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Glucocorticosteroid Effects on Liver Chromatin

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Corticosteroids induce the synthesis *de novo* of several liver enzymes. A rapid uptake of injected corticosteroids by liver nuclei and a stimulation of nuclear RNA synthesis involving the appearance of new RNA species precedes enzyme induction. Administration of inhibitors of RNA synthesis such as actinomycin D or α -amanitin prevents the rise in enzyme activity. On the basis of these findings, as well as of information obtained from studies with other hormonal systems, it has been postulated that corticosteroids act on the level of transcription in a way similar to that of bacterial inducers (Karlson & Sekeris, 1966). Subsequent work has therefore been oriented along these lines.

The stimulation of RNA synthesis by isolated rat liver nuclei by cortisol (Sekeris *et al.* 1968) points to a direct effect of the hormone within the confines of the nucleus. The high dose needed to elicit the effect (10 μ M) can be considerably lowered in the presence of a specific corticosteroid-binding protein of the cytosol (see Beato, Schmid, Braendle & Sekeris, 1970), which facilitates the intranuclear transport and binding of the hormone to nuclear proteins. The nature of the system for assaying RNA-synthetic activity eliminates problems of pool size, permeation of substrates etc. and focuses attention on the chromatin template or the RNA polymerase(s) as factors affecting the rate of transcription. Indeed an increased template activity of chromatin supporting RNA synthesis was observed either after injection (Dahmus & Bonner, 1965) or after application of the hormone *in vitro* to isolated nuclei (Beato, Homoki, Lukacs & Sekeris, 1968). Further it was shown that the effect of cortisol on the template activity of chromatin was not related to changes in the rates of acetylation, methylation or phosphorylation of the

histones. However, an increased thiol content of both histones and non-histone proteins due to cleavage of disulphide bridges was detected (Sekeris, 1969).

A direct effect of cortisol on isolated rat liver chromatin could be detected (Beato, Seifart & Sekeris, 1970) either by measuring the ability of chromatin to bind [³H]actinomycin D or by measuring its capacity to support RNA synthesis in the presence of excess of *Escherichia coli* RNA polymerase. It was considered, however, that evaluation of these results would be more meaningful if the experiments were carried out with RNA polymerase from rat liver.

From purified rat liver nuclei we isolated an RNA polymerase (Seifart & Sekeris, 1969) that, on the basis of hybridization-competition experiments, showed a much higher fidelity of transcription on rat liver DNA and chromatin than did the *E. coli* enzyme (D. Doenecke, K. H. Seifart & C. E. Sekeris, unpublished work). Our purified enzyme corresponded to enzyme II or 'nucleoplasm enzyme' in the terminology of Roeder & Rutter (1969). The enzyme transcribed rat liver chromatin much more efficiently than did the *E. coli* enzyme. Previous determinations of active chromatin performed with the *E. coli* enzyme should therefore be reconsidered. Although enzyme I or nucleolar RNA polymerase efficiently transcribed purified rat liver DNA, chromatin was transcribed very poorly. The characteristics of these enzymes are being currently studied in our laboratory as a prerequisite for further developing our chromatin system *in vitro*.

In preliminary experiments (C. E. Sekeris, K. H. Richter & W. Roewekamp, unpublished work) it was found that non-histone proteins from rat liver chromatin recombined with rat liver DNA inhibit transcription by rat liver RNA polymerase but not that by the *E. coli* enzyme. This is in contrast with previous observations that non-histone proteins act as activators of RNA synthesis, and rather support a repressor function for some of the acidic proteins. Further, this fraction binds radioactively labelled cortisol. It is tempting to speculate that the consequence of this binding is the release of repression. The non-histone proteins have been isolated and partially purified in our laboratory (K. H. Richter & C. E. Sekeris, submitted for publication). The salt-urea extraction method of Paul & Gilmour (1968) was applied, followed by removal of the DNA by ultracentrifugation, removal of the histones by binding on Bio-Rex 70 and fractionation on QAE-Sephadex A-25. The non-histone proteins were highly heterogeneous, as shown by polyacrylamide-gel electrophoresis, and, although many bands were common to several tissues, several protein components were restricted to one tissue. These fractions

are being currently tested with regard to binding to DNA, effects on transcription by the rat liver RNA polymerases and binding of [^3H]cortisol.

Independent of the existence of secondary control cycles, we consider that the initial reaction to the hormonal induction, which triggers the whole series of consequent events, is the stereospecific binding of the hormone to a non-histone regulatory protein on the DNA in a way analogous to the binding of the bacterial inducers to their specific repressors. Because of the complexity of the genome of higher organisms, the development of systems *in vitro* testing this hypothesis is still a long way off. However, the current rate of progress in the molecular biology of eukaryotes lends hope to such prospects.

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COMMUNICATIONS

Transfer Ribonucleic Acid Methylases in Normal Tissues and in Tumours

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Much suggestive evidence has accumulated to support the idea, first put forward by Srinivasan & Borek (1963), that tRNA methylases may be involved in carcinogenesis (Craddock, 1970). Much of this evidence was based on work with tumours caused by viruses, and enzyme changes could have been related to viral induction rather than to malignancy. The present experiments show that a change in tRNA methylases occurs in tumours caused by chemicals, and that certain tRNA methylases are affected more than others.

The enzymes were studied in the high-speed supernatant fraction of homogenates by incubation with *S*-adenosyl[$\text{Me-}^{14}\text{C}$]methionine and a limiting amount of *Escherichia coli* tRNA. Enzyme preparations from normal tissues methylate tRNA to a low limited extent and then stop. According to current concepts, methylation of certain sites in the tRNA molecule takes place, the sites being determined by the specificity of the enzymes, and the 'saturation curve' reaches a plateau where an increase in the amount of enzyme present does not cause further methylation to take place. However, there are other possible explanations of this effect. Preparations from tumours of liver or kidney that

had been produced by treatment of animals with nitrosamines methylate *E. coli* tRNA more extensively than do preparations from normal tissues. When tRNA that had been incubated with methylase preparations and *S*-adenosyl[$\text{Me-}^{14}\text{C}$]methionine was analysed after hydrolysis by column chromatography, the major methylated bases formed were 5/3-methylcytosine, di- N^2 -methylguanine, N^2 -methylguanine and 1-methyladenine. There was a definite difference in the relative amounts of the different methylated bases produced depending on whether the methylase preparation was from tumour or from normal tissue. This difference was found to occur also between methylases prepared from a liver tumour compared with the preparation from 'normal' liver tissue adjacent to the tumour.

Liver taken from animals 24h after partial hepatectomy gave a 'saturation level' of methylation similar to that given by normal liver, and the relative amounts of the different methylated bases formed were similar to those given by preparations from normal liver. The differences between tumours and normal liver may therefore be related not simply to growth but to altered growth.

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