The Effect of Feeding with a Tryptophan-Free Amino Acid Mixture on Rat Liver Magnesium Ion-Activated Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase

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1. The Widnell & Tata (1966) assay method for Mg^{2+} -activated DNA-dependent RNA polymerase was used for initial-velocity determinations of rat liver nuclear RNA polymerase. One unit (U) of RNA polymerase was defined as that amount of enzyme required for 1 mmol of [3H]GMP incorporation/min at 37°C. 2. Colony fed rats were found to have a mean RNA polymerase activity of 65.9 μ U/mg of DNA and 18h-starved rats had a mean activity of 53.2 μ U/mg of DNA. Longer periods of starvation did not significantly decrease RNA polymerase activity further. 3. Rats that had been starved for 18h were used for all feeding experiments. Complete and tryptophan-deficient amino acid mixtures were given by stomach tube and the animals were killed 15–120 min later. The response of RNA polymerase to the feeding with the complete amino acid mixture was rapid and almost linear over the first hour of feeding, resulting in a doubling of activity. The activity was still elevated above the starvation value at 120 min after feeding. The tryptophan-deficient amino acid mixture produced a much less vigorous response about 45 min after the feeding, and the activity had returned to the starvation value by 120 min after the feeding. 4. The response of RNA polymerase to the feeding with the complete amino acid mixture was shown to occur within a period of less than 5 min to about 10 min after the feeding. 5. Pretreatment of the animals with puromycin or cycloheximide was found to abolish the 15 min RNA polymerase response to the feeding with the complete amino acid mixture, but the activity of the controls was unaffected. 6. The characteristics of the RNA polymerase from 18h-starved animals and animals fed with the complete or incomplete amino acid mixtures for 1h were examined. The effects of Mg^{2+} ions, pH, actinomycin D and nucleoside triphosphate omissions were determined. The [Mg^{2+}]- and pH-activity profiles of the RNA polymerase from the animal fed with the complete mixture appeared to differ from those of the enzyme from the other groups, but this difference is probably not significant. 7. [5-^3H]Orotic acid incorporation by rat liver nuclei *in vivo* was shown to be affected by the amino acid mixtures in a similar manner to the RNA polymerase. 8. The tryptophan concentrations of plasma and liver were determined up to 120 min after feeding with the amino acid mixtures. Feeding with the complete mixture produced a rapid increase in free tryptophan concentrations in both plasma and liver, but feeding with the incomplete mixture did not alter the plasma concentration. The liver tryptophan concentration increased at about 45 min after feeding with the tryptophan-deficient diet. 9. There was a good correlation between the liver tryptophan concentration and RNA polymerase activity in all groups of animals. 10. It was concluded that the rat liver nucleus responded to an increase in amino acid supply by increased synthesis of RNA as a result of synthesis of RNA polymerase *de novo*. The correlation of tryptophan concentration and RNA polymerase activity appears to reflect the general amino acid concentration required to support hepatic protein synthesis and to produce new RNA polymerase. This new polymerase appears to differ from the basal RNA polymerase by its rapid synthesis and destruction, which may be a means of regulating RNA synthesis by the amino acid concentration in the liver.

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The response of the cytoplasm of rat liver *in vivo* to feeding with an amino acid mixture deficient in tryptophan has been investigated by Fleck, Shepherd & Munro (1965). Feeding with this diet led to a disaggregation of polyribosomes and an accumulation of oligoribosomes. This process was accompanied by a decreased ability of microsomes to incorporate [^{14}C]leucine *in vitro*. Pretreatment of the animals with actinomycin D, in a dose that was sufficient to stop RNA synthesis in liver, did not prevent this response, and it was concluded that the polyribosomal disaggregation was not mediated through the nucleus. This conclusion has received support from the response of rat liver polyribosomes *in vitro* to amino acid omissions (Baliga, Proneczuk & Munro, 1968).

