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The metabolism of acid-labile DNA in mammalian tissues

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Istochimica. — *The metabolism of acid-labile* DNA *in mammalian tissues*. Nota di FERNANDO DINI (*) e MARIA PIA VIOLA-MAGNI (**), presentata (***) dal Socio M. BENAZZI.

RIASSUNTO. — Ratti italici sono stati iniettati con timidina H^3 ed uccisi a varia distanza di tempo dall'iniezione. L'incorporazione è stata valutata con tecnica biochimica nel fegato e nel rene prima e dopo l'idrolisi acida, usata per la colorazione di Feulgen, la quale causa una forte perdita di ADN nucleare. I risultati hanno dimostrato che la frazione di ADN marcato resistente all'idrolisi presenta una stabilità nel tempo in accordo con il turnover cellulare, mentre la frazione di ADN marcato, che viene persa durante l'idrolisi, ha un turnover molto più veloce. Si discute il suo possibile significato in rapporto alla differenziazione e funzione cellulare.

Previous work [5] showed that a large aliquot of nuclear DNA of hepatic and kidney cells is lost during the mild acid hydrolysis (1 N HCl at 60 °C for 12 minutes) used in the Feulgen stain [6]. The amount of DNA hydrolyzed is 40.6 % for the kidney and 54.5 % for the liver according to the biochemical determinations used. Various authors [15], [8], [1], [3], have observed this phenomenon and have explained it as being due to particular physico-chemical characteristics of DNA, i.e. depolimerization [1] or a different combination with nuclear proteins [3], two situations which could modify the acid resistance of the DNA. Since these findings change in various tissues and in different state of maturation [3], it has been hypothesized that this acid sensitive DNA could be involved in cellular differentiation and function [3], [15].

In order to investigate this question, the behaviour in time of the acid lability fraction of DNA has been studied and compared with the acid resistant DNA.

MATERIALS AND METHODS

The nuclear DNA has been labelled by injecting H^3-thymidine (Radio-chemical Center, specific activity 5.000 mC_i/mM) at a dose of 1 $\mu C i/g$ body weight into Italic adult rats. The animals were killed at different time intervals from 3 hrs to 180 days after the injection; two fragments of both liver and kidney were taken: one was immediately homogenized and another was fixed in 10 % saline formalin for 24 hrs, sectioned and hydrolyzed before being homogenized according to the technique previously described [5]. The DNA was evaluated

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by the Burton [4] method and the specific activity measured by liquid scintillation counting (Mark I, Disi); this was referred to the DNA and expressed as cpm/μg.

The efficiency was 20% in all experiments.

Results and discussion

The value obtained 3 hrs after the injection represents the amount of H^3-thymidine incorporated into the nuclear DNA of liver (fig. 1) and kidney (fig. 2). This specific activity of DNA does not remain constant in the animals killed after a longer time interval from the injection of the radioactive precursor. The label decreases in time and its decay velocity is different in the same tissue before and after mild acid hydrolysis.

