SYNTHESIS AND TURNOVER OF LIVER CHROMATIN ACIDIC PROTEINS

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1. Introduction

The chromatin acidic proteins are organ specific [1–3] and are involved in the regulation of protein synthesis. By binding to histones they diminish their ability to associate with DNA [4, 5] and with RNA [6, 7]. They are heterogenous (fig. 1) and since some of them present a specific biological activity, it is of interest to investigate whether they all have identical turnover rates and whether the biosynthesis of some of them is modified in various conditions.

The turnover rates of the mouse liver chromatin acidic proteins have been compared by injecting ^{14}C-leucine and later on ^{3}H-leucine. The acidic proteins were submitted to polyacrylamide gel electrophoresis. The gel was sliced and the $^{14}C/^{3}H$ ratio established for each slice. The ratio was the same for each of them, thus it is therefore likely that the synthesis and the turnover rates were the same for all of them. A similar method was used to study modifications of the acidic protein biosynthetic patterns in various conditions. Mice submitted either to cortisone treatment or to partial hepatectomy were injected with ^{3}H-leucine and control mice with ^{14}C-leucine. The livers were mixed and acidic proteins treated as above. Cortisone does not modify the synthesis of the various acidic proteins, but some modifications occur in regenerating liver. This method applied to Zajdela hepatoma ascitis cells showed a disturbance of most of the acidic proteins.

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2. Methods

Mice of Swiss Strain were used. Zajdela hepatoma cells bearing rats were obtained from Dr. G. Zajdela. 4,5-^{3}H-leucine (31 Ci/mmole and ^{14}C-leucine (129 mCi/mmole) were obtained from the "Commissariat à l'Energie Atomique". The nuclear acidic proteins were prepared by the method of Wang [8], slightly modified [6], from nuclei isolated by the procedure of Chauveau et al. [9]. Disc electrophoreses were performed according to the method of Davis [10] with the Tris-glycine buffer, pH 8.7, described by Uriel [11]. Electrophoreses were performed at 3 mA per 8 cm long tube for 150 min at 4° and the gels were stained with Amido-Schwartz (fig. 1); they were cut into about 40 slices, 2 mm wide. Each slice was transferred into a scintillation vial, dissolved in 0.2 ml of 30% H_2O_2 at 50° [12], and 10 ml of Packard Instagel solution were added. The ^{3}H and ^{14}C radioactivities were measured in a Nuclear Chicago Mark I scintillator and either $^{3}H/^{14}C$ or $^{14}C/^{3}H$ ratio established for each slice.

3. Results and discussion

3.1. *Comparison of the turnover rates*

We have used the method of Arias et al. [13] which involves two consecutive administrations of the same amino acid labelled with different isotopes. Since the maximal incorporation occurs 1–2 hr after the injection [14, 15] and the half life of the liver acidic protein is about 1–2 days [16], we injected ^{14}C-leucine 24 hr and ^{3}H-leucine 2 hr before sacrificing the mice. No significant variations of the $^{14}C/^{3}H$ ratios have been found in the various fractions obtained from the

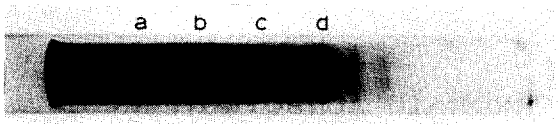

Fig. 1. Electrophoresis of liver chromatin acidic proteins on polyacrylamide gel. Electrophoresis was carried out at 3 mA for 150 min with a Tris-glycine buffer (pH 8.7). The gel was stained with Amido-Schwartz. The letters correspond to the bands in fig. 2.

electrophoretic gels (fig. 2). This result means that all the chromatin acidic proteins are synthesized and renewed at approximately identical rates. However, two types of proteins would not be detected by this method: inert proteins which would not be labelled during the time of the experiments and proteins present in very small amounts which would require a better method of fractionation.

3.2. *Cortisone treated mice* (see fig. 3a)

If it is assumed that modifications of chromatin acidic proteins could be involved in the qualitative variations of RNA and protein syntheses [17, 18], short term experiments would be the most favourable to find this type of modification.

