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ACID-DEOXYRIBONUCLEASE ACTIVITY AND LABILIZATION OF DEOXYRIBONUCLEIC ACID FROM NUCLEOPROTEIN IN THYMUS AND REGENERATING LIVER AFTER WHOLE-BODY IRRADIATION

by

R. GOUTIER, M. GOUTIER-PIROTTE
(CEN)
and A. RAFFI (Euratom)

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**Work performed at the
Nuclear Energy Research Center - CEN
Department of Radiobiology
Mol/Donk (Belgium)**

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Irradiation of regenerating liver does not result in a further increase of acid DNAase specific activity, not in a greater lability of DNA. Possible correlations between these changes and the cytological alterations after irradiation are discussed.

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Acid-deoxyribonuclease activity and labilization of deoxyribonucleic acid from nucleoprotein in thymus and regenerating liver after whole-body irradiation

R. GOUTIER, M. GOUTIER-PIROTTE and A. RAFFI†
Department of Radiobiology, Nuclear Energy Research Centre,
Mol, Belgium

(*Received* 5 *March* 1964)

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Irradiation of regenerating liver does not result in a further increase of acid DNAase specific activity, nor in a greater lability of DNA. Possible correlations between these changes and the cytological alterations after irradiation are discussed.

1. INTRODUCTION

Changes in the intracellular distribution and in the specific activity of acid deoxyribonuclease (DNAase) have often been reported in radiosensitive tissues after whole-body irradiation of mammals.

Recently, increase of the acid DNAase activity in the supernatant fractions of homogenates has been described after irradiation in the regenerating liver by Goutier-Pirotte and Goutier (1962) and in the spleen by Roth and Hilton (1963).

Earlier investigations have been reviewed by Goutier (1961).

However, the causal relationship between radiation-induced alterations in acid DNAase activity or distribution and cell-damage is still obscure. We selected two tissues for investigating the relationship : the thymus, in which cell-death occurs to a great extent after irradiation ; and regenerating liver, in which cytological alterations are much less pronounced.

In these two organs, we determined the activity and intracellular distribution of acid DNAase and the lability of DNA, as an index of the stability of the bonds between DNA and its nucleoprotein. This lability, measured by the amount of DNA extracted with 90 per cent phenol in the presence of trichloracetate, has already been shown to be altered by irradiation (Hagen 1960, 1962).

† Scholar of Euratom.

2. Methods

2.1. *Animals used*

Male Wistar rats weighing 180–200 g are used. Partial hepatectomy is performed according to the technique of Higgins and Anderson (1931) under ether anaesthesia.

2.2. *Irradiation*

A dose of 700 r x-rays whole-body irradiation is given with the following characteristics : 300 kv ; 20 ma ; filter 3 mm Cu ; dose-rate of 100 r/min.

2.3. *Tissue fractionation*

According to the technique described by de Duve, Pressman, Gianetto, Wattiaux and Appelmans (1955) for rat liver, thymus and liver are excised at different times after irradiation, blotted dry, weighed and homogenized in a Potter tube in 5 vol cold 0·25 M sucrose–0·003 M $CaCl_2$ solution. The homogenate is centrifuged at 1850 r.p.m. for 10 min ; the supernatant fraction is kept, and the pellet containing nuclei and whole cells resuspended in the same sucrose solution, rehomogenized, filtered on gauze and added to the first supernatant fraction to give a 10 per cent homogenate.

The nuclear fraction is obtained by centrifuging the homogenate at 600 g for 10 min. The nuclear pellet is washed twice, and the supernatant fluid plus the washings is spun either at 250 000 g for 30 min to sediment mitochondria and microsomes, or first at 10 000 g for 10 min to sediment the mitochondria. This last pellet is washed once, and the supernatant fraction plus washing is spun at 250 000 g for 30 min to sediment the microsomes.

2.4. *Acid DNAase determination*

This is done by viscosimetry according to Laslowski and Seidel (1945) in acetate citrate buffer as described previously (Goutier-Pirotte and Goutier 1962).

2.5. *Lability of DNA*

The extractibility of DNA is measured essentially by the method of Hagen (1960, 1962).

The method originates from the work of Kirby (1957), by which DNA can be extracted by certain anions in aqueous solutions in the presence of phenol.

The DNA is present in the aqueous phase, whereas the proteins are recovered, in a denatured state, in the phenol phase.

For thymus, water homogenates are used at a concentration of 7 per cent w/v.

For liver, reproducible results are obtained only with the nuclear fraction and not with whole homogenates. Therefore, liver homogenates are prepared in cold 0·15 M NaCl, at a concentration of 10 per cent. The nuclear pellet which sediments after 10 min centrifugation at 600 g is resuspended in cold distilled water to give a 20 per cent suspension.