Nonetheless there is a considerable body of evidence indicating that the rat liver nucleolus is responsive to dietary changes. Lagerstedt (1949) showed that starvation for 24 h produced a 50% decrease in the size of rat liver nucleoli; no further decrease occurred with more prolonged starvation. He also found that feeding starved rats with a high-protein diet resulted in a very rapid 100% increase in nucleolar size within 3 h of the feeding, and that cytoplasmic basophilia (which had decreased during starvation) began to appear, first around the nuclear membrane and then throughout the entire cytoplasm.

Stenram (1956a) showed that the increase in nucleolar size after feeding with a high-protein diet took 3 days to reach a maximum and that this was followed by a slight decrease in size over the next 15 days. He also showed that feeding with a non-protein diet led to an increase in nucleolar size that was larger than the maximum achieved by feeding with a high-protein diet. Later Stenram (1956b) showed that the increase in nucleolar size produced by the protein-free diet could also be produced by feeding with amino acid mixtures singly deficient in lysine, isoleucine, methionine, threonine, tryptophan, valine or all the essential amino acids.

The nucleolus is now known to be the site of synthesis of ribosomal precursor RNA in HeLa cells (Penman, Smith & Holtzman, 1966) and rat liver (Muramatsu, Hodnett, Steele & Busch, 1966), and Widnell & Tata (1966) have shown that rat liver nuclear Mg^{2+} -activated DNA-dependent RNA polymerase (EC 2.7.7.6) synthesizes an RNA with a ribosome-like base ratio. This enzyme activity is known to be preferentially located in the nucleolus (Siebert *et al.* 1966; Roeder & Rutter, 1969), and it was therefore decided to investigate the activity of this enzyme after feeding with amino acid mixtures, in an attempt to correlate the morphological studies with the enzyme that is thought to be involved in rRNA synthesis. A preliminary account of these

experiments has already appeared (Henderson, 1969).

MATERIALS AND METHODS

Chemicals. Puromycin dihydrochloride, cycloheximide, nor-harman hydrochloride, calf thymus DNA and all the ribonucleoside triphosphates were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. 2,5-Diphenyloxazole, 1,4-bis-(5-phenyloxazol-2-yl)benzene and *m*-Hyamine hydroxide in methanol were obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Actinomycin D was a gift from Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., U.S.A. All other chemicals were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Tetralithium [^3H]GTP (specific radioactivity approx. 1 Ci/mmol) was obtained from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A., and [^3H]orotic acid (specific radioactivity approx. 5 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Rats. The rats were male albinos from a stock bred in this Institute and weighed 140–160 g before starvation. They were maintained on Modified Diet 41B (Oxoid Ltd., London E.C.4, U.K.), which contained 15.9% crude protein and adequate mineral and vitamin supplements. All animals were handled daily for several weeks preceding the feeding experiments, and they were housed in a windowless room lit from 06.00 to 18.00 h each day. All animals used in the feeding experiments had food withdrawn at 15.00 h on the day preceding the experiments (18 h-starved), but they were allowed free access to tap water. During starvation the animals were housed in wire-bottomed cages to prevent coprophagy. 'Colony fed' rats were animals allowed free access to the stock diet until death.

Amino acid mixtures. The amino acid mixtures used for all experiments were similar to those used by Wunner, Bell & Munro (1966) except that the tryptophan supplement was not included. Two mixtures were used; the complete amino acid mixture (T+ diet) and the incomplete mixture (T- diet), which contained no tryptophan but which was otherwise identical with the complete mixture. Starved rats were fed by stomach tube between 08.00 and 09.00 h with 1.12 g of amino acids in 3 ml of the mixture.

Preparation of rat liver nuclei for the assay of DNA-dependent RNA polymerase. The method used was based on that of Muramatsu *et al.* (1966). All operations were carried out in the cold-laboratory and all solutions were ice-cold. Rats were killed by decapitation and allowed to bleed for a few minutes, and the livers were removed into 0.25 M-sucrose, transferred to 50 ml of 2.4 M-sucrose containing 5 mM-MgCl₂, minced with scissors and homogenized by six full strokes in a 200 ml glass-Teflon homogenizer with a radial clearance of 0.005 in (Glass Engineering Co., Houston, Tex., U.S.A.). A Black and Decker D900 $\frac{1}{2}$ in drill working at 900 rev./min mounted in a vertical drilling stand clamped to the bench was used as the homogenizing motor. The homogenate was filtered through eight thicknesses of white butter muslin (no. 106 obtained from Thomson Skinner and Hamilton, Glasgow C.2., U.K.) and centrifuged at 40 000 *g*_{av} for 1 h at 2°C in a type 30 rotor of a Spinco model L ultracentrifuge. The nuclear pellets were collected and suspended in 5 ml of

