Molecular Mechanisms for Changes in Hepatic Protein Synthesis Induced by Schistosomiasis Infection in Mice†

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ABSTRACT: Mice infected with *Schistosoma mansoni* and littermate controls were evaluated serially for 12 weeks. Infected mice gained weight at the same rate as controls, but starting with the sixth week their livers became enlarged with granulomas and fibrous tissue, and they developed hypoalbuminemia. To evaluate the regulation of the albumin and type I collagen gene expression, total RNA was isolated from infected and control mice and translated in an mRNA-dependent rabbit reticulocyte lysate system. Protein synthesis was decreased 1.5–3-fold with RNA from infected vs. control liver. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the cell-free products showed a reduction in albumin but an increase in type I procollagen synthesis in infected mice. Immunoprecipitation of the cell-free product confirmed that albumin synthesis was reduced in greater proportion than other liver proteins in schistosome-infected mice. Hybridization of RNA from infected liver with cloned mouse albumin cDNA (pmalb-2) demonstrated a reduction in albumin mRNA to 37% of control, while hybridization with a chick type I pro α2 collagen cDNA probe (pCg-45) revealed increased procollagen mRNA in infected liver beginning at 6 weeks postinfection. These results suggest that in murine schistosomiasis a reduction in biologically active albumin mRNA results in decreased albumin synthesis and may be responsible in part for hypoalbuminemia. In addition, increased collagen mRNA is associated with increased collagen synthesis during hepatic fibrosis.

Schistosomiasis is a major parasitic disease involving tropical regions. Although most of the 200 million people with schistosome infection show no disease, appreciable numbers have substantial morbidity and mortality from chronic liver disease (Warren, 1980). The eggs of *Schistosomiasis mansoni* cause an inflammatory response when trapped in liver sinusoids, leading to granuloma formation and ultimately to hepatic fibrosis. This fibrosis is characteristically deposited in thick bands, whereas the hepatic parenchyma is typically well-preserved (Dunn & Kamel, 1981). In some cases, however, hypoalbuminemia occurs after long-term hepatic fibrosis (Cook et al., 1974).

Mice infected with *S. mansoni* develop hypoalbuminemia (Knauft & Warren, 1969) and a 20-fold increase in liver collagen (Dunn et al., 1977). This model, therefore, is frequently used to investigate the regulation of albumin and collagen synthesis. In both human (Dunn et al., 1979) and murine schistosomiasis (Takahashi et al., 1980), increased collagen synthesis and increased collagen content have been demonstrated. Several explanations for the hypoalbuminemia of murine or human schistosomiasis have also been proposed at the pathophysiologic level (Soliman et al., 1973; Page et al., 1972; Saif et al., 1977). However, mechanisms at the molecular level which may be responsible for the increased collagen deposition and hypoalbuminemia in hepatic schistosomiasis have not been explored.

We employed the murine schistosomiasis model to investigate the effect of this condition on the translational and pretranslational levels of gene activity for hepatic albumin and collagen synthesis. Not surprisingly, these proteins of different functions were regulated in contrasting ways in response to hepatic injury.

Experimental Procedures

Animals. CF 1 female mice were infected subcutaneously with 50 cercariae of *S. mansoni*, Puerto Rican strain (received from Case Western Reserve Medical School, Department of Geographic Medicine), and littermate controls were provided by the same source. Animals were killed by cervical dislocation at 3, 6, 9, and 12 weeks postinfection. Blood was taken for evaluation of total serum proteins by employing the Bio-Rad protein assay and serum albumin by the Technicon Bromocresol Green procedure.

RNA Extraction and Protein Synthesis. RNA was isolated by 8 M guanidine hydrochloride extraction and differential ethanol precipitation by using minor modifications of the procedures of Adams et al. (1977). Further purification of the RNA was accomplished by phenol–chloroform–isoamyl alcohol extraction, high salt fractionation, and ethanol precipitation. RNA content was quantitated by spectrophotometric absorption at 260 nm, and in some cases poly(A+) RNA was prepared by oligo(dT)–cellulose chromatography.

