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Regulation of plasma acute-phase protein and albumin levels in the liver of scalded rats

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At 12 h after scalding of rats a doubling of the hepatocyte nuclear DNA content, which arose from the presence of additional complete genomes and not from amplification of genes coding for the major acute-phase proteins or albumin, was observed. Examination of relative transcription rates per control DNA mass revealed that α_1-acid-glycoprotein and cysteine-proteinase-inhibitor genes remained constitutive, α- and γ-fibrinogen and haptoglobin genes underwent transcriptional activation for 290 and 339% respectively, whereas the relative transcription rate of albumin decreased to 65% of the control level. Along with these changes, the α_1-acid glycoprotein, cysteine-proteinase inhibitor and the fibrinogen mRNA concentrations increased about 500%, haptoglobin mRNA 250%, whereas the albumin mRNA concentration fell to 86% of the control. The regulation of the mRNA levels was assessed by comparing the relative change in transcription rates expressed per control DNA content with the relative changes of mRNA concentrations. We arrived at the conclusion that the concentrations of α_1-acid-glycoprotein and cysteine-proteinase-inhibitor mRNAs were predominantly regulated by a post-transcriptional mechanism, albumin mRNA by a transcriptional mechanism, and the fibrinogen and haptoglobin mRNAs by a combination of both. The degree of change of the serum levels of the examined proteins was similar to that of their mRNA concentrations and was the result of the complete use of the available RNA templates in protein synthesis.

INTRODUCTION

In response to tissue injury or infection the liver assumes a very important role, performing a spectrum of biological activities [1], among which the increased synthesis of a subset of plasma proteins, the acute-phase reactants, is of particular importance [2]. During the acute-phase response elicited by a scalding encompassing 20% of the rat's dorsal skin surface, we have observed in the liver an enhancement of hepatocyte mitotic activity and a shift to a higher percentage of polyploid cells [3], at the ultrastructural level the appearance of invaginations of the nuclear membrane, enlargement of nucleoli, an increase in the number of nuclear pores, an accumulation of ribonucleoproteins in the nucleoplasm [4] and, by cell-free translation and Northern analysis, the several-fold accumulation of functional mRNAs coding for the major acute-phase proteins (APPs) and a decrease of albumin (Al) mRNA [5]. The APP mRNA concentrations were found to rise after the infliction of trauma and, although the induction kinetics were not the same for the various APPs (see also [6]), all of the respective mRNAs reached their highest concentrations by 24 h (see also [2]) after scalding. These levels were still maintained 48 h after the infliction of trauma, and by 56 h the mRNA concentrations began to decline towards the control values. Since the APPs fulfill a broad spectrum of essential functions [7], examination of the consecutive stages of regulation of APP levels is of considerable importance. To this end we studied the transcriptional activities of the APP and Al genes at 12 h after scalding, i.e. at the time point at which a maximal increase in transcriptional activity preceding the plateau values of APP mRNA concentrations has been observed [8]. The nuclear activity was compared with the changes of mRNA concentrations and the protein levels in the plasma.

MATERIALS AND METHODS

Materials were obtained as follows: [α-^{32}P]dCTP, L-[^{35}S]methionine, nick-translation kit and Hyperfilm MP (The Radiochemical Centre, Amersham, Bucks., U.K.); nitrocellulose filters (BA 85; 0.45 μm pore size; Schleicher und Schuell, Keene, U.S.A.); RNAase A (EC 3.1.27.5) and pBR322 (Boehringer-Mannheim GmbH, Mannheim, Germany); proteinase K (EC 3.4.21.14; E. Merck, Darmstadt, Germany); yeast tRNA and salmon testes DNA (Sigma Chemical Co., St. Louis, MO, U.S.A.); analytical-grade sucrose, Triton X-100 (Serva Feinbiochemica GmbH, Heidelberg, Germany); incomplete Freund's adjuvant (The Torlak Institute for Immunology and Virology, Belgrade, Yugoslavia); NOR-Partigen radial immunodiffusion plates (Calbiochem–Behring Corp., Lucerne, Switzerland). All other materials were of reagent grade.

