much less ammonium sulfate than is required for the ATP–nucleoside monophosphate kinase. Thus far, the nucleoside triphosphate–AMP kinase has not been further separated into a family of enzymes with similar properties.

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ALTERATIONS IN NUCLEIC ACID TURNOVERS IN SUBCELLULAR COMPONENTS DURING TRYPTOPHAN PEROXIDASE INDUCTION

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SUMMARY

Parenteral tryptophan administration to the rat leads to tryptophan peroxidase induction and to acceleration in the turnover rates of the RNA of the various liver subcellular fractions. The kinetics of these processes indicate that the stimulation in RNA turnover does not precede or accompany but, rather, is subsequent to the period of active enzyme protein synthesis. The maximum percentage increases in RNA turnover rates are: mitochondria and microsomal RNA, 260 %; soluble RNA, 154 %; nuclear RNA, 60 %. However, due to the markedly different basal turnover rates of RNA in the various subcellular organelles, the absolute increase in total amount of RNA synthesized as a result of enzyme induction are in the order: nuclear > soluble = microsomal > mitochondrial. The kinetic data are compatible with the hypothesis that enzyme induction results in extra nuclear RNA utilization with subsequent resynthesis largely in the nucleus.

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INTRODUCTION

Various aspects of the relationship between ribonucleic acid (RNA) and protein synthesis remain to be elucidated. The RNA of each subcellular component of cells of a given tissue is turned over at a different, but characteristic rate[1,2]. Although it is known that in liver the nucleus is the site of the most rapid basal RNA turnover, whereas the microsomes are the site of most rapid protein synthesis[3], the intracellular localization of accelerated RNA synthesis during experimentally enhanced protein synthesis in the mammal has not been investigated. The finding that liver tryptophan peroxides (TPO) induction is followed by an elevation in total liver RNA turnover with a characteristic kinetic pattern[4] and the availability of techniques for the isolation of subcellular entities permits the intracellular localization of the RNA synthetic activities which may be most directly related to the synthesis of this enzyme protein. This study, therefore, is designed to follow the kinetics of the fluctuations in RNA specific activity *in vivo* among the various subcellular fractions during tryptophan peroxidase induction in order to ascertain the extent to which these intracellular organelles may be concerned with the synthesis of tryptophan peroxidase.

METHODS

Adult male Sprague-Dawley rats of approximately 450 g body weight were fasted for 20 h before sacrifice to prevent spurious fluctuations in RNA turnovers due to variations in dietary intake. A graded increase in the length of the enzyme induction period was achieved by the intraperitoneal administration of *l*-tryptophan (0.45 mmoles/100 g body weight) at appropriate times preceding sacrifice of the animals. Control animals received no tryptophan. 40 μC/100 g body weight of inorganic ^{32}P (Abbott Laboratory catalog = 6710) were administered intraperitoneally to all animals precisely 2 h prior to sacrifice. In this manner, ^{32}P incorporation into nucleic acids *in vivo* was obtained for various 2-h periods during the enzyme induction process. Upon sacrifice, jugular blood from each rat was collected for inorganic phosphorus (P_i) isolation and the livers were immediately placed in chipped ice. A portion of each liver was analyzed for tryptophan peroxidase activity employing the procedure of KNOX AND AUERBACH[5].

For the isolation of subcellular fractions, 5 g of the remaining liver were homogenized in two volumes of 0.25 *M* sucrose containing $3 \cdot 10^{-4}$ *M* $KHCO_3$ using a Teflon Potter-Elvehjem homogenizer and subsequently diluted with 0.25 *M* sucrose yielding a final 16 % homogenate. Nuclei were isolated free of contaminant whole cells using 2.2 *M* sucrose[6]; mitochondria and microsomes were isolated by conventional differential centrifugation procedures at 9,000 *g* and 54,000 *g* respectively. The "soluble" RNA fraction was obtained by adjusting the final high speed (54,000 *g*) supernatant to pH 5.0 with acetic acid, thus precipitating ribonucleoprotein which was collected by centrifugation. From these four subcellular fractions, the nucleic acids were isolated, purified, quantitated and the ^{32}P was counted in the manner previously described[4].