Messenger RNA dependent rabbit reticulocyte lysate was prepared by digestion with micrococcal nuclease, according to the procedure of Pelham & Jackson (1976). Incubations in a total volume of 25 μL were performed at 26 °C for 60 min and contained 12.5 μL of lysate, 0.5 mM ATP, 0.1 mM phosphocreatine, 60 μg/mL creatine phosphokinase, 100 mM KCl, 2 mM magnesium acetate, 10 mM Hepes,[1] pH 7.4, 2.0 $\times 10^{-5}$ M 19 amino acids less methionine, 15–25 μCi of [^{35}S]methionine (specific activity 800–1200 Ci/mmol), and a saturating amount of RNA (10–20 μg). Incorporation of [^{35}S]methionine into protein was determined on a 5-μL aliquot as hot trichloroacetic acid insoluble material. Under the conditions used, amino acid incorporation increased linearly for greater than 90 min, and added RNA was the rate-limiting component in the system.

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[1] Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Me_2SO, dimethyl sulfoxide.

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Table I: Physiological Data in Murine Schistosomiasis

weeks postinfection		body wt (g) ± SD	liver wt (g) ± SD	serum protein (g %) ± SD	serum albumin (g %) ± SD
3:	control	24.4 ± 0.43	1.16 ± 0.07	6.8 ± 0.3	3.5 ± 0.10
	infected	24.5 ± 0.66	1.07 ± 0.09	7.0 ± 0.2	3.7 ± 0.09
6:	control	29.15 ± 2.0	1.25 ± 0.12	6.6 ± 0.3	3.7 ± 0.08
	infected	26.9 ± 1.9	1.47 ± 0.17	6.5 ± 0.3	3.2 ± 0.06[a]
9:	control	28.7 ± 2.9	1.25 ± 0.16	6,9 ± 0.4	3.5 ± 0.10
	infected	30.8 ± 1.2	3.12 ± 0.33[a]	7.0 ± 0.2	2.9 ± 0.07[a]
12:	control	30.1 ± 1.7	1.46 ± 0.05	7.0 ± 0.3	3.5 ± 0.05
	infected	29.3 ± 4.5	3.80 ± 0.86[a]	7.2 ± 0.2	3.0 ± 0.10[a]

[a] $p < 0.001$. $n = 6$.

Immunoprecipitation of the Products Synthesized in the Cell-Free System with Anti-Albumin IgG. Immunoprecipitation was performed with protein A–Sepharose according to the method of Bhargava (1983). Briefly, the cell-free translation product was incubated for 12 h at 4 °C in 1.5% bovine serum albumin, 2% glycerol, 2 mM methionine, anti-albumin IgG, and immunoprecipitation buffer (50 mM Tris-HCl, pH 8.0, 0.5% sodium deoxycholate, 0.5% Triton X-100, 0.75 M NaCl, and 0.05% $NaDodSO_4$). This material was then mixed with 20% Sepharose which had been preabsorbed with wheat germ extract overnight. The mixture was then washed extensively with immunoprecipitation buffer and the immunoprecipitated material extracted from the Sepharose by heating to 86 °C in sample buffer. Aliquots were evaluated by liquid scintillation spectroscopy of trichloroacetic acid precipitated material or by $NaDodSO_4$ gel electrophoresis and autoradiography.

Preparation and Labeling of Cloned Albumin and Collagen DNA Probes. Plasmid pBR 322 containing mouse albumin DNA (pmalb-2) was a kind gift of Dr. S. Tilghman, and chicken pro α2 (I) collagen DNA (pCg-45) in plasmid pBR 322 was generously provided by Dr. H. Boedtker. Albumin and collagen gene sequences were prepared from their respective recombinant plasmids essentially according to the method of Villa-Komaroff et al. (1978). Briefly, the plasmids were grown in bulk in *E. coli* HB101, isolated by CsCl banding, purified, ethanol precipitated, and digested with restriction enzyme *Hin*dIII. The digests were then electrophoresed in 1% preparative agarose slab gels with Dingman & Peacock buffer (1968) to separate the purified inserted gene sequences from the residual linearized plasmid band. The recombinant DNA sequences were reisolated from the gel and were nick translated with [^{32}P]dCTP and [^{32}P]TTP to a specific activity of (3–5) × 10^7 cpm/μg of DNA by a modification of the procedure of Rigby et al. (1977).

