KINETICS OF LIVER NUCLEIC ACID TURNOVERS DURING ENZYME INDUCTION IN THE RAT

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(Received June 3rd, 1958)

SUMMARY

The parenteral administration of tryptophan in the rat produces a transient elevation in liver protein synthesis and in liver tryptophan peroxidase activity which results in an increased incorporation of inorganic ^{32}P and [^{14}C]glycine into RNA. The relative kinetics of these phenomena indicate that there is a burst of RNA synthetic activity subsequent to the period of active enzyme protein synthesis and that this increased RNA synthesis persists for 8 h and then returns to control rates.

A working hypothesis is proposed in which it is suggested that increased enzyme protein synthesis utilizes and consumes pre-existent RNA and that this RNA is subsequently replenished. This hypothesis implies that RNA serves as a reactant which is consumed, rather than as a perfect catalyst of protein synthesis.

INTRODUCTION

Extensive evidence on nucleic acid and protein inter-relationships of cytological, nutritional, isotope tracer and enzymic natures have led to the current hypothesis that nucleic acids, and in particular RNA, may serve as templates for the synthesis of proteins in the cell[1,2]. Compatible with this hypothesis is the demonstration that nucleic acid synthesis is a necessary pre-condition for maintenance of enzyme induction in micro-organisms[3,4]. To elucidate further the role of RNA in the intimate mechanism of enzyme protein synthesis, it would be of value to ascertain whether induced enzyme formation, which is generally accepted as representing *de novo* synthesis of enzyme protein[5–8] must be preceded by the synthesis of its nucleic acid template. Thus, a role might be assignable to the nucleic acids in the sequence of events leading to enzyme induction by its substrate.

For a study of the general relationships between protein synthesis and nucleic acid metabolism, induced enzyme formation provides a unique tool, which has been employed in bacterial studies and which is herein extended to mammalian systems. Parenteral substrate administration to mammals induces in certain instances[9] homologous enzyme formation. This phenomenon occurs *in vivo* and is presumed to represent stimulation in the synthesis of the enzyme protein whose concentration may be readily ascertained by measurement of its catalytic activity. Tryptophan peroxidase (TPO) induction may be initiated at will by its substrate and is of brief duration

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in the rat[10]. This system provides an acute situation, permitting the investigation of kinetic relationships between nucleic acid and protein syntheses in an attempt to gain further insight into the precise mechanisms linking these two biochemically important classes of compounds. The current study investigates these inter-relationships *in vivo*, employing ^{32}P and ^{14}C precursors of nucleic acids to assess the turnovers of RNA and DNA preceding, during and following tryptophan peroxidase induction in the rat.

METHODS

Food was withdrawn 20 h before sacrifice from adult male Sprague-Dawley strain rats of approximately 400 g body weight. Tryptophan peroxidase induction was initiated by the intraperitoneal injection of 0.45 mmoles of L-tryptophan per 100 g body weight as a 0.25 *M* suspension in 0.9 % saline. To vary the enzyme induction period, animals were injected with tryptophan at spaced intervals and were then sacrificed together. Twenty μC of inorganic ^{32}P (Abbott Laboratory) and 5 μC of [2-^{14}C]glycine (Isotopes Specialties Company) per 100 g body weight were administered intraperitoneally as indicated in the text. The ^{32}P and [2-^{14}C]glycine were administered in all cases precisely two hours preceding sacrifice of the animal. In this manner, a 2-h isotope incorporation period took place at various times post-tryptophan injection, and the extent of RNA, DNA and total protein turnovers during this 2-h interval, as well as the magnitude of enzyme activity at the end of this period were assessed.

The livers were promptly removed from the decapitated rats, chilled in chipped ice and weighed. After homogenization in a semi-micro Waring blendor for 2 min at 2° with 7 vol. of 0.14 *M* potassium chloride containing 0.0025 *N* sodium hydroxide, aliquots of the homogenates were assayed for TPO employing the procedure of KNOX AND AUERBACH[11]. To measure ^{32}P incorporation into the nucleic acids as an indication of their net synthetic rates, RNA, DNA and inorganic phosphorus (I.P.) isolations were undertaken. Five ml of cold 50 % trichloroacetic acid (TCA) were added to 35 ml of the chilled homogenates. Following a 5-min extraction period at 0° and centrifugation in the cold, I.P. was isolated from the TCA supernatants, as described by DAVIDSON AND FREEMAN[12]. The TCA-extracted liver residues were placed in centrifuge tubes and washed with 40 ml of cold 95 % ethanol to remove the TCA and then extracted three times with 40-ml portions of boiling 95 % ethanol and once with boiling absolute ethanol. The ethanol-extracted residues were then re-extracted three times with 40 ml of 10 % sodium chloride solution for 15 min at 100° with constant mixing. In the ^{14}C experiments, these residues were reserved for protein isolation. The three sodium chloride extracts were pooled and the sodium nucleates precipitated by the addition of 2.5 vol. of cold 95 % ethanol. The mixtures were then permitted to stand overnight at 5°. The sodium nucleate precipitates were recovered by centrifugation and dissolved in 10 ml of water. The solutions were twice deproteinized by shaking with 2 ml of 4:1 chloroform–octanol mixture, centrifuged, and the gels discarded. Acidification of the supernatants with 0.3 *N* HCl and addition of 2 vol. of cold 95 % ethanol reprecipitated the nucleic acids as the free acids. After centrifugation, the precipitates were dissolved in 3 ml of 0.4 *N* sodium hydroxide and allowed to incubate overnight at 30°, during which time hydrolysis of the RNA

