According to the results, ionizing radiation seems to have a sensitizing effect on DNA subjected to the action of Natulan. This may be explained by the formation of unstable peroxides during irradiation[9], which yield free radicals as a result of their decomposition[10]. SCHOLES et al.[11] have demonstrated that the organic peroxides formed by X-irradiation of DNA in the presence of oxygen show a marked post-irradiation decay which mainly takes place within a few hours after termination of the irradiation. The free radicals formed during this decay may act as initiators[12] of the autoxidation of cytotoxic methylhydrazine compounds[1]. The higher rate of autoxidation will result in an increase of both the formation[1] and of the activation[13] of hydrogen peroxide and thus enhance the degradation of DNA.

Zusammenfassung. Die kombinierte Einwirkung von ionisierender Strahlung und N-Isopropyl-*p*-(2-methylhydrazinomethyl)benzamid hydrochlorid (Natulan®) führt zu einem wesentlich stärkeren Abbau von Desoxyribonucleinsäure (DNS) als auf Grund der linearen Superposition zu erwarten wäre. Der Synergismus ist am ausgeprägtesten, wenn der Zusatz von Natulan unmittelbar nach der Bestrahlung erfolgt. Dieser Effekt kann als Folge der Bildung instabiler Peroxide während der Bestrahlung erklärt werden. Bekanntlich zerfallen die bei der Bestrahlung entstandenen organischen Peroxide zum grössten Teil innerhalb der ersten zwei bis drei Stunden nach der Bestrahlung unter Bildung freier Radikale. Diese können als Startradikale bei der Autoxydation von Natulan wirken und daher den Abbau von DNS beschleunigen.

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Determination of the Dry Mass of Nervous and Glial Cell Nuclei by Interference Microscopy[1]

BRATTGARD and HYDEN[2,3] and HYDEN[4] measured the dry mass concentration of nerve cells by means of X-ray microscopy and calculated indirectly the total dry mass of the same cells on the basis of the area.

This method is laborious and does not seem well suited for the determination of the dry mass of the nuclei. Interference microscopy seems to show marked advantages for this purpose, and in this paper the values obtained in the measurements of the dry mass of nervous and glial cell nuclei of albino rats are described.

Samples were taken from white matter (corona radiata, lumbar enlargement of the spinal cord) and from grey matter (lumbar enlargement of the spinal cord).

The tissue (5 mg) was dispersed in anhydrous glycerol (Merk) after previous freeze-drying, or directly in BARNES, ESNOUF, and STOCKEN fluid[5]. For preparation of the dry nuclei, the tissue was dropped immediately after dissection into semifrozen Freon 12 and rapidly transferred to the histological freeze-drying apparatus (TD2, Edwards Crowley). After completion of the drying, the tissue was dispersed in anhydrous glycerol by Potter-Elvejhem apparatus.

In the second case, the nuclei were dispersed directly in BARNES, ESNOUF, and STOCKEN[5] solution and the nuclear fraction was separated from the homogenate by centrifugation in a refrigerated centrifuge (Eispirouette, Phywe), which was rapidly accelerated to 3000 *g* and then left to decelerate until it stopped.

The nuclei prepared according to this method undergo a notable loss of the soluble proteins and their dry mass values correspond substantially to the content of insoluble proteins, whereas the nuclei prepared in anhydrous glycerol possess their full complement of proteins. The difference in the values obtained with the two methods gives an indication of the nuclear content of soluble proteins[6].

Nuclei were measured in a chamber about 40 μ thick (obtained by interposing thin mica foils between slide and coverslip) in order to maintain their spherical shape, which is altered by compression when they are placed directly between slide and coverslip.

Dry mass measurements were made with a Smith-Baker interference microscope using a × 100 'shearing' objective and 'half-shade' eyepiece. The formula used for the calculation of the total dry mass was:

$$M = KQA/100\alpha,$$

where M is the total dry mass, K is a constant (calculated by PELLEGRINO et al.[7]), Q is the optical path difference,

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Total dry mass, volumes and concentrations of glial nuclei in anhydrous and aqueous medium, determined by microinterferometry (Average values over 100 nuclei)