Hybridization Analysis of Total RNA Transferred to a Membrane Filter ("Northern Transfer"). Total RNA was extracted from infected and control mouse liver as described above. Ten micrograms each of infected and control total RNA was denatured for 1 h at 50 °C in buffer containing 1.5 M glyoxal–75% Me_2SO–10 mM NaH_2PO_4–10 mM Na_2HPO_4 (McMaster & Carmichael, 1977), placed in separate lanes of a 0.8% agarose slab gel, and electrophoresed in Peacock and Dingman buffer for 4–5 h at 65 V. After electrophoresis, the RNA was transferred to Gene Screen (New England Nuclear), as described by the manufacturer, and hybridized with a "nick-translated" ^{32}P-labeled probe. After hybridization, the membrane was exposed for autoradiography at −70 °C on Kodak XAR-5 film.

Spotting of RNA onto Nitrocellular Filter Paper ("Dot Blot Assay"). Total RNA from infected and control mouse liver was serially diluted and dotted, without being denatured, onto nitrocellulose paper which had been pretreated with 3 M NaCl/0.3 M sodium citrate (Thomas, 1980). The "dot blots" were then baked for 2 h at 80 °C, prehybridized for 6–20 h, hybridized for 20–36 h, and washed essentially as described by Thomas (1980), with minor variations. The blots were exposed to Kodak XAR-5 film at −70 °C. Quantitative evaluation of the dots on exposed film was performed by densitometry.

Table II: In Vitro Protein Synthesis Using mRNA Extracted from Infected vs. Control Liver[a]

	[^{35}S]methionine incorporation [cpm × 10^5/μg of poly(A+) RNA]			
week:	3	6	9	12
control	5.3	6.6	5.5	5.4
infected	3.5	3.3	2.2	1.7

[a] Four separate experiments gave similar results.

Results

Several studies have demonstrated the deleterious effect of protein–calorie malnutrition on protein synthesis or albumin mRNA content (Yap et al., 1978; Pain et al., 1978; Quartey-Papafio et al., 1980). For this reason, we utilized only those infected animals whose growth approximated those of controls. Although their weights did not differ significantly from controls at any stage (Table I), the liver of the mice infected with *S. mansoni* became increasingly larger than controls beginning with the sixth week (Table I). This difference in liver weight was caused by the presence of granulomas and fibrous tissue. Table I also demonstrates that while total serum proteins of infected mice were similar to controls, serum albumin levels decreased significantly in infected animals, and the albumin/globulin ratio became reversed beginning with the sixth week.

To evaluate whether hypoalbuminemia was related to decreased hepatic albumin synthesis, we isolated total liver RNA, prepared poly(A+) RNA, and translated it in a rabbit reticulocyte mRNA-dependent lysate. There was no difference in the total RNA or poly(A+) RNA content per liver between control and infected mice at any stage postinfection. However, as shown in Table II, there was a persistent decrease in [^{35}S]methionine incorporation into protein in the cell-free system when the same amount of poly(A+) RNA from infected mice was compared to control. This ranged from a 33% decrease in in vitro protein synthesis at the third week postinfection to a 68% decrease at the 12th week. These results suggest that some change in the mRNA from infected liver had caused it to be less biologically active in protein synthesis than was mRNA from control liver.

