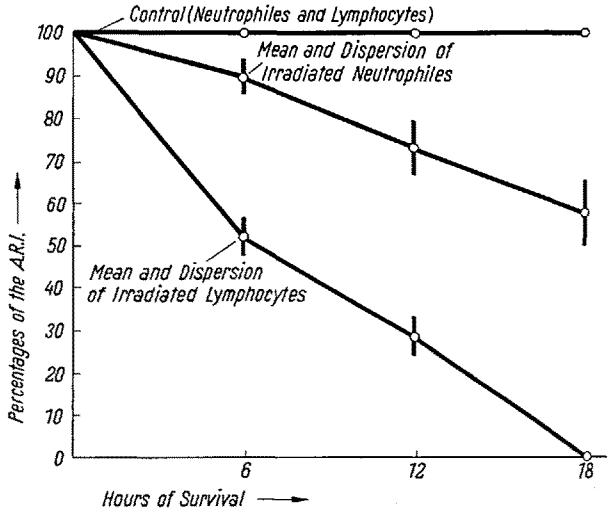
Experimental Investigations on the Influence of X-Rays on Glycogen Content in Surviving Leucocytes*

With regard to the influence that antimitotic agents may have upon normal tissues and cells during antineoplastic therapy, we have studied the influence of X-rays on the glycogen content of leucocytes.

The radiation was carried out directly on the cells surviving *in vitro*. In fact, during survival leucocytes keep their biological activities for a time, including the metabolic ones. In particular, with reference to the glycogen metabolism, glycogenolysis occurs in leucocytes during survival and they utilize their glycogen content (ASTALDI, BERNARDELLI, RONDANELLI[1]). Moreover, when leucocytes survive in a medium enriched in glucose, glycogenesis occurs and, as a consequence, leucocytes increase their glycogen content (ASTALDI, BERNARDELLI, RONDANELLI, and GORINI[2]). On the other hand, X-rays can diminish the A.P.S. positive structures of the connective tissue when patients are irradiated (PISANI and ROMANINI[3]), and also some cytotoxic substances can interfere with the glycogen content of the liver cells and of leucocytes, too (CARDINALI, CAPRINO and PISCITELLI[4]). Finally, regarding the influence of X-rays on the biological properties of surviving leucocytes, ASTALDI and MASSA[5] have shown an increase in their osmotic fragility.

The researches were carried out on leucocytes from normal human beings (10 subjects). Leucocytes were kept in homologous serum at 37° C, and a part was kept as control, another was irradiated with 1000 r. A big dose like this was given to increase the probability of seeing the effect, if any.



Glycogen content of irradiated leucocytes in relation to the control (control = 100).

The irradiation was made according to the following conditions: unic dose, kV 140, mA 10, filter 3 Al, distance cm 14, r/m' 600. At this point, special thanks are due to Dr. B. UGGERI and Dr. A. GIORDANO from the Hospital of Tortona, for their radiological assistance.

At the beginning of the experiment, and periodically to the 6th, 12th and 18th h of survival, samples of leucocytes were taken and smeared. To demonstrate the

Table I.—Neutrophils

Hours after irradiation		Cases										Mean and Dispersion	't' Student		
		1	2	3	4	5	6	7	8	9	10	$m \pm \sigma m$	Calculated	Snedecor's	
														P=5%	P=1%
0		3·9	3·8	4·0	4·0	3·8	3·9	3·7	3·9	5·0	4·0	3·9			
6	contr.	3·5	3·6	3·4	3·3	3·1	3·5	3·7	3·5	3·0	3·3	3·3 ± 0·20			
	irrad.	3·1	3·2	3·0	3·2	2·8	2·8	2·9	3·2	2·5	2·8	2·95 ± 0·017	6·92	2·26	3·25
12	contr.	2·8	3·2	2·9	2·7	2·5	2·9	2·7	2·8	3·0	2·6	2·75 ± 0·2			
	irrad.	2·2	2·0	1·9	2·1	2·1	1·8	2·1	2·0	2·2	1·9	1·95 ± 0·2	9·74	2·26	3·25
18	contr.	1·5	1·0	1·4	1·4	0·9	1·5	1·3	1·2	1·4	1·6	1·32 ± 0·07			
	irrad.	1·2	0·4	0·8	1·1	0·5	0·7	0·9	0·7	0·8	0·9	0·79 ± 0·07	9·64	2·26	3·25

Table II.—Lymphocytes

Hours after irradiation		Cases										Mean and Dispersion	't' Student		
		1	2	3	4	5	6	7	8	9	10	$m \pm \sigma m$	Calculated	Snedecor's	
														P=5%	P=1%
0		0·8	0·7	0·5	0·8	0·8	0·6	0·7	0·9	0·8	0·6	0·72			
6	contr.	0·5	0·4	0·3	0·6	0·6	0·4	0·5	0·6	0·4	0·3	0·46 ± 0·04			
	irrad.	0·3	0·15	0·15	0·3	0·3	0·2	0·15	0·3	0·25	0·2	0·23 ± 0·003	6·63	2·26	3·25
12	contr.	0·1	0·15	0·10	0·40	0·20	0·20	0·15	0·10	0·20	0·20	0·18 ± 0·008			
	irrad.	0·0	0·05	0·03	0·20	0·10	0·0	0·05	0·0	0·10	0·0	0·053 ± 0·004	3·60	2·26	3·25
18	contr.	0·0	0·10	0·0	0·20	0·05	0·0	0·05	0·0	0·05	0·05	0·05 ± 0·004			
	irrad.	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0 ± 0·0	2·44	2·26	3·25

* These researches have been performed with the help of the «Lega italiana per la lotta contro i Tumori».