To 2 ml homogenate or nuclear suspension are added 2 ml of sodium trichloracetate (TCA Na) of different concentrations (0, 1, 1·5, 2, 2·5, 3 and 5 per cent). The mixture is allowed to stand for 30 sec, after which 4 ml of 90 per cent phenol are added. After shaking for 30 min, the mixture is centrifuged at 15 000 g for 15 min. The DNA in the aqueous phase is precipitated in 0·5 N $HClO_4$ and re-extracted after 20 min heating at 80°c in 0·5 N $HClO_4$.

2.6. *DNA and protein determination*

Protein-content of homogenates and subcellular fraction is determined by the Folin reaction (Lowry, Rosenbrough, Farr and Randall 1951). DNA-content is measured by the Dische-Burton test (Burton 1956).

3. Results

3.1. *Thymus*

Table 1 gives the results of several fractionations of thymus of normal and irradiated rats. The increase of acid-DNAase activity in the supernatant fraction at the expenses of the granules is observed from the second hour after irradiation onwards.

Time after 700 r	Nuclei	Particulate fraction	Supernatant fraction
Controls	20·6 ± 2	54 ± 0·9	22·6 ± 1
45 min	24·9 ± 4	55·2 ± 4·2	21·0 ± 0·5
2 hours	23·1 ± 5·0	48·8 ± 1·7	29·2 ± 4·0
3·5 hours	19·0 ± 2	50·3 ± 2·1	31·0 ± 1·5
5·5 hours	28 ± 1·5	42 ± 1·1	30 ± 1·5

Table 1. Intracellular distribution of acid DNAase in rat thymus. Values are means of three to four determinations and given in percentage of the total homogenate activity, ± S.E.

Table 2 shows that a small increase in specific activity of acid DNAase is observed 2 hours after irradiation, when the free DNAase activity has already risen. The specific activity increases further 5·5 hours after irradiation.

The amount of DNA extracted in 1 and 1·5 per cent TCA is already higher than in the controls 2 hours after irradiation, when the proportion of free acid DNAase is also increased, but rises more markedly at 5·5 hours after irradiation, when the specific activity of acid DNAase goes up. The increased lability of DNA in irradiated thymus has been described by Hagen (1960, 1962).

Time after irradiation	Acid DNAase		DNA extracted, in per cent of total DNA	
	Free activity (per cent of total activity)	Specific activity per mg protein	1 per cent TCA-Na	1·5 per cent TCA-Na
Controls	22 ± 1·5	77 ± 3·2	1·5 ± 0·2	4·5 ± 0·5
45 min	21 ± 2	84 ± 4·0	1·5 ± 0·12	4·5 ± 0·4
2 hours	29 ± 3	82 ± 3·8	2·5 ± 0·3	8 ± 0·6
3·5 hours	31 ± 2·8	82 ± 3·1	9 ± 0·7	13·5 ± 0·9
5·5 hours	30 ± 3·1	98 ± 3·9	19·5 ± 1·5	25·5 ± 1·8

Table 2. Specific activity (per milligram protein) and free activity of acid DNAase and lability of DNA in rat thymus homogenates after 700 r whole-body x-irradiation (means of two to four determinations).

Table 3 shows that in control thymus, 90–95 per cent of the DNA extracted in neutral TCA is still precipitable in cold-acid medium.

The proportion of short deoxypolynucleotides is therefore small. Irradiation does not seem to increase this proportion to a great extent, since 6 hours after 700 r, 80 to 90 per cent of the extracted DNA is still acid-insoluble.

Concentration of TCA-Na (per cent)	Control animals		Irradiated animals	
	DNA extracted in per cent of total DNA	DNA precipitable in 0·2 N $HClO_4$	DNA extracted in per cent of total DNA	DNA precipitable in 0·2 N $HClO_4$
0·5	1·2	1·0	7·66	6·7
1	2·0	1·8	21·6	18·7
1·5	7·9	7·5	37·7	34·0
2	82·0	76·5	85·5	70·0
5	100	96	100	82

Table 3. Lability of DNA in thymus 6 hours after 700 r whole-body x-irradiation.

3.2. *Regenerating liver*

The results given in table 4 are obtained from nuclear suspensions and not from whole homogenates. They show that free acid DNAase activity is significantly increased only when irradiation is given before hepatectomy (see also Goutier-Pirotte and Goutier 1962). Although the specific activity of acid DNAase rises during normal regeneration (see also Goutier and Leonard 1962), no difference is observed between the specific activity of irradiated and non-irradiated animals.

