Alteration of Template Activity of Chromatin from Livers of Pneumococcus-Infected Mice[1] (35633)

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Stimulation of protein synthetic activity in mouse liver cells is an early response to the stress of an experimental infection with *Diplococcus pneumoniae* (1). This stimulation was demonstrated by increased incorporation of isotope-labeled amino acids into total liver microsomal proteins in both *in vivo* and *in vitro* systems. Also, it has been suggested that total ribonucleic acid (RNA) synthesis in the liver is stimulated at a time approximating the time of increased protein synthesis (2).

To determine the involvement of the control of transcription in the early, infection-induced changes in liver protein synthesis, we have examined the ability of isolated liver chromatin to serve as template for *in vitro* RNA synthesis. Since the transcription of the genetic material is the first characteristic of new gene expression, it might logically follow that the changes in protein synthesis are related to changes in transcription. To date there is no evidence bearing on the question of whether the infection-related increase in protein synthesis represents a synthesis of new kinds of proteins.

It has been demonstrated that, without the capacity for glucocorticoid secretion, infected mice no longer respond with an early increase in liver protein synthetic activity (3). Accordingly, we have also determined the response of infected, adrenalectomized mice in terms of chromatin template activity.

Methods. Infection. CD-1 male mice (Charles River Laboratories, Wilmington, Mass.), 28–35 g, were housed in our facilities at least 2 weeks prior to infection and were fed (Rockland Mouse/Rat Diet, Teklad Inc., Monmouth, Ill.) and watered *ad libitum* throughout the conditioning and experimental periods. The mice were inoculated intraperitoneally (ip) between 0830 and 0900 hr with 18–22 *D. pneumoniae* (type I) organisms in the late log phase suspended in 0.5 ml of normal saline. Blood titers of pneumococci rose to over 10^3 organisms/ml in 14 hr, with death of over 90% of the population occurring within 36–48 hr. Controls were inoculated with saline alone. The bacterial culture was maintained by repeated transfer through mice to maintain virulence, followed by growth in brain–heart infusion broth supplemented with serum, and subsequent freezing in 15% glycerol at —70°.

Chromatin preparation. Groups of infected and control mice were killed at various intervals following injections. Livers were promptly removed, washed in cold 0.025 *M* citric acid solution, and frozen at —20°. Chromatin was prepared the following day by a modification of the method of Marushige and Bonner (4). Quantitation was by the deoxyribonucleic acid assay of Burton (5). The chromatin was stored at 0° prior to the template activity assay.

Template activity assay. Chromatin served as the template for RNA synthesis *in vitro* using RNA polymerase purified from *Escherichia coli* (6). Details of the assay are described in a recent paper (7). The quantity of RNA synthesized from the chromatin template *in vitro* represents, at least in a relative fashion, the degree of availability of sites on the templates for transcription *in vivo* (7–10).

Binding of ^{3}H-actinomycin D to chroma-

[1] In conducting the research reported herein the investigators adhered to "Guide for Laboratory Animal Facilities and Care" established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

tin. One μg of DNA as chromatin, isolated as noted previously, was incubated on ice in 0.01 *M* Tris buffer, (pH 8.0) for 40 min with a saturating quantity of ^{3}H-actinomycin D (Schwarz Bioresearch, Inc. Orangeburg, N.Y.). Total radioactivity in each mixture was 1 μCi, and the total volume was 0.5 ml. Following incubation, chromatin was precipitated with cold 95% ethanol, collected on membrane filters, and washed with more ethanol. The dried filters were placed in scintillation fluid for counting ^{3}H-disintegration. Background radioactivity, determined from a mixture lacking chromatin, was subtracted from all counts. This drug binding assay is essentially that developed by Beato *et al.* (11).

Results and Discussion. Our analysis of the template activity of isolated chromatin indicates a change in activity related to the experimental pneumococcal infection. Figure 1 shows an early, significant rise in the ability of the chromatin from infected mice to serve as template for RNA synthesis. This rise in activity, amounting to about 140% of the control, occurred 2–5 hr after infection. However, the activity falls to about 70% of the control levels at 14 and 23 hr. As shown in Fig. 1, at 14 hr the decrease is largely due to absence of the normal nighttime rise in the control which maintains its periodicity in activity (7). The transcription characteristics of the chromatin, therefore, appear to vary in a pattern similar to the alterations in liver microsomal protein and RNA synthetic activity in the same model (1, 2). It is proposed that, in the liver, a generalized infection by *D. pneumoniae* demands a gene expression *sui generis.*

Since mice lacking the capacity for glucocorticoid secretion no longer respond with the characteristic, infection-related early rise in protein synthetic activity (3), it seemed reasonable that the early rise in chromatin template activity would be absent in infected, adrenalectomized mice if the phenomena were related. Accordingly, adrenalectomized male mice (Adrex, CD-1 strain, Charles River Laboratories), housed in our facilities for 2 weeks following surgery, were used in a chromatin template analysis following ip in-

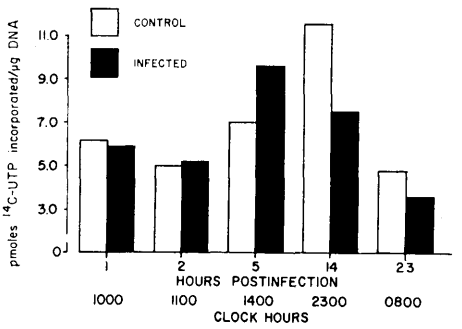

FIG. 1. Template activity of chromatin isolated from livers of infected and control mice: Chromatin, isolated at the postinfection intervals indicated, was added in 4 to 6 different limiting quantities to otherwise identical mixtures, and the ^{14}C-UTP incorporation into RNA was determined for each mixture. The ^{14}C-UTP incorporation was plotted versus chromatin (DNA) concentration. The slopes from these plots determined by linear regression analysis for each chromatin preparation, are presented here. Analysis of variance between infected and control mice yielded $p<0.01$, $p<0.05$, and $p<0.001$ for postinfection hours 5, 14, and 23, respectively.

fection with *D. pneumoniae* organisms. The mice were provided normal saline for their liquid intake.