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to RNA mononucleotides took place, whereas the DNA remained polymerized[13,14]. The supernatants were reserved for plating and estimation of RNA nucleotides. The DNA was precipitated by the addition of 2 vol. of 0.33 *N* alcoholic HCl and separated from the RNA nucleotides by centrifugation at 0°. The DNA precipitates were dissolved in 3 ml of 0.4 *N* sodium hydroxide and incubated at 30° for 1 h. The DNA was then reprecipitated by the addition of alcoholic HCl. This process was repeated twice to minimize contamination of the DNA by ribonucleotides. Aliquots of each sample of the isolated RNA nucleotides and DNA were taken for nucleotide quantitation by ultraviolet spectrophotometry[15] and for phosphorus analyses following digestion with sulphuric acid and hydrogen peroxide[12]. Aliquots of isolated I.P. samples were also assayed for phosphorus. Other aliquots of RNA nucleotides, DNA and I.P. samples were plated infinitely thin for ^{32}P radioactivity measurements and counted, employing a thin end-window Geiger tube. Sufficient counts were made to reduce the probable counting error to below $\pm$ 5 %. All the ^{32}P data were corrected for background, coincidence and decay back to the day on which the isotope was injected into the animals, and the RNA and DNA specific activities are expressed as relative to the specific activities of the I.P. pools.

In the [2-^{14}C]glycine experiments, the ^{14}C-labeled RNA adenines were isolated on Dowex-50 ion exchange columns following acid hydrolysis of the isolated ribonucleotides in 1 *N* hydrochloric acid for 1 h at 100°. Aliquots of the isolated adenine samples were placed into liquid scintillation counting bottles and taken to dryness under a jet of dry air. Each adenine sample was then dissolved in 0.3 ml of hyamine hydroxide[16] and diluted with 5 ml of a toluene–phosphor solution containing 0.3 % diphenyloxazole and 0.03 % *p*-bis-[2-(5-phenyloxazolyl)]-benzene and the ^{14}C radioactivity was quantitated with a Packard Tricarb liquid scintillation counter[17]. Under our counting conditions, the counting efficiency of this system is 47 %. For expression as specific activity, separate aliquots of the isolated adenine were quantitated spectrophotometrically[18].

To assess the incorporation of [^{14}C]glycine into total liver proteins, the liver proteins were isolated as follows: the sodium chloride extracted protein residues obtained during the nucleic acid isolation were suspended in 20 % mercaptoethanol and allowed to stand overnight under nitrogen to remove bound glutathione[19], the precipitates were harvested by centrifugation and then washed successively with 5 % TCA, 95 % ethanol, twice with absolute ethanol and twice with ether; the dehydrated protein samples were then dried *in vacuo* under an infra red lamp and ground to a fine powder. Replicate portions of the isolated protein were then weighed into a scintillation counting bottle, suspended in 15 ml of a 2.5 % thixcin[20], 0.3 % diphenyloxyzole solution in toluene and then placed into the liquid scintillation counter for radioactivity measurements. By the use of two different amplifications on the photomultiplier tubes of this instrument, one can distinguish between and quantitate the ^{14}C and ^{32}P isotopes present in a doubly-labeled sample.

RESULTS

The *in vivo* incorporation of inorganic ^{32}P and [2-^{14}C]glycine into nucleic acids during 2-h periods at various times during tryptophan peroxidase induction is shown in Table I. Control or zero hour animals received no tryptophan. Although tryptophan