[1] G. ASTALDI, E. BERNARDELLI, and E. G. RONDANELLI, Boll. Soc. Ital. Biol. sper. *28*, 286 (1952).

[2] G. ASTALDI, E. BERNARDELLI, E. G. RONDANELLI, and P. GORINI, Boll. Soc. Ital. Ematol. *3*, 87 (1955).

[3] G. PISANI and A. ROMANINI, Tumori *40*, 410 (1954).

[4] G. CARDINALI, G. CAPRINO, and M. PISCITELLI, Gazz. int. Med. Chir. *59*, 1331 (1954); Tumori *40*, 176 (1954).

[5] G. ASTALDI, *6th International Congress of Haematology* (Boston 1956). – G. ASTALDI and B. MASSA, to be printed.

glycogen in cells, the smears were submitted to the P.A.S. reaction, according to HOTCHKISS. To valuate the average glycogen content per cell, the average reaction index (A.R.I.) according to ASTALDI *et al.*[6] was determined separately for neutrophils and lymphocytes.

The results obtained from neutrophils are given in Table I, those regarding lymphocytes in Table II. The Figure expresses graphically the quantitative behaviour of the glycogen content of the irradiated leucocytes in relation to the control taken as 100.

The differences between the control and the irradiated leucocytes were submitted to statistical analysis, using Student-Fisher t test. The differences resulted in a significant degree, applying the *t* value to the differences in pairs.

From the results taken as a whole, we may conclude that X-rays carried out directly on surviving leucocytes cause an evident increase of glycogenolysis in these cells. Such a phenomenon occurs to a still more marked degree in lymphocytes than in neutrophils.

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Riassunto

Sono stati irradiati direttamente *in vitro* leucociti sopravviventi ed è stato studiato il loro contenuto in glicogeno durante la sopravvivenza.

I risultati dimostrano un aumento significativo della glicogenolisi nei leucociti irradiati rispetto ai controlli. Riguardo alla perdita del loro contenuto in glicogeno, i linfociti risultano più sensibili dei neutrofili alla irradiazione Roentgen.

[6] G. ASTALDI, E. BERNARDELLI, and E. G. RONDANELLI, Haematology *36*, 749 (1952).

The Influence of Lowering the Body Temperature on Postural Orientation of the Organism

It is well known that the postural orientation of the organism as a whole and of separate parts of the body is realized by the righting reflexes of Magnus, the centres of which are localized in the reticular formation of the brain-stem at the level of the nucleus ruber. Stereotaxic lesions are a common cause of elimination of these reflexes. This causes irreversible damage, which usually effects simultaneously the centres of several reflexes. It is possible to obtain a successive, and at the same time a relatively quick, functional elimination of these reflex centres, which is reversible, and which was obtained in our experiments by a lowering of the body temperature.

A considerable drop of temperature of the environment was used for lowering the animal body temperature (30 rabbits). The loss of a prompt and exact body righting response was denoted as the stage of a decreased response. As can been seen from the Table, lowering of the body temperature first leads to the extinction of Magnus' body righting reflexes acting on body, while the labyrinthine righting reflexes are the last to be lost. The recovery of reflexes takes place in the reverse order, i.e. first to appear, are the labyrinthine reflexes. The order

The order in which the postural reflexes are eliminated after lowering the body temperature

Rectal temperature °C	Percentage of animals, in which a postural reflex response was seen					
	I		II		III	
	a	*b*	*a*	*b*	*a*	*b*
25–24	30					
23–22	70	20	30	10	10	
21–20	20	80	60	40	30	20
19–18		100	10	90	40	50
17–16				100	20	80
15–14						100

I Body righting reflexes acting on body (Magnus)
II Neck righting reflexes
III Labyrinth righting reflexes

a Decreased reflex response, *b* Extinction of reflexes.

in which the elimination of reflexes progresses is quite constant and also stresses the importance of the labyrinths for spatial analysis, which makes possible the general postural orientation of the organism.

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Zusammenfassung

Durch starke Unterkühlung des Warmblüterorganismus kann man die Stellreflexe sukzessiv und reversibel ausschalten. Zuerst erlöschen die Magnusschen Körper-Stellreflexe und zuletzt die labyrinthären Stellreflexe.

Versuche zur biologischen Bestimmung der Sedimentationskonstanten kleiner neurotroper Viren in der präparativen Ultrazentrifuge «ZF 3»

BEYERLE, MOHRING und BÜCHER[1] berichteten 1954 über eine neue, elektromagnetisch angetriebene Präparierzentrifuge (Typenbezeichnung «ZF 3»), deren besondere Eigenart darin besteht, dass das gesamte Einsatzvolumen in einer einzigen, radial zur Drehachse gerichteten Zelle zusammengefasst ist. Ein Gerät dieser Bauart wurde in unserem Laboratorium erstmalig für virologische Studien eingesetzt und hat sich seit längerer Zeit beim präparativen Arbeiten mit kleinen neurotropen Viren bewährt. Die dabei gesammelten Erfahrungen veranlassten uns zu prüfen, ob die Zentrifuge auch zur Bestimmung von Sedimentationskonstanten auf biologischem Wege, das heisst aus der Aktivitätsabnahme im Überstand der Zelle, herangezogen werden kann. Als Modell für unsere Untersuchungen wählten wir das Encephalomyocarditis-(EMC-)Virus der Maus, dessen Sedimentationskonstante von WEIL *et al.*[2] mittels

[1] K. BEYERLE, D. MOHRING und TH. BÜCHER, Chem. Ing. Technik *26*, 94 (1954).

[2] M. L. WEIL, J. WARREN, S. S. BREESE, S. B. RUSS und H. JEFFRIES, J. Bacteriol. *63*, 99 (1952).