Experimental mice were sacrificed 3 hr after the injection of cortisone and 2 hr after the injection of ^{3}H-leucine. Control mice were injected with ^{14}C-leucine 2 hr before the sacrifice. Livers from experimental and control mice were mixed, acidic proteins extracted and submitted to electrophoresis, as described above. No significative modifications of the $^{3}H/^{14}C$ ratios have been found in any of the fractions obtained from the gel. This observation is in apparent opposition with recent results of Shelton and Allfrey [19] who found in adrenalectomized animals an acidic protein whose synthesis is increased by the injection of cortisone. This discrepancy could be explained in several ways: we are not using adrenalectomized mice, since cortisone has been found to be fully effective in stimulating the synthesis of specific RNAs and enzymes in intact animals [20]. The maximal

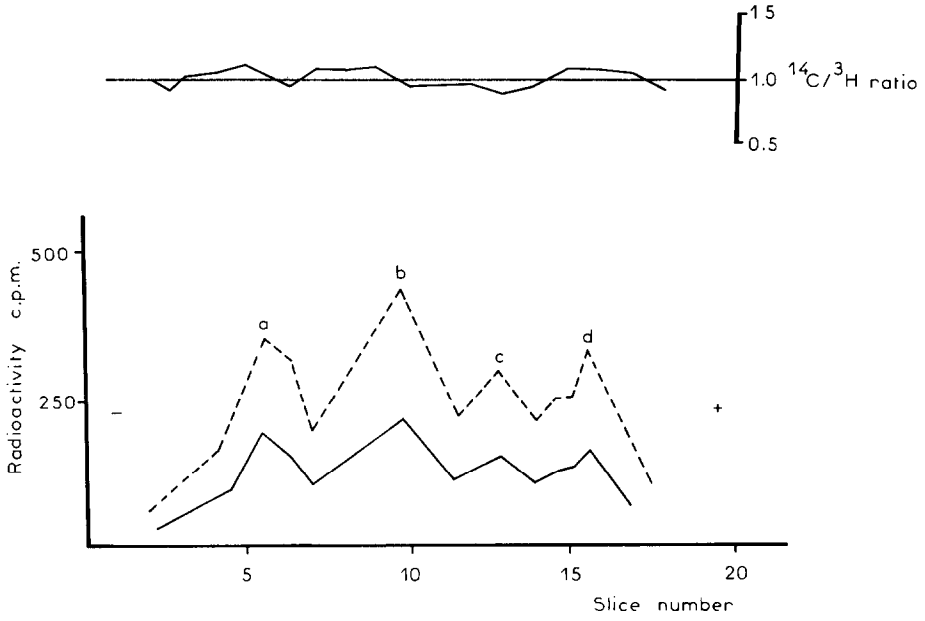

Fig. 2. Turnover rate chromatin acidic proteins. Mice were injected with 30 μCi of ^{14}C-leucine (——), 22 hr later with 120 μCi of ^{3}H-leucine (- - - -) per 10 g of body weight and sacrificed 2 hr later. Extracted acidic proteins were submitted to electrophoresis. The gels were sliced, ^{3}H and ^{14}C were measured in each slice and the $^{14}C/^{3}H$ ratio established. 1.0 is the ratio found with the bulk of the acidic proteins.