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TABLE I

THE EFFECTS OF TRYPTOPHAN PEROXIDASE INDUCTION ON THE TURNOVERS OF NUCLEIC ACIDS

Hours post-tryptophan administration	*Tryptophan peroxidase μmoles kynurinine/h/g liver*	*Specific activity counts/min/μg P*			*Relative specific activity**		*Specific activity RNA–Adenine counts/min/μmole adenine*
		Inorganic phosphate	*RNA*	*DNA*	*RNA*	*DNA*	
0	3.7	405	6.47	1.24	1.60	0.306	
2	9.5	458	8.09	0.98	1.77	0.214	
5	26.9	505	9.86	0.90	1.95	0.178	
10	6.4	574	26.4	1.61	4.60	0.280	
0	3.3	554	6.27	0.605	1.13	0.109	16
5	31.7	644	7.17	0.557	1.11	0.086	92
10	13.5	462	13.5	0.917	2.92	0.198	201
15	4.6	591	18.1	1.25	3.06	0.211	172
0	3.0	525	8.75	0.57	1.66	0.109	72
5	27.2	793	13.2	1.30	1.66	0.163	126
10	12.2	588	25.0	0.71	4.25	0.121	304
15	6.0	428	24.3	0.95	5.67	0.222	370
20	3.1	454	10.1	0.62	2.22	0.137	92

* Relative specific activity $= \frac{\text{Specific activity of sample}}{\text{Specific activity of inorganic phosphate}} \times 100.$

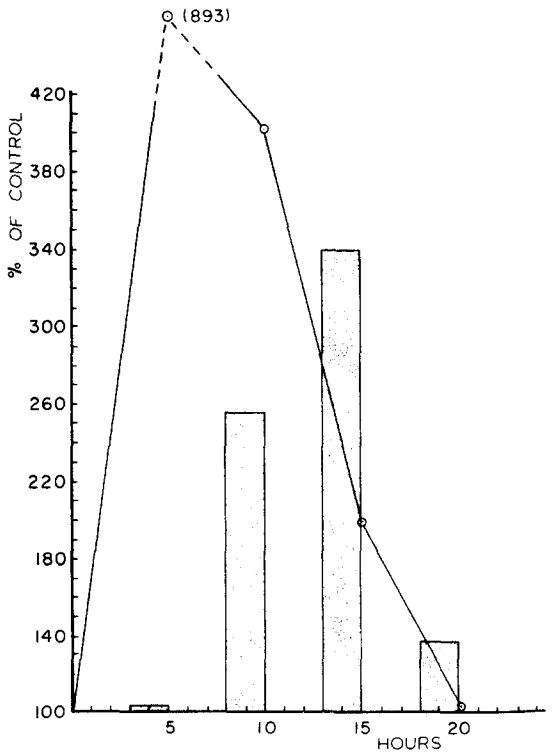

Fig. 1. Kinetic relationships between liver tryptophan peroxidase induction (curve) and the incorporation of ^{32}P into ribonucleic acid (bars) as a function of time following tryptophan administration.

administration caused no significant consistent alterations in the specific activity of the liver inorganic phosphorus pool, the data are, nevertheless, expressed as relative specific activities to correct for any minor variations in the *in vivo* I.P. pool size. In accordance with previous findings, the liver tryptophan peroxidase activity rises following intraperitoneal tryptophan administration reaching a maximum value at 5 h and then decreases exponentially approaching control values at about 15 h. It is to be noted that whereas the liver tryptophan peroxidase data indicate the level of the enzyme at the time of sacrifice, the isotope incorporation data reflect events which occurred during the 2-h isotope incorporation period preceding the time of sacrifice. As tabulated in Table I and more clearly evidenced in Fig. 1, where the data are expressed relative to control animals, during the period of active enzyme synthesis (0–5 h) no stimulation in ^{32}P incorporation into RNA is observed. However, 8 to 15 h post-tryptophan administration, subsequent to the period of accelerated TPO synthesis, during which time the liver TPO concentration is falling toward basal values, there is a burst of RNA synthesis as evidenced by threefold elevations in the absolute and relative specific activities of the RNA. This accelerated synthetic rate persists for approximately 7 h and by 20 h post-tryptophan administration the ^{32}P incorporation into RNA approaches its basal rate.

The kinetics of the incorporation of [^{14}C]glycine into RNA adenine differs somewhat from the incorporation of ^{32}P into RNA. Unlike the ^{32}P, the incorporation of [^{14}C]glycine into RNA adenine is significantly elevated at 3 to 5 h post-tryptophan administration, simultaneous with the period of active enzyme protein synthesis. However, both tracers indicate that the maximum stimulation in RNA synthesis occurs subsequent to the period of active tryptophan peroxidase enzyme protein synthesis.

The detected alterations in DNA turnover (Table I) are relatively small and although repeatable, are of uncertain experimental reliability. In spite of the indicated precautions against contamination of the DNA, a very minor contamination by the higher specific activity RNA would produce the observed effects. It is therefore not possible to assess with certainty the physiological import of the detected stimulation in DNA turnovers at 15 h post-tryptophan administration.