Plasmids encoding the following rat plasma proteins were used (the insert size in base-pairs at the restriction-endonuclease-*Pst*I site of pBR322 is given in parentheses): pIRL21, α_1-acid glycoprotein (AGP) (850);

Abbreviations used: APP, acute-phase protein(s); AGP, α_1-acid glycoprotein (= orosomucoid); CPI, cysteine-proteinase inhibitor (= major acute-phase protein = α_1-acute-phase globulin = T-kininogen); Fb, α- and γ-fibrinogens; Hp, haptoglobin; Al, albumin.

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pIRL25, haptoglobin (Hp) (1300); pIRL28, cysteine-proteinase inhibitor (CPI) (1600); pIRL14, α-fibrinogen (α-Fb) (975); pIRL20, γ-fibrinogen (γ-Fb) (1650); these were all obtained from Dr. H. Baumann (see Baumann *et al.* [9]) of the Roswell Park Memorial Institute, Buffalo, NY, U.S.A.; pRSA57, Al (1175), was obtained from Dr. T. D. Sargent (see Sargent *et al.* [10]) from the California Institute of Technology, Pasadena, CA, U.S.A.

Male adult Wistar rats weighing between 220 and 250 g were used.

Induction of the acute-phase response by scalding was performed by the method of Arturson [11]. About 20% of the total skin surface was shaved on the dorsal side of the rat 24 h before the experiment and exposed to hot water (83 °C) for 30 s under ether anaesthesia. The animals were maintained without food and water after the infliction of the trauma and were killed either 12 or 24 h after scalding.

Rat liver nuclei were isolated by homogenization in 0.25 M-sucrose/50 mM-Tris/HCl (pH 8.0)/10 mM-$MgCl_2$/1 mM-dithiothreitol and purified by ultracentrifugation in the same buffer containing 2.2 M-sucrose. Nuclei were counted in a Bürker–Türk counting chamber.

The nuclear-transcription assay was performed as described by Vannice *et al.* [12], except that the nuclei were incubated with 18.5 MBq of [α-^{32}P]UTP (9.25 MBq/mmol)/ml. The ^{32}P-labelled RNA was hybridized to nitrocellulose filters containing 3 μg of linearized denatured plasmid DNAs containing inserts complementary to rat mRNAs for AGP, CPI, Hp, Fb and Al, as well as pBR322. The filters were hybridized with all of the '*in vitro*'-labelled RNA isolated from 10^8 nuclei of control and scalded rats, i.e. with about 10^8 and $(2–3) \times 10^8$ d.p.m. respectively. The conditions of pre-hybridization, hybridization and washing were as described [5], except that the hybridization was carried out for 42 h. The transcription assays were quantified by scanning the autoradiograms in a linear range with a Beckman Microzone densitometer (model 110).

DNA was isolated from the purified nuclei. The nuclear envelope and attached ribosomes were removed by incubating nuclei (10^9/ml) for 10 min in homogenization buffer containing 1% Triton X-100 [13] as an addition and centrifugation for 10 min at 1500 *g*. The nuclear pellet was resuspended in 10 mM-Tris/HCl (pH 7.8)/5 mM-EDTA, SDS was added to a final concentration of 0.5%, and the nuclei were lysed by gentle mixing; proteinase K was added to a final concentration of 50 μg/ml [14] and then incubated overnight at 37 °C [15]. The mixture was extracted with a phenol/chloroform/3-methylbutan-1-ol mixture (1:1:0.24, by vol.) twice; RNAase A was added to the aqueous phase to a final concentration of 100 μg/ml and the mixture incubated at 37 °C for 1 h. The mixture was extracted twice more with phenol/chloroform/3-methylbutan-1-ol and once with chloroform and dialysed in 10 mM-Tris/HCl (pH 8.0)/1 mM-EDTA.

The relative number of copies of the examined APP and Al genes per μg of total DNA was quantified by densitometry of the autoradiograms obtained after Southern transfer and dot-blot analysis as described in the Schleicher und Schuell Specification Sheet 352–354.

Total liver RNA was extracted by the guanidinium hydrochloride procedure [17,18], separated on agarose gels [19], transferred to nitrocellulose and hybridized to nick-translated plasmids encoding the APPs and Al [5].

Plasma proteins were labelled *in vivo* for 90 min before 12 and 24 h post-scalding after an intraperitoneal injection of L-[^{35}S]methionine (9.2 MBq; 1160–1460 TBq/mmol).

Proteins were separated electrophoretically in the first and second dimensions as described by O'Farrell [20] and the gels were prepared for fluorography by the method of Bonner & Laskey [21].