Blood inorganic phosphate was isolated following deproteinization by the addition of one part of 50 % trichloroacetic acid (TCA) to 9 parts of chilled whole jugular

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TABLE 1

THE EFFECTS OF TRYPTOPHAN PEROXIDASE INDUCTION ON NUCLEIC ACID TURNOVERS IN SUBCELLULAR COMPONENTS

Hours post-tryptophan	Tryptophan peroxidase		Specific activity* counts/min/μg P						Relative specific activity**				
	μmoles/ Kynurinine/ h/g	% of control	Serum inorganic phosphate	RNA microsomes	RNA nucleus	RNA soluble	RNA mitochondria	DNA nucleus	RNA microsomes	RNA nucleus	RNA soluble	RNA mitochondria	DNA nucleus
0	2.92	100	1,087	6.01	198	24.0	8.9	4.91	0.552	18.2	2.21	0.819	0.452
5	17.7	605	1,190	6.28	180	19.7	9.3	3.59	0.528	15.1	1.66	0.781	0.302
7.5	25.9	888	988	10.5	235	37.8	10.3	4.17	1.06	23.8	3.83	1.04	0.422
15	4.84	166	927	18.5	270	52.0	26.9	5.73	1.99	29.1	5.61	2.90	0.618
20	4.34	149	1,006	10.6	199	34.4	12.9	5.48	1.05	19.8	3.42	1.28	0.545
0	3.19	100	1,192	8.60	163	23.8	—	—	0.720	13.7	2.00	—	—
5	33.3	1043	1,064	7.94	183	23.8	10.0	—	0.746	17.2	2.24	0.940	—
7.5	13.9	438	1,160	14.1	226	38.9	14.8	—	1.22	19.5	3.35	1.28	—
10	6.56	206	1,132	31.3	348	66.4	31.9	—	2.77	30.7	5.87	2.82	—
15	2.85	89	948	18.4	212	47.6	19.4	—	1.94	22.4	5.02	2.05	—
20	4.95	155	923	12.1	229	29.7	13.0	—	1.31	24.8	3.22	1.41	—

* Specific activities refer to ^{32}P incorporation into each nucleic acid fraction during a 2-h incorporation period preceding the indicated hour post-tryptophan administration.

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

blood. After centrifugation in the cold, inorganic phosphorus was isolated from the TCA supernatants employing procedures previously described[4].

The results are expressed as means of duplicate animals.

RESULTS

As shown in Table I and in confirmation of previous findings[4,7], following parenteral tryptophan administration the rat liver tryptophan peroxidase activity rises rapidly reaching maximum levels of approximately ten times control values at 5–7 ½ h and returns to basal levels by approximately 15–20 h post-tryptophan injection. Measurements of the specific activity of serum inorganic phosphate indicate that tryptophan administration results in no consistent significant alteration in the radioactivity of the serum inorganic phosphate pool. Table I further reveals that the turnover rates of RNA isolated from the various subcellular fractions differ and are characteristic for each fraction. This is most readily observed in the animals receiving no tryptophan (0 h post-tryptophan), where it is shown that nuclear RNA is synthesized approximately 20–30 times more rapidly than is microsomal or mitochondrial RNA, whereas the specific activity of the soluble RNA is intermediate between these extremes. It is also evident from the relative specific activities of these control animals that the specific activity of nuclear RNA is about 15 % that of the serum inorganic phosphate, thus emphasizing the great rapidity with which nuclear RNA and its nucleotide precursors are derived from this inorganic phosphate pool.

In Table I and more clearly in Fig. 1, where the percentage changes in ^{32}P incorpora-

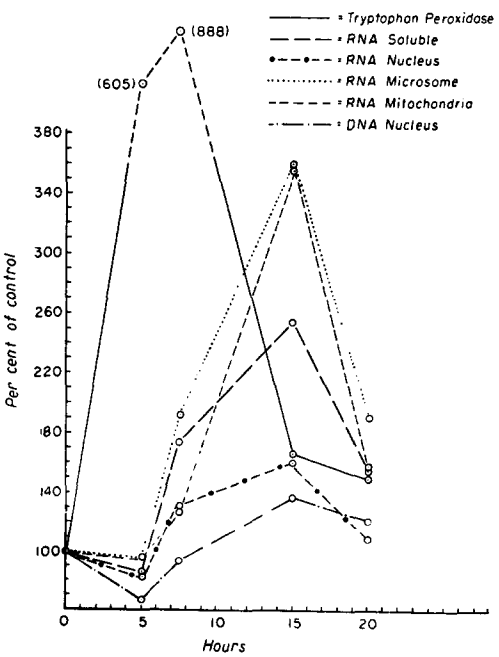

Fig. 1. Percentile elevations of tryptophan peroxidase and nucleic acid turnovers in subcellular components relative to control animals following tryptophan administration.