When equal amounts of trichloroacetic acid insoluble product of the 6-week postinfection translation reaction were electrophoresed in an $NaDodSO_4$–polyacrylamide gel, there were obvious differences in the relative amounts of albumin

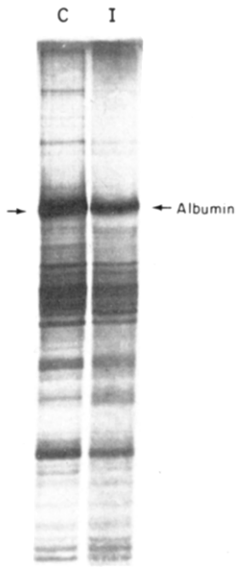

FIGURE 1: Identification of albumin in the cell-free reaction products with RNA isolated from control vs. infected liver 6 weeks postinfection. In vitro protein synthesis was performed as described under Experimental Procedures. Equal amounts of trichloroacetic acid insoluble material were applied to a 12% $NaDodSO_4$–polyacrylamide slab gel and electrophoresed according to the procedure of Shields & Blobel (1977). Slot C represents proteins synthesized from RNA of control liver and slot I from infected liver.

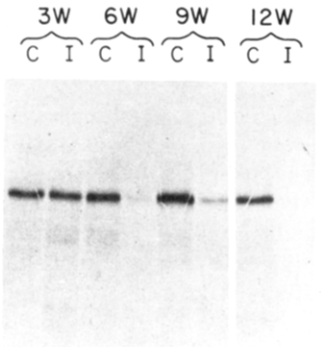

FIGURE 2: Immunoprecipitation of albumin from products of cell-free protein synthesis with RNA isolated from control vs. infected liver. In vitro protein synthesis was performed as described under Experimental Procedures, and the products were immunoprecipitated with anti-mouse albumin immunoglobulin by using protein A–Sepharose as recently described by Bhargava (1983). The immunoprecipitated material was subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis and autoradiographed. Control lanes (C) alternate with infected lanes (I) for each of the four periods of observation, 3, 6, 9, and 12 weeks, respectively.

synthesis (Figure 1). Lane C represents products of the lysate under the influence of mRNA from control liver and lane I from infected mouse liver. There is clearly more albumin synthesis with the mRNA from the liver of control animals when the same amount of total cell-free products was analyzed. Similar results were obtained at 9 and 12 weeks postinfection. However, there was no significant difference compared to controls in the amount of albumin obtained by cell-free translation of RNA obtained from livers at 3 weeks postinfection (data not shown). These findings were confirmed when the reaction products were immunprecipitated with anti-albumin immunoglobulin (Table III and Figure 2). Whereas the percentage of total proteins synthesized as albumin remained relatively constant in control animals during the entire 12 weeks (5.2–6.8%), it decreased to half this value from 6 weeks onward in infected mice (Table III). This difference

Table III: Immunoprecipitation of Albumin from in Vitro Protein Synthesis Products of RNA from Infected vs. Control Liver

	albumin synthesis (% of total [^{35}S]methionine incorporation)			
week:	3	6	9	12
control	5.8	6.2	6.8	5.2
infected	5.6	3.0	2.7	2.4

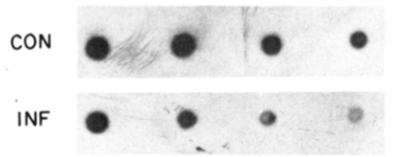

FIGURE 3: "Dot blot" analysis of RNA isolated from liver of control and 6-week infected mice on nitrocellulose paper, hybridized with an albumin cDNA probe. RNAs from control (CON) and infected (INF) liver were spotted on nitrocellulose paper as previously described (Thomas, 1980) and summarized under Experimental Procedures. Serial dilutions of the control RNA show more intense signals when hybridized with nick-translated pmalb-2 than do dilutions of RNA from 6-week infected liver.