Antiserum was prepared by subcutaneously injecting into rabbits 150 μl of rat serum isolated 24 h after the injection of turpentine (250 μl/rat). Rat serum was diluted with saline (0.9% NaCl, 350 μl) and mixed with complete Freund's adjuvant. Over a period of 2 weeks, three to five more injections were given with incomplete Freund's adjuvant.

Crossed immunoelectrophoresis was performed by the method of Laurell [22].

The relative changes in concentration with respect to the controls of AGP and CPI were established by quantification of the areas under the immunoprecipitation peaks after immunoelectrophoresis. The concentration of Hp was obtained by radial immunodiffusion, of Fb by the method of Koj & McFarlane [23] and of Al by precipitation with poly(ethylene glycol) [24], followed by the method of Lowry *et al.* [25] for protein determination.

The DNA content of liver nuclei was determined by the method of Burton [26].

RESULTS

When isolated 12 h after scalding, rat liver nuclei contained (±S.E.M.) 24.1±2.56 μg of DNA/10^6 nuclei, this was 2.04 times higher than the value of 11.8±0.21 μg of DNA/10^6 nuclei determined in the controls.

Results obtained after densitometry of the autoradiograms from the Southern blots of total nuclear DNA (Fig. 1) unambiguously demonstrated that the number of copies of these genes remained unchanged per unit mass of DNA and that the net result of the scalding was the doubling of a complete hepatocyte genome rather than amplification of particular sequences.

In order to correlate the establishment of the plateau levels of APP and Al mRNAs at 24 h post-scalding [5] with the nuclear events that precede them, the transcription of APP and Al genes was examined by '*in vitro*' transcription in isolated nuclei at 12 h post-scalding. The amount of *in vitro*-labelled RNA which hybridized to each dot containing cDNAs for the APPs and Al was quantified by scanning the autoradiograms (Fig. 2). As Table 1, column 1, shows the relative transcriptional activity of 10^8 nuclei had increased for all the examined genes 12 h after scalding. However, when the average increase of the DNA content in the nuclei of traumatized rats was taken into account and the relative rates of transcription per control DNA template content calculated for the genes in question, it appeared that the AGP and CPI genes were constitutively transcribed throughout and the Fb and Hp genes showed respectively a 284 and a 332% increase in relative transcription rate; whereas the Al-gene transcription rate, however, was decreased to 65% of the control value (Table 1, column 2).

The relative changes in concentration of APP and Al mRNAs were established by Northern analysis (Fig. 3). The results (Table 1, column 3) show that the concentrations of the examined APP mRNAs increased (AGP,

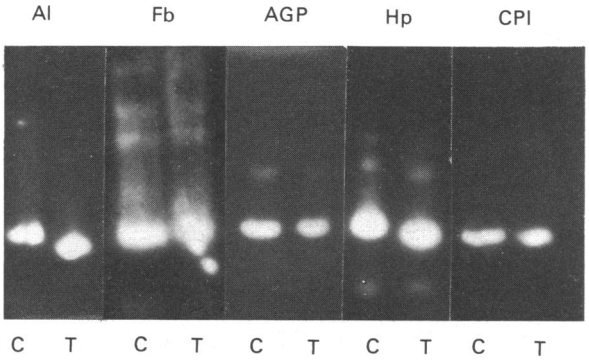

Fig. 1. Estimation of relative amounts of complementary APP and Al DNA sequences in total DNA isolated from control and treated animals

A portion (10 μg) of total endogenously digested liver DNA from control (C) and rats killed 12 h after the infliction of scalding (T) were electrophoretically separated in 0.8% agarose, transferred to nitrocellulose filters and hybridized with nick-translated plasmids bearing sequences complementary to Al, Fb, AGP, Hp and CPI mRNAs.

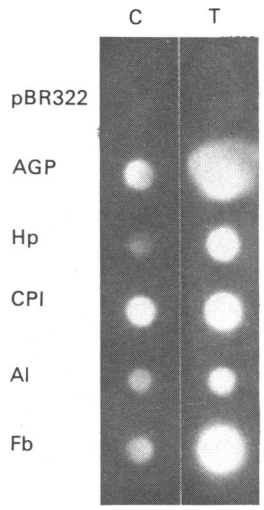

Fig. 2. Nuclear transcription assay of APP and Al genes

Purified nuclei (10^8) from livers of control (C) and rats killed 12 h after scalding (T) were incubated with [α-^{32}P]UTP. Filters containing 3 μg of linearized, denatured plasmid DNAs bearing sequences complementary to the mRNAs of the indicated proteins were hybridized for 42 h with the *in vitro*-labelled RNA, washed and autoradiographed.