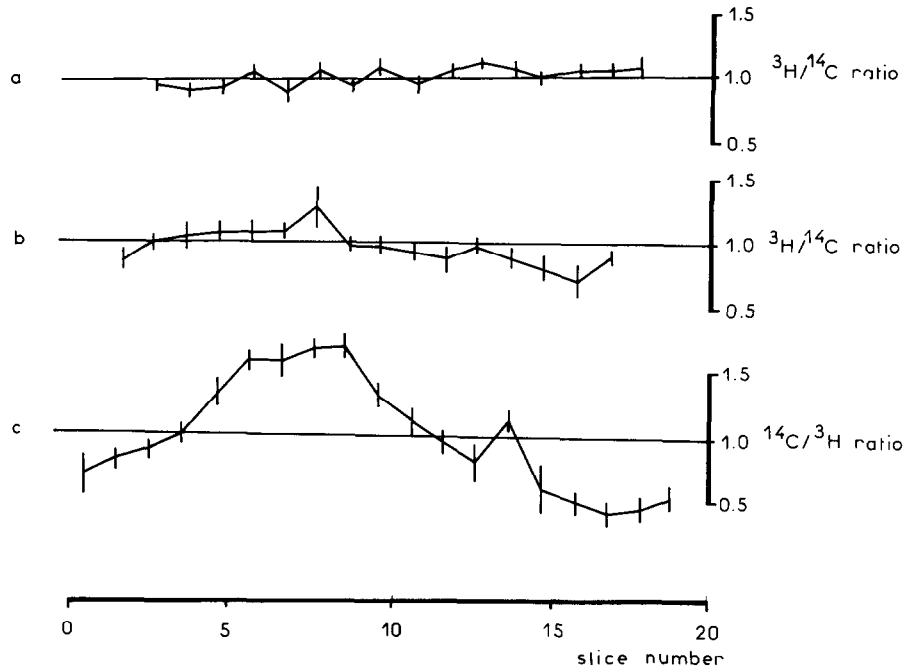

Fig. 3. Relative biosynthetic rates of chromatin acidic proteins. Three experiments were made in each case: (a) Mice were injected intraperitoneally with 500 μg of cortisone and 1 hr later with 120 μCi of ^{3}H-leucine per 10 g of body weight. The control mice were injected with 30 μCi of ^{14}C-leucine per 10 g. The mice were sacrificed 2 hr later, the livers were mixed, acidic proteins extracted and treated as above. The figure represents the $^3H/^{14}C$ ratio for each slice. (b) Similar experiments were carried out with mice submitted to 60% hepatectomy 10 hr before the injection of ^{3}H-leucine and with mice sham-operated and injected with ^{14}C-leucine. They were sacrificed 2 hr after the injections. (c) Rats with Zajdela hepatoma ascitis cells were injected in the ascitis liquid with 200 μCi of ^{14}C-leucine, the cells isolated 2 hr later and mixed with liver of rats injected 2 hr earlier with ^{3}H-leucine.

effect observed by these authors was obtained 7–8 hr after the injection of the hormone, whereas the maximal effect of cortisone at the gene level occurs much earlier and is over at this time [21, 22]. In the limits of the method used it can be concluded that the variation of RNA synthesis is not directly related to a modification of the rate of an acidic protein synthesis. In fact, the relative similarity of the turnover rates of all the acidic proteins makes it unlikely that under physiological conditions normal homeostasis would proceed via drastic alterations in the turnover rate of an individual protein. The action of the hormone at the gene level could proceed by a binding with a chromatin protein. Actually the association of glucocorticoid with chromatin acidic protein has been observed [23].

3.3. *Liver regeneration* (see fig. 3b)

Experimental mice were sacrificed 12 hr after a 60% hepatectomy and 2 hr after the injection of ^{3}H-leucine. Control mice were sham-operated and injected with ^{14}C-leucine. Twelve hr correspond to the maximal increase of RNA synthesis [21]. We have found some modifications of the $^3H/^{14}C$ ratio in several fractions, the synthesis of some of the acidic proteins is increased (fraction 8), while the synthesis of some others is decreased (fraction 16), in regenerating liver. After hepatectomy, as during rapid cell proliferation, there is an increase in the synthesis of RNA and of the bulk of the acidic proteins [21, 24, 25]. These observations favour a nuclear control of the repair mechanism after hepatectomy.

3.4. *Zajdela hepatoma ascitis cells* (see fig. 3c)

Rats with Zajdela hepatoma ascitis cells [26] were injected in the ascitis liquid with ^{14}C-leucine, with

control rats being injected intraperitoneally with ^{3}H-leucine. They were sacrificed 2 hr later and the ascitis cells were mixed with the normal liver. The chromatin acidic proteins were extracted and submitted to gel electrophoresis. In the cancer cells the rates of synthesis of the various acidic proteins were very different from those found in normal liver cells. The $^{14}C/^{3}H$ ratios varied from 0.4 to 1.6. The abnormality of the cancer cells might be at least partly related to modifications occuring in the chromatin proteins.

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