques et le vieillissement hépatique.

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Fluorescein Staining of Guinea-Pig Lymphocytes Induced by *Echis colorata* Venom

In the course of a study[1] on the action of *Echis colorata* venom on the blood-brain barrier, it was found to affect the permeability of guinea-pig lymphocytes. Guinea-pigs, weighing 250–300 g, were injected intracardially with a mixture of 100 μg (protein content[2]) *Echis colorata* (EC) venom and 25 mg fluorescein (Fluorescite, Moore Kirk Laboratories Inc., Worcester, Mass.) in 1 ml of saline solution. Such animals died within 15–30 min. Blood samples were obtained by cardiac puncture 5, 10 and 15 min after injection of the mixture. Figure 1 shows fluorescein-stained lymphocytes from an animal injected with venom and fluorescein. The fluorescence-inducing action of the venom was manifest at all 3 time intervals. Fluorescein-staining of lymphocytes was observed in all animals in which lethal venom doses, i.e. a minimum of 20 μg of venom, were administered. It should be noted that in the control non-venom-treated animals the platelets became strongly fluorescent (Figure 2). The absence

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