Similarly, an increase in the lability of DNA can be observed in the non-irradiated regenerating liver, compared with normal liver, especially 6 and 21 hours after partial hepatectomy. But no significant difference is noted between the irradiated and control groups.

4. Discussion

It has often been observed that whole-body irradiation provokes an increase in acid DNAase activity of the lymphoid organs (see references in Goutier 1961). A major cause of this phenomenon seems to be a change in cell population. Thymocytes are small and rather poor in acid DNAase (Gordon, Gassner, Okada and Hempelmann 1959). Since they are very radiosensitive, they disappear rather rapidly after irradiation and leave in the tissue an increasing proportion of radioresistant reticular cells which are richer in acid DNAase (Aldridge, Hempelmann and Emmel 1960) and in lysosomes (Rahman 1962 a, 1962 b, 1963, Balner, Old and Clarke 1961).

No change in any hypothetical enzyme inhibitor seems to occur after irradiation (Okada, Gordon, King and Hempelmann 1957). However, the possibility of a synthesis of new enzyme molecules cannot be excluded (Weymouth 1958, Aldridge *et al.* 1960). According to Hagen and Braun (1961), pycnotic nuclei already appear two hours after irradiation in rat thymus and may represent 77 per cent of the total number of thymus nuclei 6 hours after 1000 r.

	Acid DNAase		DNA extracted, in per cent of total DNA					
	Free activity (in per cent of total activity)	Specific activity per mg protein (arbitr. units)	Concentration of TCA-Na (per cent)					
			1	1·5	2	2·5	3	5
Normal liver	15	30	2·5	4·5	22·5	80	94	100
Regenerating liver								
6 hours controls	16	39·8	—	10·5	38	87	—	100
700 r at 0 hours	25	40	—	9·6	35	87	—	100
21 hours controls	15	44	1·3	9·8	27	100	100	100
700 r at 18 hours	19	44	5·3	9	33	100	100	100
24 hours controls	11	39·6	5·2	7·2	20	91	100	100
700 r at 18 hours	12	37·5	—	8	23	90	100	100

Table 4. Specific activity (per mg protein) and free activity of acid DNAase and lability of DNA in regenerating rat-liver after irradiation.

The increase in acid DNAase specific activity (table 2) seems therefore to be parallel to the evolution of the cytological lesion. Much larger increases of enzyme specific activity can be observed in the thymus at later times after irradiation (Weymouth 1958, Kurnick, Massey and Sandeen 1959) : they are most probably associated with phagocytoses of cell debris, which is an important function of the lysosome acid hydrolases (de Duve 1959).

An increase of the acid DNAase in the supernatant fraction had already been described in the spleen of irradiated rats (Roth and Hilton 1963). We found the same phenomenon in the thymus (tables 1 and 2) where the maximum increase seems to occur by 2 hours after irradiation, and no further changes are observed at 6 hours, despite the marked increase in specific activity.

The extractibility of DNA in neutral TCA is a function of the lability of the bond between DNA and its protein and not a test for measuring the extent of DNA-breakdown.

The fact that most of the DNA extracted from irradiated thymus in neutral TCA is still precipitable in cold-acid medium (table 3) only means that if degradation of DNA has taken place, the amount of acid-soluble nucleotides must nevertheless be small. That some degradation occurs is proved by the observation of a 50 per cent decrease in the viscosity of thymus DNA 2 to 4 hours after 1000 r (Kuzin, Strajevskaia and Struchkov 1961). But changes in the elution pattern of thymus DNA on ion-exchange columns seem to occur only at a later time (24 hours) after irradiation with 1000 r (Struchkov and Kuzin 1961). Small changes in the chromatographic pattern of DNA from irradiated regenerating liver were described by Foster and Ord (1962).

We confirmed the results of Hagen (1960, 1962) and of Bauer, Dreyer and Kurnick (1963) on the increased lability of DNA in irradiated thymus, but it is impossible to say, from our observations, whether this increased lability precedes the appearance of pycnoses. In irradiated isolated thymocytes Scaife and Alexander (1961) did not detect any alteration of the deoxyribonucleoprotein before pycnosis appeared.

But the relationship between increased acid DNAase specific activity and increased lability of DNA, which seems to exist in the irradiated thymus, clearly appears from the observations made on regenerating liver.