Glia nuclei from:	Frozen dried tissue			Fresh tissue		
	Dry mass pg	Volumes μ^3	Concentration pg/μ^3	Dry mass pg	Volumes μ^3	Concentration pg/μ^3
Grey matter of spinal medulla	30 ± 0.5	164 ± 4	0.18 ± 0.002	19 ± 0.1	147 ± 4	0.12 ± 0.002
White matter of spinal medulla	23 ± 0.4	147 ± 3	0.16 ± 0.003	17 ± 0.3	157 ± 3	0.12 ± 0.002
Corona radiata	21 ± 0.4	139 ± 3	0.15 ± 0.003	16 ± 0.2	140 ± 3	0.11 ± 0.002

A is the area of the nucleus, and α is the specific refractometric increment for proteins (in anhydrous glycerol 0.00095, in water 0.0018).

The formula used for the calculation of the volumes was:

$$4/3\ \pi\ a^2 b\ ,$$

where a = cross radius and b = longitudinal radius of the nucleus. (The radius was measured with an ocular micrometer.)

The glial nuclei were distinguished from neuronal nuclei according to the criteria established by NURNBERGER and GORDON[8].

The glial nuclei are grouped in a single class. Little difference is noticeable between glial nuclei of grey and white matter when the measurements are made in aqueous solution, while, in anhydrous glycerol, the dry masses of glial nuclei grey matter are larger than those of the nuclei from white matter (Table).

The loss of soluble substances (proteins) is 30% for glial nuclei from grey matter and 25% for those from white matter.

This difference is likely to represent a difference in function. A larger amount of soluble proteins in the glial nuclei from the grey matter can be assumed to be an expression of the involvement of these glial cells in the metabolism of the nerve cells[9].

Volume variations are negligible and there is no noticeable difference between nuclei in aqueous and anhydrous medium.

The neuronal nuclei show wide scattering both of dry masses and volumes (Figures 1 and 2). Histologically, volume differences have been demonstrated in the cat's lumbar enlargement by AITKEN and BRIDGE[9] and in the anterior horns of the cervical enlargement of the rat's spinal cord by SAKLA[10].

The dry mass concentration is light (0.20 pg/μ^3) in homogenated nuclei in anhydrous medium as reported by HYDEN[11]. The dry mass concentration in aqueous medium is 0.09 pg/μ^3; therefore the loss of soluble substances corresponds to 50% of total dry mass.

The distribution of values of the dry mass of neuronal nuclei remained constant through several experiments; they can be considered therefore as a representative sample of the lumbar enlargement of the spinal cord.

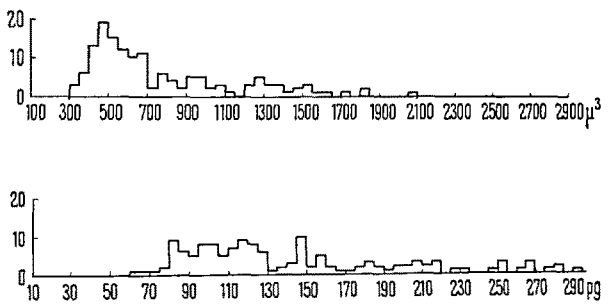

Fig. 1. Dry mass (pg) and volume (μ^3) of nervous cell nuclei of lumbar enlargement grey matter. Frozen-dried tissue dispersed in anhydrous medium. (Baker-Smith interference microscopy.)

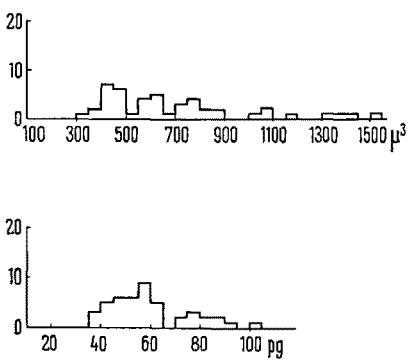

Fig. 2. Dry mass (pg) and volume (μ^3) of nervous cell nuclei of lumbar enlargement grey matter. Fresh tissue dispersed in aqueous medium. (Baker-Smith interference microscopy.)

Riassunto. In questo lavoro si descrive una nuova metodica per la determinazione della massa secca dei nuclei delle cellule nervose o gliali mediante il microscopio ad interferenza. Sono riferiti i valori della massa secca, dei volumi e della concentrazione dei nuclei isolati sia da tessuto liofilizzato che da tessuto fresco.

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