CPI and Fb by 500% and Hp by 250%), whereas the Al mRNA concentration was 24% lower than in the control.

The mode of regulation of APP and Al synthesis can be assessed by two criteria: either by comparing the extent of the relative changes of the mRNA levels with the nuclear transcriptional activities or with the transcriptional activities per control nuclear DNA content. In the case of CPI and AGP, where the relative transcription rates per control DNA content remained unchanged after scalding (Table 1, column 2) and the increase in mRNA levels exceeded the increase of the nuclear transcriptional activities (Table 1, column 1), the regulation of the mRNA concentrations could be primarily ascribed to post-transcriptional mechanisms. However, differences implicated in the two criteria became more evident in the cases of Fb, Hp and Al. Thus the same magnitude of increase of the level of Fb mRNA and the nuclear transcriptional activities (Table 1, columns 1 and 3) suggested that the concentration of Fb mRNA was predominantly under transcriptional control, whereas a

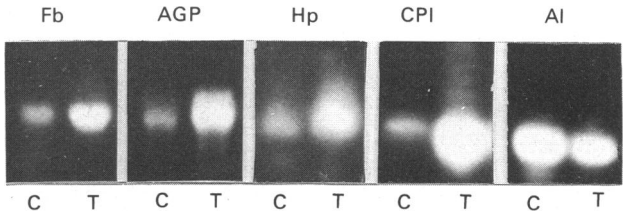

Fig. 3. Estimation of the relative changes of liver mRNA concentrations 12 h after scalding

A portion (20 μg) of total liver RNA from control (C) and animals killed 12 h after the infliction of scalding (T) were separated in 0.8% agarose, transferred to nitrocellulose and hybridized with nick-translated plasmids bearing sequences complementary to Fb, AGP, Hp, CPI and Al mRNAs.

Table 1. Scalding-induced changes in the relative transcriptional activities and mRNA and protein concentrations with respect to the control values (100%)

The concentrations were established as described in the Materials and methods section. The values are averages ± S.E.M. from three to six different experiments.

	Relative change in transcriptional activity		Relative change in concentration		
				Plasma protein	
Protein	Per nucleus	Per control DNA content	mRNA	12 h	24 h
Al	130±25	65	86±3.93	85.7±2.56	87.7±3.04
AGP	230±51	113	499±63	360±35.8	495±16.6
CPI	213±32	104	475±37.9	213±26.1	423±32.3
Fb	580±99	290	477±65.8	346±36.8	511±25.2
Hp	678±40	339	250±39	157±8.49	231±19.5

comparison with the relative increase in the transcription rate on the DNA template (Table 1, column 2) implied the involvement of post-transcriptional mechanisms as well. When the relative increase in concentration of Hp mRNA was compared with the increase in the rate of Hp-gene transcription (Table 1, column 2), it appeared that the concentration of this mRNA was essentially under transcriptional control, whereas an evaluation with respect to the increase in the nuclear transcriptional activity (Table 1, column 1) pointed to a considerable influence of post-transcriptional mechanisms. In the case of Al, the criteria based on the alteration of the relative transcription rate (Table 1, column 2) argued for a transcriptional mode of Al mRNA control, whereas that based on the net increase of the nuclear transcriptional activity (Table 1, column 1) implied the involvement of both transcriptional and post-transcriptional mechanisms. Here it must be pointed out that our interpretations were based on observations of relative changes of transcriptional activities and mRNA concentrations. Therefore we arrived at an estimate of the contribution of the relative transcriptional and post-transcriptional mechanisms to the changes in APP levels. A definitive measure of these contributions would require an assessment of absolute changes in the number of nuclei active in APP-gene transcription and in the amount of individual RNAs per liver.