In non-irradiated regenerating liver, the specific activity of acid DNAase increases markedly after hepatectomy (this observation has been discussed in Goutier and Leonard, 1962) and this increase in enzyme specific activity is paralleled by an increase in the lability of the DNA-protein bonds. On the other hand, there is no change in free DNAase activity during normal liver regeneration. The temporary increase of free acid DNAase activity observed after irradiation does not coincide with any change in the enzyme specific activity. Since irradiation does not alter the lability of the DNA-protein bonds in regenerating liver, this lability therefore seems to be related to increased specific activity of acid DNAase and not to increased amount of free enzyme activity.

After irradiation, regenerating liver cells undergo cytological alterations, which differ from those observed in the thymus.

Whereas thymocytes display early pycnosis and cytolysis after whole-body irradiation (Hagen and Braun 1961), regenerating liver cells do not show any significant degree of cellular degeneration, at least in the early stages of regeneration (Albert and Bucher 1960), nor changes in cell-population due to cell-death or mitotic arrest (Howard 1956). Mitotic abnormalities are observed at all

phases of the mitosis, but do not seem to result in cell-degeneration leading to a change of cell-population, since they persist several weeks after irradiation, at a time when, despite irradiation, the mass of hepatic tissue is completely restored (Leong, Pessotti and Krebs 1961).

In conclusion, the lack of extensive cell-degeneration during the early stages of liver regeneration in irradiated rats coincides with a normal specific activity of the acid DNAase and a lability of the DNA-protein bonds similar to that of the control group.

In the irradiated thymus, on the other hand, the marked degeneration processes are associated with a pronounced rise in acid DNAase specific activity and an increased lability of the DNA-protein bonds.

It is difficult to state whether these effects can be ascribed only to the changes in thymus cell-population after irradiation. One indirect argument which points to this conclusion is that in irradiated regenerating liver, in which no change in cell-population occurs in the time limits of our observations, we did not detect changes in acid DNAase specific activity or in extractibility of DNA in neutral TCA.

It should be remembered that hydrolases other than DNAase also display increases of activity and changes in intracellular distribution. For example, in spleen from irradiated rats, the activity of β-glucuronidase and acid phosphatase is increased, without any marked change in intracellular distribution (Roth, Bukovsky and Elchel 1962) ; acid RNAase, on the other hand, displays both increased specific activity and changes in distribution, as does acid DNAase (Roth and Eichel 1959). In irradiated mouse thymus, acid and alkaline RNAases both have an increased activity (Weymouth 1958).

Laying stress on DNAase activity rather than on other enzymes in our work is a mere assumption that DNAase is the enzyme most likely to affect the stability of the deoxyribonucleoprotein.

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Dans le thymus de rat irradié, la hausse de l'activité spécifique de la DNAase acide s'accompagne d'une augmentation de la quantité de DNA extractible dans des solutions de trichloracétate de sodium de concentrations croissantes. Dans le foie en régénération non irradié, l'activité spécifique de la DNAase acide augmente également et, parallèlement, on observe une plus grande labilité du DNA. L'irradiation ne provoque pas de hausse plus grande d'activité de la DNAase et n'augmente pas la labilité du DNA. On discute les corrélations possibles entre ces changements et les altérations cytologiques après irradiation.

Freie und spezifische Aktivität der sauren DNSase wurde in Thymus und regenerierender Leber von Ratten nach 700 r Ganzkörperbestrahlung bestimmt.

Labilisierung der DNS dieser Organe wurde mittels der durch ansteigende Konzentrationen von Natriumtrichloracetat extrahierbaren DNS Menge gemessen.

Der Anstieg der spezifischen Aktivität der DNSase in bestrahltem Thymus steht in Zusammenhang mit der zunehmenden Labilität der DNS Protein Bindung.

In normaler regenerierender Leber nimmt die spezifische Aktivität der sauren DNSase ebenfalls zu, und diesem Anstieg entspricht wiederum eine erhöhte Labilität der DNS.

Bestrahlung der regenerierenden Leber führt zu keiner weiteren Zunahme der spezifischen Aktivität der sauren DNSase oder einer vermehrten Labilisierung der DNS. Die möglichen Beziehungen zwischen diesen biochemischen und den cytologischen Veränderungen nach Bestrahlung werden besprochen.

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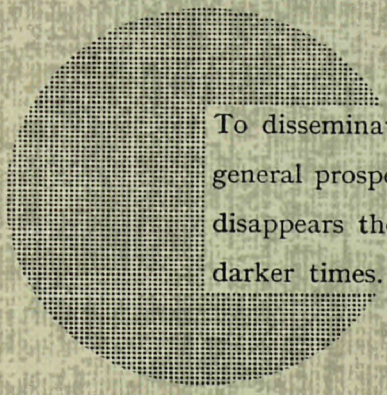

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel