EFFECTS OF X-RAY IRRADIATION IN ASCITES TUMOR CELLS: PARTIAL RESTORATION OF DPN CONTENT AND DNA SYNTHESIS BY NICOTINAMIDE

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ROITT (1956) found that nicotinamide (NA) will prevent the inhibition of glycolysis induced by triethylenimine melamine (TEM) in ascites cells. HOLZER and coworkers (196o) extended these studies and showed that NA inhibits the radiomimetic effects of a number of ethylenimines and H_2O_2, whose action was thought to inhibit DPN synthesis. MAASS et al.(1958) showed that high doses of X-rays also produced a drop in DPN and inhibited glycolysis. Both effects could be reversed by NA. In this communication, we want to report on an apparent relationship between DPN content and thymidine incorporation into DNA of Ehrlich ascites tumor cells. Both parameters are affected similarly by X-rays and by nicotinamide respectively.

When cells[1)], after suspension in ascites serum and irradiation with 3ooo r, are incubated in 65% serum (fortified with glucose, $KHCO_3$ and H-3-thymidine) under air/CO_2 in the Warburg apparatus, the DPN content drops to 2o-5o % of the control value and the incorporation of labelled thymidine into DNA is inhibited to a similar degree (table I). The addition of NA to the incubation mixture (after irradiation)

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TABLE I

The influence of irradiation and nicotinamide on DPN content and thymidine incorporation into DNA.

NA	↯	DPN Σ mμM.	DPN % of control	DNA Σ cpm	DNA % of control
-	—	39.0	100 %	9 900	100 %
	3000 r	14.5	37 %	4 200	42 %
+	—	45.5	117 %	12 000	121 %
	3000 r	35.0	90 %	8 300	84 %

0.50 ml cells in 6.00 ml ascites fluid were irradiated when indicated at 2-5° with 3000 r. 1.30 ml of the suspension were pipetted into Warburg vessels (0°) containing 0.7 ml water with 50 μM. glucose, 50 μM. $KHCO_3$, 1 μC H-3-thymidine (1.9 μC/μM!) and 80 μM. nicotinamide when indicated. The vessels were incubated at 37° with shaking and gazing with air/CO_2 (95/5) for 10 min. Gas exchange was recorded for further 80 min, the vessels then placed in ice, the whole content transferred into small, all-glass homogenizer tubes with the aid of 2 x 2 ml ice-cold isotonic sucrose, and homogenized with 0.4 ml 70% $HClO_4$. After 10 min in ice, the tubes were centrifuged in the cold. In the supernatant, DPN was detd. after neutralization using cryst. alcohol dehydrogenase. The acid-insoluble precipitate was homogenized 3 x with ice-cold 4% $HClO_4$ and finally suspended in 2 ml water. Aliquots were counted on alu planchets in a gas flow counter or on small filter papers in a liquid scintillation counter.

counteracts these effects, restoring the values of the irradiated cells to nearly normal. In the non-irradiated control, addition of NA brings about an increase in DPN content and a correspondingly higher DNA synthesis. These values indicate a dependency of DNA synthesis (as measured by the incorporation of thymidine) on the DPN level in the cells. Similar findings are obtained if the cells are incubated with a variety of radiomimetic compounds like ethylene imines, H_2O_2 and organic peroxides including the carcinolytic bis-hydroxymethylene peroxide, active in vivo on Ehrlich ascites tumor cells (Weitzel et al.1961).

There is also a strong influence of glucose on the biochemical consequences of X-irradiation (table II). If irradiated cells are incubated under O_2/CO_2 in ascites serum with the addition of glucose, the cells show a 5o-9o % reduction[2] of DPN content and thymidine incorporation, whereas the same cells, incubated without glucose, are affected much less (a lo-25 % drop in DPN content and DNA synthesis). Under these latter conditions, the cells are forced to oxidize endogenous substrates and substrates in the ascites serum, which contains negligible amounts of glucose (WARBURG et al.1957). There must be sufficient sources to provide the required energy since gas exchange and DPN content remain nearly constant in contrast to anaerobic conditions without added glucose. Here, DPN is slowly degraded and DNA synthesis is negligible. On the other hand,