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tion into the RNA of each fraction are comparable, since they are plotted as per cent of control values, it can be seen that during the 3–5 h period post-tryptophan administration, while TPO is most rapidly synthesized, there is no accelerated rate of nucleic acid turnover in any cell fraction. However, subsequently (*viz.* between 7½–15 h) marked increases in the specific activities of most of the subcellular RNA fractions are found. The maximum rate of RNA turnover in all fractions is observed during the 13–15 h period post-tryptophan administration by which time the enzyme level has fallen close to control levels. These kinetics are in substantial agreement, with findings on nucleic acid turnovers of unfractionated liver[4]. The magnitude of stimulation of RNA turnover subsequent to enzyme induction differs widely amongst the various cell fractions; the most marked percentage acceleration in RNA synthesis is observed in the microsomes and mitochondria, where 250 % increases in RNA turnovers are found; soluble RNA is elevated 150 %; nuclear RNA is elevated approximately 60 % over control animals. The nuclear DNA synthetic rate is also significantly elevated by 30 % over the control rates. Thus the accelerations in RNA turnover in subcellular fractions expressed as per cent of the basal rates occur in the following orders microsomal = mitochondrial > soluble > nuclear RNA.

TABLE II

SYNTHESIS OF RNA IN SUBCELLULAR FRACTIONS PRECEDING AND FOLLOWING TRYPTOPHAN PEROXIDASE INDUCTION IN RAT LIVER

	*(a)**	*(b)***	*(a × b)*	*(c)****	*(a × b × c)*
Cell fraction	*Relative basal synthetic rate*	*Basal RNA content*	*Relative basal amount RNA synthesized*	*Increase in RNA synthetic rate after TPO-induction*	*Relative increment in RNA synthesized after TPO induction*
	counts/min/ μgRNA-P	*mg RNA-P/ g liver*		*Fractional increase over controls*	
Nucleus	198.0	0.114	22.6	0.60	13.5
Soluble	24.0	0.192	4.61	1.54	7.09
Microsome	6.0	0.435	2.61	2.60	6.79
Mitochondria	8.9	0.060	0.53	2.54	1.36

* From Table I.
** W. C. SCHNEIDER, *J. Biol. Chem.*, 176 (1948) 259.
*** From Fig. 1.

Consideration of the total quantities of RNA synthesized in each cellular fraction provides quantitative insight into the relative importance of these subcellular sites for RNA synthesis as related to enzyme induction. From a typical experiment as reported in Table II, it can be seen that the product of the basal turnover rates, as reflected by the specific activities in the control animals (a), and the basal RNA content of each subcellular fraction (b), indicate, in a relative manner, the total amount of RNA synthesized in each subcellular fraction preceding enzyme induction (a × b). It is evident from this calculation that the greatest quantity of the cellular RNA is synthesized in the nucleus. The relative amount of RNA synthesized in each fraction (a × b) multiplied by the observed increase in RNA turnover in each fraction during the 13–15 h ^{32}P incorporation period (c) indicates the relative increment in the amount of RNA synthesized in each fraction due to tryptophan administration (a × b × c).

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Thus the absolute increments in RNA synthesized in each subcellular component subsequent to TPO induction may be ranked in the following order: nucleus > soluble = microsomes > mitochondria.

It is to be noted in Table I that microsomal and mitochondrial RNA have similar basal turnover rates, and, furthermore, during all phases of tryptophan peroxidase induction, the turnover rates of the mitochondrial and microsomal RNA are altered identically, suggesting either a functional or complete identity of the RNA in these two fractions.

DISCUSSION

The nucleic acid turnover of all of the subcellular fractions are altered following parenteral tryptophan administration. The observed alterations in RNA turnovers are compatible with the hypothesis that RNA is synthesized in the nucleus and transported to the cytoplasmic organelles where it is utilized and consumed in protein synthesis. Although these data are compatible with this proposed hypothesis, much additional information is necessary before acceptance is possible. For example, since these data do not reveal the actual source of the RNA found in each fraction, it is not known whether the RNA found in a subcellular fraction has been synthesized therein or has been transported there having been produced at another subcellular site. In addition, it must be noted, that it has been assumed that isotopic turnover is equivalent to synthetic rate and, furthermore, that the nucleotide precursors of RNA in each cell fraction have the identical specific activities such that the observed specific activity of RNA reflects the true rate of incorporation of these precursors rather than alterations in the specific activities of the nucleotide precursors.

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