The data shown in Fig. 2 confirmed our expectation. The infection-related rise in template activity normally detectable at 5 hr was absent in chromatin from infected, adrenalectomized mice. Single analyses at 1 and 3 hr postinfection also showed no difference between infected and control animals. At 14 hr, when intact controls showed normal rhythmic increases (7), the infected animals remained unchanged from the 5-hr level. The partial rhythmic increase in adrenalectomized controls at this time has been discussed elsewhere (7). At 23 hr the infected level remained the same as at 5 and 14 hr unlike intact, infected which were decreased below the controls at this time (Fig. 1). Since infected, adrenalectomized mice become moribund earlier than intact mice, it is difficult to conclude anything on this latter point.

The results at the 5-hr interval suggest that glucocorticoids serve some mediating function in early increase in template activity as they appear to influence protein synthetic activity following infection. Such data lend

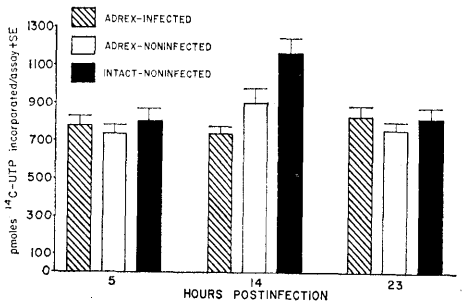

FIG. 2. Adrenalectomy and the early template activity response to infection: Liver chromatin was isolated from adrenalectomized (Adrex) infected and noninfected control mice as well as from intact controls at the postinfection intervals indicated. To permit greater replication of assays, chromatin was added to template activity assay mixtures in a constant quantity (100 μg of DNA), and the absolute levels of ^{14}C-UTP incorporation were compared. The results plotted represent the means (+ standard error of mean) from triplicate assays of 9 chromatin preparations, each preparation from 3 pooled livers.

further support to the notion that at least part of the infection-induced response of the protein synthetic machinery in the liver is related to changes in transcription characteristics of the chromatin. The actual role of glucocorticoids in these responses remains unknown.

To investigate further the properties of liver chromatin isolated 5 hr after infection, the binding of ^{3}H-labeled actinomycin D to chromatin was examined. Since the drug has been shown to bind similarly to naked DNA and regions of chromatin which are unrestricted by chromosomal proteins (12, 13), other workers (11) have proposed the use of its binding as a measurement of the amount of DNA in chromatin which is unmasked and available for transcription. The data in Table I indicate that at 5 hr the labeled actinomycin binds to liver chromatin from infected animals in greater amounts than to chromatin from controls, suggesting again that an alteration of gene expression at the level of transcription is an early hepatic response to generalized infection. An analysis of variance for each experiment shows the differences in binding efficiency to be significant (p values given in Table I).

The possibility that self-imposed starvation is responsible for the lack of the normal, nighttime rise in template activity of chromatin from infected mice is disproved by recently published data which show that experimentally controlled starvation does not eliminate the nocturnal rise in template activity (7).

Evidence relating all of these infection-related changes in chromatin template activity to early changes in protein synthetic activity is, at this time, only circumstantial. Characteristics of gene expression other than transcription may be partly, or even primarily, involved in the alterations in protein synthesis; efficiency of translation, availability of substrates, or nuclear permeability, to name the obvious. Our results at this time do not answer the question of whether infection-

TABLE I. Binding of ^{3}H-Actinomycin D to Chromatin Isolated 5 hr Postinfection.

Expt. no.		^{3}H bound (cpm)	Mean ± standard error of mean
1.	Infected	8760, 7974, 10492, 7861, 8471, 9638	8866 ± 417
			$p < 0.01$
	Control	4518, 3970, 8920, 6582, 7269, 4661	5987 ± 787
2.	Infected	6926, 5454, 6565, 5541	6122 ± 368
			$p < 0.05$
	Control	6007, 5514, 3432, 3542, 3124	4324 ± 596
3.	Infected	8434, 9032, 9095, 9509, 7970, 8385	8738 ± 232
			$p < 0.01$
	Control	6879, 7866, 6979, 6545, 3816, 6349	6406 ± 543

related changes in chromatin template activity represent changes in the kinds of RNA molecules (and perhaps the kinds of proteins) being synthesized in the liver. Duplication of genetic information in eukaryotes makes it particularly difficult to solve this problem. Analysis of RNA molecules synthesized *in vivo* and *in vitro* should further our understanding of the early responses to infection.

Summary. The template activity of mouse liver chromatin is elevated when examined 5 hr after infection of the animal with *D. pneumoniae*. At 14 hr, the activity falls below control values. These changes demonstrate an early influence of generalized infection on the control of transcription in the liver. Infected, adrenalectomized mice do not respond with the early rise in chromatin template activity, suggesting the role of glucocorticoid mediation. It is proposed that these changes in transcription characteristics are related to changes known to occur in protein synthesis during the course of bacterial infection.

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