m-sucrose containing mM-MgCl₂ and homogenized by hand in a 5 ml glass-Teflon homogenizer (radial clearance 0.01 in) with five full strokes. The homogenate was centrifuged at 800g_{av} for 5 min at 2°C in an MSE Mistral 2L centrifuge and the pellet suspended in 1 ml of m-sucrose containing mM-MgCl₂.

Determination of [5-³H]orotic acid uptake by rat liver nuclei. Livers were removed into and washed in ice-cold 0.25 M-citric acid, transferred to 20 vol. of the same citric acid, minced with scissors and homogenized by five full strokes of a 20 ml glass-Teflon homogenizer. The homogenate was filtered through eight thicknesses of white butter muslin and centrifuged at 1500g_{av} for 5 min at 20°C. The supernatant was discarded, and the precipitate was resuspended in 10 vol. of citric acid and recentrifuged. The procedure was repeated once more, the nuclear pellet was suspended in 50 ml of distilled water and duplicate 1 ml portions were removed for radioactivity measurement by drying in a scintillation vial under an infrared lamp and then incubating in the dark for 6 h at 37°C with 0.25 ml of m-Hyaminate hydroxide. Then 10 ml of the toluene-2,5-diphenyloxazole-1,4-bis-(5-phenyloxazol-2-yl)benzene mixture was added, and the whole was mixed and left in the dark overnight. Radioactivity was determined as described below and the results were expressed as pmol of orotic acid incorporated/mg of DNA.

Determination of DNA. The indole procedure of Ceriotti (1952, 1955) was applied to the acid-insoluble precipitate obtained after alkaline digestion of the nuclei in the modified Schmidt-Thaunhauser method of Fleck & Munro (1962). The DNA standard was calf thymus DNA purified by the method of Kay, Simmons & Dounce (1952).

Determination of tryptophan. The fluorescence method of Denckla & Dewey (1967) was used for the determination of tryptophan in rat plasma and liver. An Aminco-Bowman spectrofluorimeter was used with a 1 cm quartz cuvette. The photomultiplier microphotometer was set to 100% (multiplier 0.1) with a 5 nmol nor-harman standard. Blood was removed by tail section, after light ether anaesthesia, collected in a heparinized tube and plasma obtained by centrifugation. Livers were then rapidly removed, weighed and homogenized in 7 vol. of ice-cold 150 mM-NaCl, made up to 50 ml and sampled immediately. All determinations were carried out in duplicate.

The daily (in-batch) precision, expressed as coefficient of variation, was 3% (*n*=10) for plasma and 4.9% (*n*=10) for liver homogenate. Between-batch precision was 7% (*n*=38) determined by using a 5 nmol tryptophan sample. Recovery of added tryptophan from plasma or liver homogenate was quantitative within the standard deviation of the method.

Assay of DNA-dependent RNA polymerase. The assay mixture was based on that of Widnell & Tata (1966). The reaction mixture contained, in a volume of 1 ml: 250 μmol of sucrose, 100 μmol of tris-HCl buffer pH 8.5, 3.75 μmol of MgCl₂, 10 μmol of L-cysteine hydrochloride, 3 μmol of NaF, 0.4 μmol each of ATP, CTP and UTP, 2.3 nmol of [³H]GTP and 0.1 ml of nuclear suspension containing about 200 μg of DNA. The mixture, with the nuclear suspension omitted, was preincubated for 5 min at 37°C, and the nuclear suspension (preincubated at 37°C for no longer than 1 min) was then added and the assay was carried out for 3 min in a 37°C shaking water-bath. Linearity of [³H]GMP incorporation was obtained by