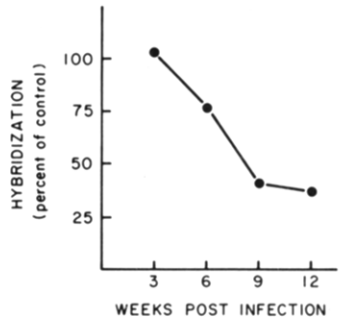

FIGURE 4: Dot blot hybridization analysis of albumin mRNA sequence content of infected liver compared to controls. RNAs from control and infected liver from each of the four time periods (3, 6, 9, and 12 weeks) were spotted on nitrocellulose and hybridized with the ^{32}P-labeled albumin cDNA probe as described under Experimental Procedures. The autoradiographs were then scanned by densitometry. For each of the four extraction periods, the desitometry reading (hybridization) of the infected RNA is represented as a percentage of the control.

was also demonstrated by $NaDodSO_4$–polyacrylamide gel electrophoresis of cell-free products immunoprecipitated with a monospecific antibody to albumin (Figure 2). The amount of albumin immunoprecipitated is similar in lanes 3WC (3-week control) and 3WI (3-week infected), but there is a marked decrease when 6-week control (lane 6WC) is compared to 6-week infected (lane 6WI) and also at 9 and 12 weeks. In fact, albumin synthesis was undetectable in lane 12WI (12-week infected) when total protein synthesis was very low.

Albumin mRNA sequence content was evaluated by hybridizing the various liver RNA extracts with a purified mouse albumin cDNA probe (pmalb-2). Figure 3 shows the results obtained after dotting progressively decreasing amounts of RNA from control and infected mouse liver 6 weeks postinfection onto nitrocellulose filter paper and hybridizing it with purified ^{32}P radiolabeled recombinant albumin sequence from plasmid pmalb-2. There is significantly greater hybridization with the RNA from control liver as compared to RNA from infected liver. This was repeated with samples at 3, 9, and 12 weeks postinfection, and the relative amount of albumin mRNA in the various dots was quantitated by densitometry scanning. Figure 4 shows a progressive decrease in the albumin mRNA sequence content as a percent of control at 6, 9, and 12 weeks. Figure 5 shows the size of the hybridizing RNA

FIGURE 5: Identification of specific albumin mRNA in total RNA isolated from liver of control and 6 weeks postinfection mice by Northern transfer. Total RNA was extracted from liver, denatured, electrophoresed, transferred to Gene screen filter sheets, hybridized with pmalb-2, and autoradiographed as described under Experimental Procedures. There is more full-size 17S albumin mRNA per microgram of control RNA (lane C) as compared to infected liver RNA (lane I).

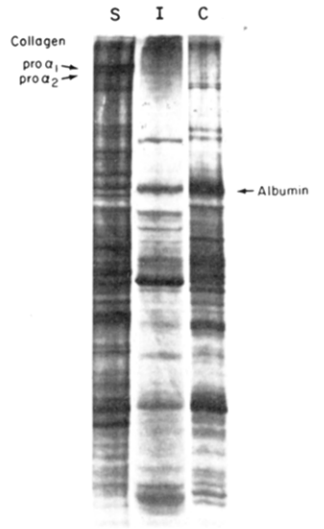

FIGURE 6: Autoradiograph of an $NaDodSO_4$–polyacrylamide gel of translation products from reticulocyte lysate with control vs. infected liver RNA 9 weeks postinfection. In vitro protein synthesis was performed as described under Experimental Procedures. Lane S represents protein products of RNA extracted from chick embryo sterum, lane I products of RNA from infected mouse liver, and lane C the same amount of trichloroacetic acid insoluble products from control mouse liver. The locations of the type I collagen bands and albumin are indicated.

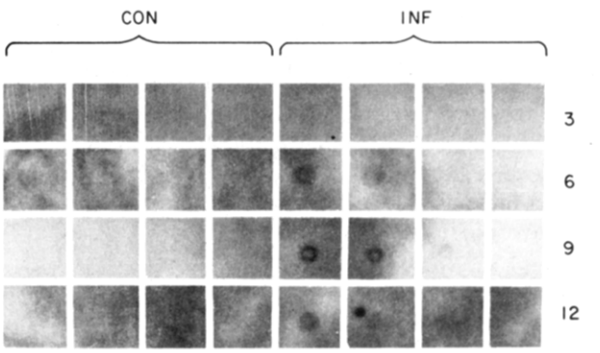

FIGURE 7: Dot blot assay of RNA from liver of control and infected mice hybridized with a collagen cDNA probe. Total RNA was extracted from liver at the four time periods postinfection and spotted on nitrocellulose paper as described under Experimental Procedures. Hybridization with pCg-45 revealed the presence of collagen sequences in liver RNA at later stages of infection but not in the RNA extract from control animals.