To ascertain whether tryptophan administration was responsible for any gross changes in the overall liver protein metabolism which might in turn influence the nucleic acids turnovers, [2-^{14}C]glycine incorporation into total liver protein was measured, during 2-h incorporation periods, at various times post-tryptophan ad-

TABLE II

THE EFFECT OF TRYPTOPHAN PEROXIDASE INDUCTION ON INCORPORATION OF [2-^{14}C]GLYCINE INTO TOTAL LIVER PROTEINS

Time post-tryptophan h	*Tryptophan peroxidase activity μmoles Kynurinine/ h/g liver*	*Specific activity liver protein counts/min/mg protein*
0	3.1	673
5	29.5	978
10	12.9	679
15	5.4	660

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ministration. The specific activities of the liver proteins freed of soluble, lipid nucleic acid and sulfhydryl bound contaminants were measured and the mean results of two such experiments are shown in Table II. The sole detectable effect of tryptophan administration is an increase of 45 % in the incorporation of [^{14}C]glycine into total liver protein during the 3- to 5-h period post-tryptophan administration. This is concurrent with the period of rapid tryptophan peroxidase synthesis and may reflect both generalized increased protein synthesis as well as accelerated TPO enzyme protein synthesis resulting from tryptophan administration. These data indicate no alterations in total liver protein metabolism during the 10- to 15-h post-tryptophan period, which is concurrent with the observed increase in RNA turnovers.

DISCUSSION

This study extends to an animal system, the findings observed in bacterial systems, that induced enzyme formation leads to stimulation in the incorporation of labeled RNA precursors into RNA[4, 21]. Whereas, CHANTRENNE, in studies on enzyme induction in resting yeast, observed markedly increased incorporation of [^{14}C]adenine into DNA as well as RNA[21], in this study suggestive, although no definitive, evidence indicating increased turnover of DNA was obtained.

Tryptophan peroxidase induction in the rat differs in several respects from enzyme induction in bacteria. Tryptophan peroxidase is a constitutive enzyme being normally present at a basal level in the rat liver; this level is transiently elevated approximately 10-fold subsequent to tryptophan administration. In the rat, the elevated rate of enzyme protein synthesis persists only for the 5 h during which time the blood tryptophan level is elevated[10]. After the blood tryptophan level returns to normal, the increased synthetic rate of tryptophan peroxidase disappears and the liver enzyme level rapidly falls towards control values. This situation differs from that of the usual bacterial enzyme induction experiment, in which the bacteria are maintained continuously in the nutrient medium containing the substrate towards which adaptation is to take place. In this case, the induced enzyme level rises and maintains a high plateau level. In a kinetic sense, the bacterial systems permit study only of the initial phases of enzyme induction and of the steady state condition, whereas the transient stimulation of enzyme protein synthesis, which is characteristic in the mammals, permits evaluation of the events which occur subsequent to the brief period of accelerated enzyme protein synthesis.

Inorganic ^{32}P incorporation indicated no acceleration in RNA phosphate turnover during the period of active enzyme synthesis 0–5 h post-tryptophan; with [^{14}C]glycine as the tracer, stimulation in the incorporation of radioactive carbon into RNA adenine is observed during this period. Possible mechanisms underlying the variations in incorporation patterns of nucleic acids using different precursors have been advanced[22]. However, with either isotope as indicators of nucleic acid metabolism, the major stimulation in RNA turnover, which is interpreted as an increase in RNA synthesis, occurs during the 8- to 15-h period; this is subsequent to the accelerated total liver protein and tryptophan peroxidase protein syntheses. This elevated RNA synthetic rate continues for 8 h and then returns toward normalcy. It is clear that maximum RNA synthetic rates do not accompany but rather follows the period of stimulated protein synthesis. Assuming that a casual relationship exists between

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nucleic acid turnovers and the observed alterations in protein syntheses as reflected in [^{14}C]glycine incorporation into liver protein and liver TPO levels and these data are interpreted as suggesting, as a current working hypothesis, that RNA, whether a template or not, serves not as a perfect catalyst for protein synthesis, but as a reactant which is consumed in the process, and that during the period subsequent to the stimulated protein synthesis, the liver cells accelerate RNA synthesis to replenish the utilized RNA.

Since elevations in liver tryptophan peroxidase commence with no demonstrable latent period following tryptophan injection, whereas the major increases in RNA turnovers occur many hours later, this study does not support the view that accelerated RNA turnover need precede induced enzyme formation. The findings of PARDEE[3] and GALE[4] indicating the dependency of continued induced enzyme formation upon RNA synthesis may be interpreted in the light of these data as indicating that RNA is consumed in the process of enzyme protein synthesis and that the RNA need be replaced by resynthesis in order to perpetuate enzyme protein synthesis. Both the observed requirement for RNA precursors and the increased incorporation of such labeled precursors into RNA during continued enzyme induction are compatible with this hypothesis.

ACKNOWLEDGEMENT

This study was supported in part by grants from the National Science Foundation and the National Cancer Institute, National Institutes of Health, United States Public Health Service.

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