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Pharmacological inhibition of hormonal tyrosine amino transferase induction

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WHILE much knowledge has been gained on the mechanism of mRNA translation by ribosomes, little is known about its processing, i.e. the events which occur from its origin until its appearance in the cytoplasmic polysomal complexes.

We have sought to examine here the role of ribosomal RNA processing on mRNA expression by determining the effect of selective nucleolar poisons on the inducibility of tyrosine amino transferase (TAT). TAT activity, which is decreased in fasted animals, is readily brought up to normal levels, either by refeeding or by steroid hormone injection.[1] This effect is mediated through stimulation of specific mRNA synthesis,[2] which in turn brings about enhanced translation.[3] One such poison is actinomycin D, a potent inhibitor of RNA synthesis,[4] which can, when administered in sublethal doses, specifically suppress rRNA synthesis without affecting mRNA production.[5–8] Thioacetamide, which induces profound changes in nuclear and especially in nucleolar physiology,[9,10] such as enhancement of nucleolar RNA synthesis,[11] increased RNA polymerase activity[12] and suppression of a 45S RNA-cleaving RNase,[13] is another good probe.

TABLE 1. EFFECT OF ACTINOMYCIN D AND THIOACETAMIDE ON THE INDUCIBILITY OF TYROSINE AMINO TRANSFERASE (TAT)*

Type of sample	N	$\bar{x}$	S. D.	PU
Induced	6	3·80	1·00	
Induced + thioacetamide	5	1·14	0·31	0·002
Induced + actinomycin D	4	1·02	0·24	0·005

* N = number of experiments. Each experimental value is obtained from a pool of three to five rats. $\bar{x}$ = Induction coefficient (enzyme units in experimental animals/enzyme units in noninduced control). S. D. = standard deviation. PU = probability (Mann–Whitney U test[15]). TAT activity was determined by the Diamondstone procedure.[14]

Male, adrenalectomized, Wistar-strain albino rats received intraperitoneal injections of either thioacetamide (50 mg/kg of body weight) for 7 days or actinomycin D (0·55 mg/kg of body weight) once, 3 hr before hormonal induction (hydrocortisone, 150 mg/kg of body weight). The animals were sacrificed 3 hr after induction and TAT activity[14] was assayed in the 105,000 *g* supernatant of liver homogenates.[8]

Table 1 shows that experimental animals present a 4-fold increase of TAT activity over basal values, whereas in animals given either thioacetamide or actinomycin D injections no significant inductions occurred (induction coefficients were 1·14 and 1·02, respectively, as opposed to 3·8 in the control group).

In order to check the selective inhibition of rRNA synthesis by actinomycin D, as used in the present experiments, 10 mCi ^{32}P-orthophosphate was injected intraperitoneally 3 hr after drug administration. After 4 hr, the rats were sacrificed, their livers were removed and ribosomes were precipitated from the deoxycholate-treated (final concentration, 0·5 per cent) post-mitochondrial supernatant by centrifugation at 105,000 *g* for 90 min. The RNA was extracted by a phenol method[16] and used for acrylamide gel electrophoresis[17] and base composition analysis. For the latter purpose, RNA samples were hydrolysed with 0·3 M KOH for 18 hr at 37° and their base composition was determined by thin-layer chromatography on PEI-cellulose plates.[18] Eluted spots were assayed for their absorbancy and radioactivity. Figure 1 shows that the radioactivity profile does not follow that of the absorbancy on gel electrophoresis, displaying a single peak at about 17S. Base composition analysis (Table 2) shows that the radioactive RNA has a $G + C$ content consistent with that reported for mRNA by other workers[19–21] and much lower than that observed for rRNA ($G + C$, 61·04 per cent).

Ribosomal RNA and ribosome formation have been implicated in the regulation of growth and development of mammalian cells by partially controlling mRNA transport and expression.[22] The results are

therefore suggestive that rRNA processing is important for induction of TAT activity, even though other interpretations cannot be excluded.