sequence after gel electrophoresis of RNA extracted from infected and control mouse livers 6 weeks postinfection and hybridized with the albumin cDNA probe. There was a significant decrease in 17S albumin mRNA molecules in the liver of infected mice. Figure 5 also shows that there was no evidence for enhanced albumin mRNA degradation, detected as lower molecular weight hybridizing bands in the gels, in RNA extracts from infected liver tissue.

In contrast to the decrease in serum albumin levels in murine schistosomiasis, liver collagen content increases markedly during development of hepatic fibrosis. The molecular mechanism responsible for this increased collagen content was investigated by in vitro protein synthesis and molecular hybridization. Figure 6 shows an autoradiograph of an $NaDodSO_4$–polyacrylamide gel of translation products in the reticulocyte lysate system with control vs. infected liver RNA. Lane S represents protein products of RNA extracted from chick embryo sternums, showing strong type I pro α1 and pro α2 collagen bands (the RNA was a kind gift of Dr. S. Adams, University of Pennsylvania). Lane I represents products of RNA from mouse liver at 9 weeks postinfection showing the presence of these collagen bands. Lane C shows the pattern obtained with the same amount of hot trichloroacetic acid insoluble translation products of RNA from control mouse liver. Despite evidence for considerable albumin synthesis, no collagen bands are present. Collagen mRNA sequence content was evaluated further by dot hybridization. As can be seen in Figure 7, hybridization of RNA with the chick type I pro α2 collagen probe (pCg-45) from infected and control liver at 3 weeks was undetectable. However, at 6, 9, and 12 weeks postinfection, collagen sequences were clearly present in RNA from infected liver, but not from controls. It also appears that more collagen mRNA is present per microgram of RNA in the liver of mice infected for 9 weeks than in those infected for 6 or 12 weeks.

Discussion

We have employed a murine schistosomiasis model to evaluate the steady-state level of mRNAs for two exported hepatic proteins, albumin and collagen. In this system, hypoalbuminemia is associated with a decreased albumin mRNA, and increased liver collagen is associated with an increase in collagen mRNA.

In evaluating the effect of a pathophysiological state on the protein synthesis machinery of the liver, a major concern is the possible coexistent effect of protein–calorie malnutrition. The infected mice used in the present study were not different in weight from their littermate controls. In addition, the changes we observed were different from the typical changes of protein–calorie malnutrition in that fasted animals usually show a generalized reduction in total RNA compared to fed controls (Yap et al., 1978). Some studies have also shown an increase in ribonuclease activity in starved animals (Enwonwu & Sreebny, 1971), but neither of these effects was observed in schistosomiasis-infected mouse liver as compared to controls. In the present study, there was a significant decrease in total protein synthesis activity when RNA extracted from the liver of infected mice was compared to equal quantities of RNA from control liver in an mRNA-dependent reticulocyte lysate system. This change occurred as early as 3 weeks postinfec-

tion, before marked changes were seen histologically and before hypoalbuminemia occurred. One might, therefore, postulate that subtle changes in the properties or functional behavior of mRNA can be identified by very sensitive in vitro protein synthesis techniques before these changes are observed by other methods. There is no evidence that the decreased protein synthesis activity is due to mRNA degradation, as "Northern blots" showed no increased degradation in the albumin mRNA from infected liver when compared to controls (see Figure 5). In addition, the presence of high molecular weight collagen protein products in $NaDodSO_4$ gels of RNA from infected liver also suggests that nonspecific degradation of mRNA is not a major problem, as larger mRNAs such as collagen mRNAs would presumably be more susceptible to such degradation.