Scalding-induced changes in the composition of plasma proteins were studied by two-dimensional polyacrylamide-gel electrophoresis and fluorography of ^{35}S-labelled proteins (Fig. 4) as well as by crossed immunoelectrophoresis (Fig. 5) with a polyspecific antiserum to the plasma of turpentine-treated rats. The percentage changes in concentration relative to the control levels (100%) of the APPs and Al are presented in Table 1. They show that the increase in concentration of APPs at 12 h post-scalding was about 30–50% lower (column 4) and at 24 h similar (column 5) to the relative increases of the respective mRNAs at 12 h (column 3), which suggests that all the available mRNA templates were translated into proteins.

DISCUSSION

In the present paper we have examined the levels of control of APP and Al concentrations during the acute-phase response. We have established that hepatocytes responded to trauma with a shift to a higher percentage of polyploid cells and have ruled out an APP gene-dosage effect per unit mass of DNA. This doubling of the DNA templates resulted in a general increase of nuclear transcriptional activity, thus masking the changes of transcription rates on the DNA templates. Therefore, in order to establish the mechanisms which regulated APP and Al RNA synthesis, the extents of change of the mRNA concentrations (Table 1, column 3) were compared with the extents of change of the respective transcriptional activities per control DNA content (Table 1, column 2). Our results suggest that the relative transcriptional and post-transcriptional contribution to changes in RNA concentrations differed between the APPs, i.e. that their RNA concentrations were regulated by different mechanisms. In the cases of CPI and AGP, where the relative transcription rates remained virtually unchanged (104 and 113% respectively) and where the mRNA concentrations rose 5-fold (475 and 499%

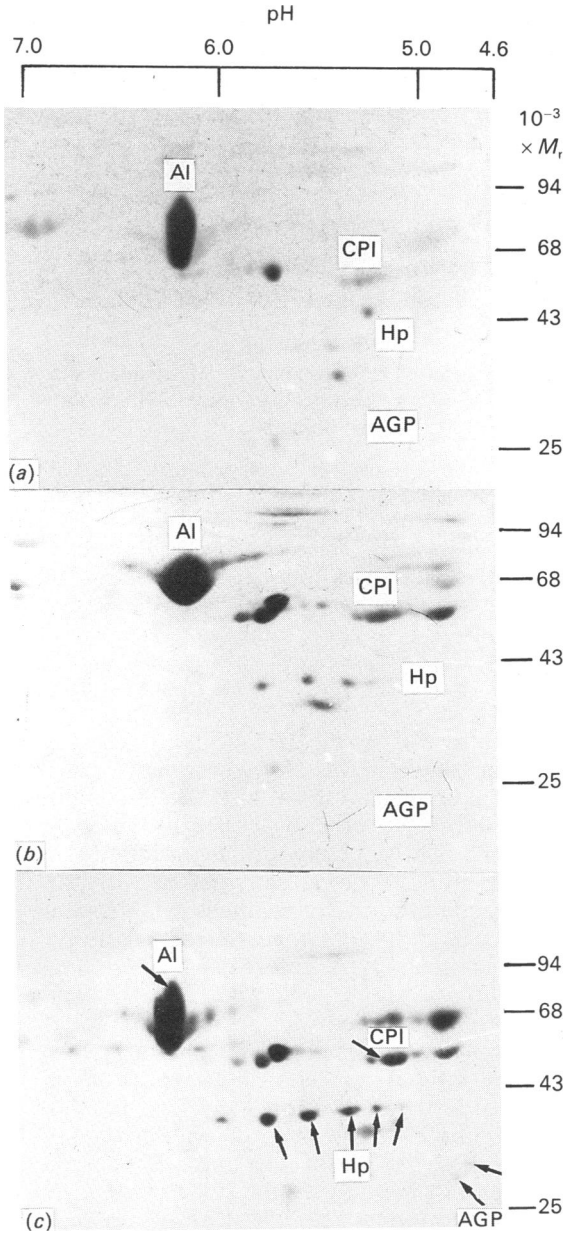

Fig. 4. Two-dimensional electrophoretic analysis of plasma proteins

The ^{35}S-labelled proteins (600 μg) isolated from control (*a*) and rats killed 12 h (*b*) and 24 h (*c*) after scalding were separated in the first and second dimensions and the polyacrylamide gels prepared for fluorography. Only the proteins whose relative transcriptional activities and mRNA concentrations were studied are indicated on the fluorograms. The proteins were identified on the basis of the previously determined pI and M_r values [30].