TABLE II

The influence of irradiation and glucose on DPN content and thymidine incorporation into DNA under different conditions

glucose	ϟ	DPN Σ mμM.	DPN % of control	DNA Σ cpm	DNA % of control
a)under O_2/CO_2:					
-	—	37.9	loo %	34 5oo	loo %
	3ooo r	29.6	78 %	27 6oo	8o %
+	—	35.7	loo %	11 8oo	loo %
	3ooo r	lo.o	28 %	2 7oo	32 %
b)under N_2/CO_2:					
-	—	27.2	loo %	(<8oo)	-
	3ooo r	6.6	24 %	(<4oo)	-
+	—	29.7	loo %	lo 4oo	loo %
	3ooo r	9.1	33 %	3 9oo	37 %

Experimental details and analyses as outlined in table I.

[2] depending on unknown factors related to age of cells and serum

under the conditions of anaerobic glycolysis with glucose added, the cells are able to preserve the DPN and to synthesize DNA at least for one hour. Surprisingly, glucose inhibits thymidine incorporation in non-irradiated cells to a considerable degree (table II). It is not known yet, if this is the result of a repression mechanism comparable to the action of glucose on enzyme synthesis in bacteria (MONOD 1941, COHN 1956, ROSEN_ BERGER 1961) or merely an isotope dilution effect.

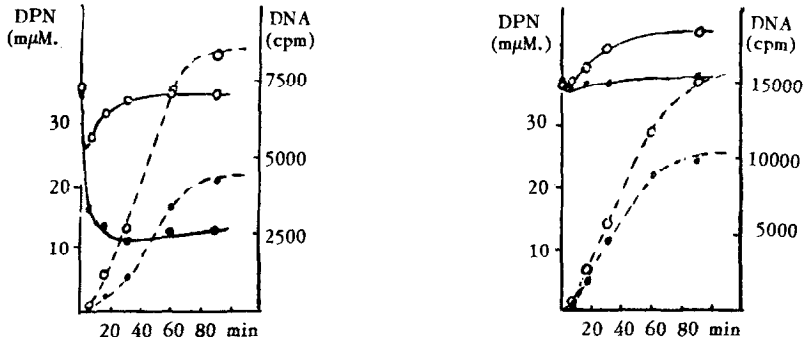
A kinetic study of irradiated cells reveals (fig.Ia) that the DPN content immediatly (1 min) after irradiation (at 2-4°) is identical with the control, but drops to its low level within 5-1o min after incubation. Even in the presence of NA, the initial fall of the DPN after irradiation becomes visible, but soon is overcome by an increased resynthesis of cozymase. Concomitantly with the restituted level of DPN, there is again a correspondingly higher incorporation of thymidine into DNA. Apparently, the rapid fall of the DPN content cannot be the result of an impaired DPN synthesis as suggested by HOLZER et al.(196o) for the action of ethylene imines, since there is a net increase of DPN between 5 and 3o min after addition of nicotinamide (fig.Ia). Nevertheless, with increasing irradiation values, the irradiated cells loose gradually the capacity to restaure the normal DPN levels on addition of NA, indicating a secondary and irreparable radiation effect on the DPN synthesizing system (HILZ and SCHOLZ 1961).

In another strain of Ehrlich ascites cells and in Yoshida ascites cells, the effect of irradiation on the mentioned parameters is less pronounced then in our strain. Still, a parallel behavior of DPN content and thymidine incorporation is visible again (Hilz et al. 1961). This parallelism is not dependent

FIGURE I

Kinetic study of DPN level and DNA synthesis ± nicotinamide.

DPN: -NA •——• +NA o——o DNA: -NA •---• +NA o---o



a) with irradiation b) without irradiation

on thymidine being the DNA precursor. Irradiation experiments with C-14-labelled ribose and orotic acid or with P-32-phosphate indicate the same relationship between DPN level and incorporation into DNA. It is not clear, if these findings are related to the DPNH-dependent formation of deoxycytidine phosphate from cytidine phosphate reported by ABRAMS and coworkers (1960).

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