Several studies have evaluated possible causes for the hypoalbuminemia of murine or human schistosomiasis. They include nutritional factors (Soliman et al., 1973), increased albumin catabolism (Page et al., 1972), diminished albumin synthesis (Saif et al., 1977), and liver damage or dysfunction (Soliman et al., 1973). Our results explore this question at the molecular level. They show a marked decrease in functionally active albumin mRNA in the liver of schistosomiasis-infected mice beginning with the sixth week postinfection, corresponding to the period of hypoalbuminemia. When these data are considered together with our findings using in situ hybridization that isolated hepatocytes from infected mice have less albumin mRNA than controls (Saber et al., 1983), it seems that the hypoalbuminemia in infected mice is influenced by a reduction in the albumin messenger content by either transcriptional or posttranscriptional control (including possible alterations in messenger RNA half-life). It should be pointed out, however, that these effects were not unique to albumin, as other proteins were altered by the infection as indicated by gel electrophoresis.

It is not surprising that albumin mRNA content is decreased in this state of chronic hepatic injury, for one might expect a "down regulation" of an exported serum protein under conditions in which limited cellular resources are available to maintain essential functions. In this regard, albumin mRNA levels have been shown to be decreased in several other types of hepatic injury, including amino acid deprivation (Yap et al., 1978), triiodothyronine supplementation (Siddiqui et al., 1981) and following acute inflammation (Morrow et al., 1981; Princen et al., 1981). During these pathophysiological stresses, there are reciprocal increases in the mRNA levels of proteins which increase during these various insults, e.g., acute phase reactants during inflammation (Morrow et al., 1981; Princen et al., 1981) or increased malic enzyme when triiodothyronine is added (Siddiqui et al., 1981).

The increased collagen content in hepatic schistosomiasis can similarly be explained by biochemical and molecular mechanisms. An increase in the hepatic proline pool size has been associated with increased collagen synthesis (Dunn et al., 1978), and increases in prolyl hydroxylase have also been demonstrated (Dunn et al., 1978). In addition, the presence of a fibroblast-stimulatory factor has been proposed as an alternative mechanism for increased collagen synthesis based on fibroblast activation (Wyler et al., 1978). Our data demonstrate that increased hepatic type I collagen in infected liver is associated with increased type I collagen mRNA content. Whereas type I collagen protein synthesis could only be demonstrated by using RNA extracted from liver at 9 weeks postinfection, dot hybridization studies showed an increase in collagen mRNA content in livers of infected mice at 6, 9, and 12 weeks as compared to controls. In this model, it appears that the collagen mRNA content was highest at 9 weeks postinfection, the same time at which Takahashi et al. (1980) have shown collagen synthesis to be at its peak. It seems, therefore, that type I collagen mRNA content parallels the increase in hepatic collagen synthesis, which in turn parallels hepatic collagen content in murine schistosomiasis.

Current evidence suggests that transcription is the most common level of gene regulation in eukaryotic cells (Darnell, 1982), and there is evidence for transcriptional control of albumin synthesis during development (Tilghman & Belayew, 1982). Transcriptional control of procollagen mRNA levels has also been reported. Rous sarcoma virus infection of fibroblasts leads to a decrease in type I procollagen mRNA levels, and this decrease has been shown to be regulated at the transcriptional level (Sandmeyer et al., 1981; Avvedimento et al., 1981). Therefore, transcriptional regulation as a possible cause of the albumin and collagen mRNA changes in murine schistosomiasis warrants further evaluation.