respectively), the major form of regulation was post-transcriptional. On the other hand, definite increases in transcription were found for the Fb and Hp genes. Since the rise in Fb mRNA concentration (477%) exceeded the relative increase in transcription rate (290%), we concluded that post-transcriptional mechanisms contributed to the establishment of its mRNA levels. Post-transcriptional mechanisms were also involved in Hp regulation. Here the increase of the relative trans-

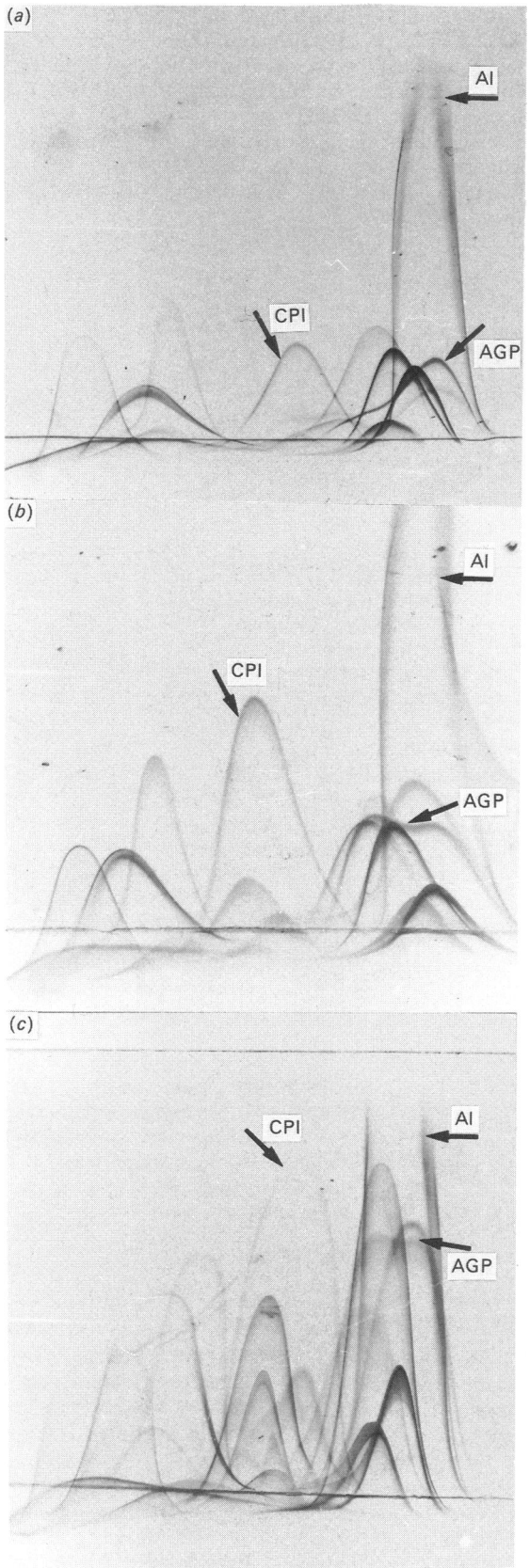

Fig. 5. Immunoelectrophoretic identification of the major APPs in the sera of scalded rats

Crossed immunoelectrophoresis was performed with sera isolated from control (*a*) and rats killed 12 h (*b*) and 24 h (*c*) after scalding. Proteins were stained with Coomassie Blue. AGP was identified with a monospecific antibody and CPI by re-electrophoresis and two-dimensional SDS/polyacrylamide-gel electrophoretic analysis of the immune precipitate on the basis of its pI and M_r [30].

criptional activity (339%) exceeded the rise in mRNA concentration (250%). This may have been the result of synthesis of nucleus-confined Hp sequences which were not a part of stable mRNAs [27]. Lastly, the principal mechanism of Al regulation was transcriptional; its behaviour as a 'negative' APP [1] was the result of a decrease in transcriptional activity, since its transcriptional activity and mRNA concentration underwent a near-proportional decrease.

The changes in concentration of APP and Al at 24 h post-scalding, when the highest plasma levels of APP were reached, closely paralleled the maximal accumulation of the respective mRNAs. From these results the conclusion can be drawn that the APP and Al concentrations in plasma were controlled by their mRNA concentrations. This is in agreement with previous findings [6,28,29] that AGP, CPI and Al mRNA concentrations and rates of protein synthesis change coordinately during acute inflammation.

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