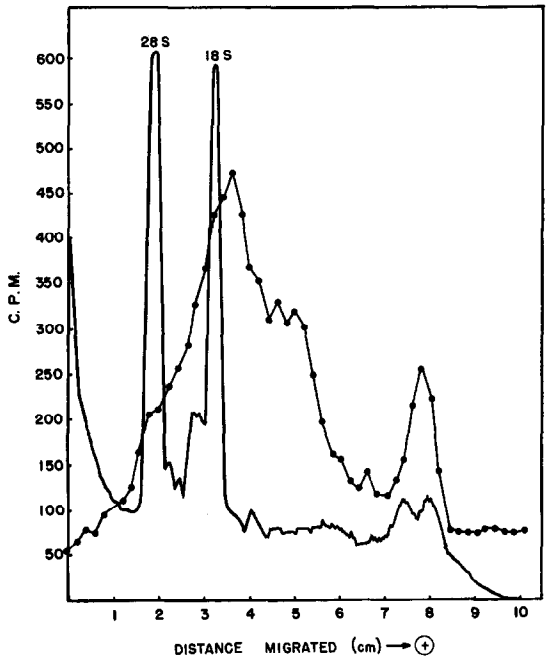

FIG. 1. Electrophoretic profile of RNA synthesized in the presence of actinomycin D. RNA extracted from rat liver ribosomes was run on 2·7% acrylamide gels for 3 hr at 5 mA/gel. After u.v. scanning, the gels were sliced and the radioactivity was determined. Experimental animals received intraperitoneal injections of actinomycin D (0·55 mg/kg of body weight) and 10 mCi ^{32}P-orthophosphate, 7 and 4 hr before sacrifice respectively.

TABLE 2. BASE COMPOSITION ANALYSIS OF RNA SYNTHESIZED IN THE PRESENCE OF ACTINOMYCIN D*

	Radioactivity	DNA†	Absorbancy
GMP	24·93 ± 1·74 (5)	23·7	36·65 ± 1·93 (5)
UMP	23·48 ± 1·66 (5)	26·4	21·31 ± 2·1 (5)
AMP	28·92 ± 1·67 (5)	28·4	17·55 ± 1·46 (5)
CMP	22·67 ± 1·05 (5)	20·7	24·39 ± 0·24 (5)
GMP + CMP	0·90	0·81	1·57

* The same RNA employed in the experiment depicted in Fig. 1, was hydrolysed with 0·3 M KOH for 18 hr at 37°. After neutralization and removal of insoluble $KClO_4$, individual nucleotides were separated by thin-layer chromatography,[18] eluted and the radioactivity and absorbancy were determined. The numbers in parentheses refer to the number of determinations.

† Taken from Wilson and Hoagland.[6]

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Device for controlled drug release—Application to methotrexate infusion in mice

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IN THE COURSE of our research to relate the pharmacokinetics of methotrexate* to its biochemical effect *in vivo*,[1] we developed a small diffusion cell for constant release of this drug in mice. The cell is easily fabricated and implanted subcutaneously. Because it is adaptable for the controlled slow release of numerous other chemicals, we report our technique for its fabrication, its behavior *in vitro*, and plasma concentrations of methotrexate in normal and tumor-bearing mice after its implantation.

Controlled release of drugs from implants has been discussed widely. The most commonly used material for control of release rate is silicone rubber. Folkman and Mark[2] have reviewed the application of this material for release of a variety of drugs and cited a number of examples of sustained pharmacologic effect. Schmidt *et al.*[3] employed silicone rubber capsules for release of the lipophilic antineoplastic drug BCNU.†

* *N*(*p*-[(2,4-diamino-6-pteridinylmethyl)methylamino]benzoyl) glutamic acid, a strong inhibitor of dihydrofolate reductase.

† 1,3-Bis (2-chloroethyl)-1-nitrosourea.