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5-Bromodeoxyuridine-Induced Amplification of Prolactin Gene in GH Cells Is an Extrachromosomal Event†

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ABSTRACT: Treatment of a 5-bromodeoxyuridine-resistant (brdUrd^r) and prolactin-nonproducing (Prl$^-$) subclone of GH cells with this drug led to amplification of the prolactin (Prl) gene and induced Prl synthesis. Withdrawal of the drug treatment reversed both of these processes. In normal rats, the increased Prl synthesis observed during late pregnancy and lactation does not seem to be mediated via amplification of the gene. Amplification of the Prl gene and induction of Prl synthesis can also be observed in the Prl$^-$, brdUrd-sensitive (brdUrd^s) GH cell strain. Prl gene amplification thus does not seem to be associated with the mechanism that confers the brdUrd^r phenotype to these cells. brdUrd-induced amplification of the Prl gene can be identified with the low molecular weight, extrachromosomal, supernatant DNA fraction, isolated by Hirt's method. Southern blot analysis of Hirt's supernatant DNA (undigested) from brdUrd-treated cells generated a distinct band following hybridization with [^{32}P]$_p$DNA$_{Prl}$-insert. The size of this band is greater than 23 kb but smaller than chromosomal DNA. Growth hormone (Gh) and albumin (Alb) gene sequences can be detected in the chromosomal DNA preparation but are absent in the extrachromosomal DNA prepared from Hirt's supernatant. The levels of Gh and Alb sequences are unaffected by brdUrd treatment of these cells. Results presented here suggest that in rat pituitary glands as well as in GH cells, hormonally controlled increased Prl synthesis is not caused by gene amplification. However, the brdUrd-induced expression of the Prl gene seems to be linked to the mechanism of drug-induced amplification of the Prl gene, mediated via an extrachromosomal event.

Different GH[1] (rat pituitary tumor) cells in culture synthesize and secrete different amounts of prolactin (Prl) (Brennessel & Biswas, 1980). Prl synthesis cannot be detected in the GH subclone GH_12C_1. Two 5-bromodeoxyuridine-resistant (brdUrd^r) strains were derived from GH_12C_1 by stepwise exposure of these cells to drug concentrations higher than the sublethal dose (Biswas et al., 1977). Prl synthesis can be induced in one of the brdUrd derivatives, $F_1BGH_12C_1$, and in the parent strain, GH_12C_1, following treatment of the cells with the drug (Biswas et al., 1977), whereas the second brdUrd^r derivative, $F_2BGH_12C_1$, does not synthesize Prl either in the absence or in the presence of the drug. Our recent results demonstrate that treatment of $F_1BGH_12C_1$ cells with brdUrd increases the level of Prl gene sequences in conjunction with the increased Prl production (Biswas & Hanes, 1982).

In eukaryotes, amplification of specific genes has been observed in several systems during normal cellular developmental processes in parallel with the overproduction of the gene product. The rRNA gene sequences increase several thousand fold during oogenesis in *Xenopus* (Brown & Dawid, 1968) and during macronuclear formation in *Tetrahymena* (Yao et al., 1979). Amplification of chorion protein genes during oogenesis in *Drosophila melanogaster* (Spradling & Mahowald, 1980) and amplification of actin genes during myogenesis in chicken (Zimmer & Schwartz, 1982) are two other examples of such gene amplification processes during normal development. Gene amplification is also observed in several instances in eukaryotic cells, which utilize this mechanism to resist the toxic effects of certain drugs. Overproduction of dihydrofolate reductase was observed in methotrexate-resistant mouse and

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[1] Abbreviations: GH cells, rat pituitary tumor cells in culture; Prl, prolactin; Alb, albumin; Gh, growth hormone; brdUrd, 5-bromodeoxyuridine; r, resistant; s, sensitive; Prl$^+$, prolactin-producing cells; Prl$^-$, prolactin-nonproducing cells; $_pDNA_{Prl}$, cloned (in plasmid pBR322) DNA complementary to mRNA$_{Prl}$ (Gubbins et al., 1979); $_pDNA_{Gh}$, cloned DNA complementary to mRNA$_{Gh}$ (Seeberg et al., 1977); $_pDNA_{Alb}$, cloned DNA complementary to rat mRNA$_{Alb}$ (Gorin & Tilghman, 1980); $_pDNA_{Prl}$-insert, $_pDNA_{Prl}$ sequence released by *Pst*I from the plasmid DNA.

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