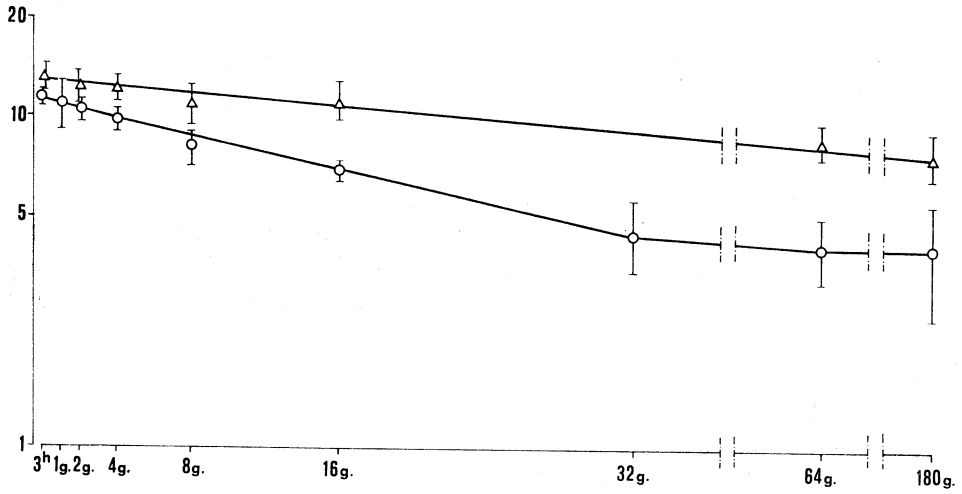

Fig. 1. - Behaviour in time of labelled DNA in adult rat liver (○: untreated tissue; △: hydrolyzed tissue). On the abscissa, time intervals from the H^3-thymidine injection. On the ordinate, specific activity of DNA expressed as cpm/μg.

In untreated tissue the specific activity per μg of DNA shows a greater decrease in time with respect to the hydrolyzed tissue and is characterized by three different kinetics: the decay is faster during the first period of 16 days; this is then followed by a slower rate which continues until the 64th day after the injection; from 64 days to 180 days the label remains constant (figs. 1 and 2). The decrease of labelled DNA in the hydrolyzed tissue is slow and shows the same rate until 180 days.

The half life (i.e. the period in which the specific activity/μg of DNA is half of the value obtained three hours after the injection) is 24 days in the untreated liver and 300 days after hydrolysis (fig. 1). The 300 days value agrees satisfactorily with the cellular turnover time calculated on the basis of

the liver mitotic activity in adult rats [9]. A similar result is also described in the kidney in which the DNA half life is 58 days after hydrolysis and is only 12 days before hydrolysis (fig. 2).

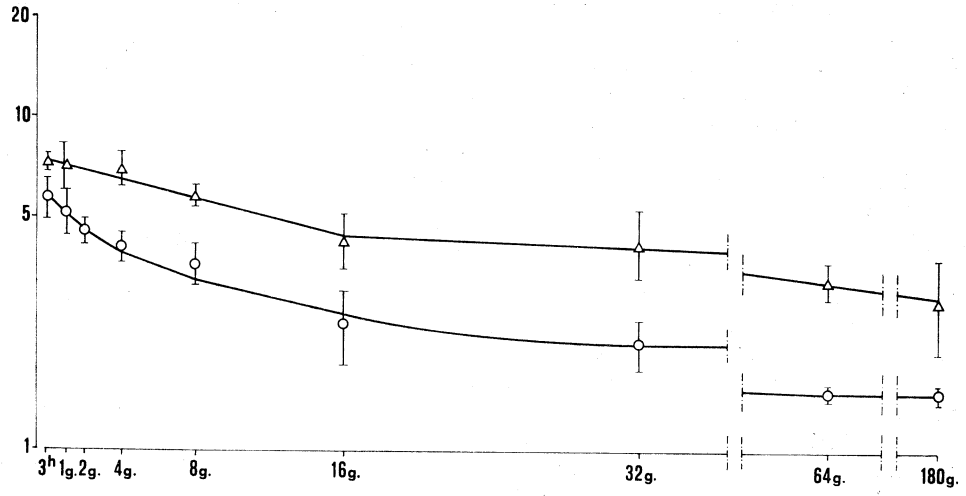

Fig. 2. – Behaviour in time of labelled DNA in adult rat kidney (○: untreated tissue; △: hydrolyzed tissue). On the abscissa, time intervals from the H^3-thymidine injection. On the ordinate, specific activity of DNA expressed as cpm/μg.

The values obtained in untreated tissue are in agreement with the biochemical findings of Gerber *et. al.* [7] and with the autoradiographic results of Pelc and Gahan [11] and Pelc [10]. As a consequence, the labelled DNA in intact tissues has a life which is shorter than the cellular life. This discrepancy is not evident when only the acid resistant DNA is considered. Therefore the presence of two nuclear DNA fractions characterized by different turnover, suggested by Sampson and Davies [13], Stroun *et al.* [14] and, more recently, by Bibbiani and Viola-Magni [2] seems to be confirmed by the present findings.

It is possible that the instability of the acid sensitive fraction not explained by the cellular turnover is related to the rôle of these fractions during cellular differentiation [2] and function [12].

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