using this time-period and DNA concentration. Each assay was carried out in duplicate together with duplicate zero-time determinations. The reaction was stopped by the addition of 1 ml of ice-cold 0.2 M-Na₄P₂O₇ (adjusted to pH 7.5 with m-HCl and containing 1 mg of bovine serum albumin/ml) followed by 1 ml of 0.6 M-HClO₄. The pyrophosphate solution diminishes the non-specific binding of nucleotides (Kammen, Klemperer & Canellakis, 1961). The tubes were stood in ice for 10 min and then centrifuged at 600g_{av} for 10 min at 20°C, and the supernatant was discarded. The pellet was washed and centrifuged a further four times with 2 ml of ice-cold 0.2 M-HClO₄. The final acid-insoluble precipitate was allowed to drain overnight and then dissolved in 0.5 ml of m-Hyaminate hydroxide by incubation in the dark for 6 h at 37°C, mixed with 10 ml of 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis-(phenyloxazol-2-yl)benzene in toluene, left at room temperature in the dark overnight and its radioactivity counted the following morning. This procedure was found necessary to avoid spurious counts due to chemiluminescence in Hyamine hydroxide-protein solutions described by Herberg (1958). Samples were counted in a Nuclear-Chicago model 725 liquid-scintillation spectrometer, the channels-ratio method of quench correction being used. All counts were corrected for an adequately determined background (38 c.p.m.). The efficiency of ³H counting was about 30% and the counting error did not exceed 1%. Results were expressed as pmol of GMP incorporated/mg of DNA calculated from the specific radioactivity of the [³H]GMP used and the DNA content of a sample of nuclei. Zero-time incorporations were less than 1 pmol/mg of DNA and 3 min incorporations agreed to within 2% of each other. Assays exceeding these limits were rejected. The duplicate determinations were regressed against incubation time and the gradient of the line thus obtained was the initial enzyme velocity expressed as pmol of GMP incorporated/min per mg of DNA. One unit (U) of DNA-dependent RNA polymerase activity is defined as the amount of enzyme that catalyses the incorporation of 1 mmol of GMP/min at 37°C and the initial enzyme velocity is finally expressed at μU/mg of DNA.

Statistical methods. Analysis of means of determinations and their variances were made by *t*-test when the variances were equal and by a modified *t*-test when they were unequal (Sokol & Rohlf, 1969). Regression lines were calculated by the method of least squares (Model I regression) or by Bartlett's three-group method (Model II regression) as described by Sokol & Rohlf (1969).

RESULTS

Effect of starvation on DNA-dependent RNA polymerase. The studies by Fleck *et al.* (1965) and Wunner *et al.* (1966) were carried out on 18h-starved animals. They were unable to determine the effects of starvation on polyribosome patterns because of the large amounts of liver glycogen, which affects the isolation of polyribosomes in the fed animals. However, liver glycogen does not affect the isolation of nuclei and the nuclear DNA-dependent RNA polymerase activities of colony fed animals and those starved for 18h or 40h were determined (Table 1). It is clear that there is a significant

Table 1. *Effect of starvation on the activity of rat liver nuclear Mg^{2+} -activated DNA-dependent RNA polymerase*

Rats were allowed free access to the stock diet or were starved for 18 or 40 h as described in the Materials and Methods section. The rats were decapitated between 08.00 and 09.00 h and liver nuclei isolated. Mg^{2+} -activated DNA-dependent RNA polymerase activity was determined as described in the Materials and Methods section. Results are expressed as means $\pm$ S.E.M., with the numbers of animals given in parentheses. The enzyme activity of the colony fed group was significantly different from that of the 18 h-starved group ($P < 0.001$), but there was no significant difference between those of the two starved groups.

	RNA polymerase activity (μ U/mg of DNA)
Colony fed rats	65.9 $\pm$ 2.5 (6)
18 h-starved rats	53.2 $\pm$ 1.2 (9)
40 h-starved rats	49.2 $\pm$ 2.2 (6)

Fig. 1. Response of Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei to feeding with the complete and tryptophan-free amino acid mixtures. Animals that had been starved for 18 h were fed with the T+ diet (○) or with the T- diet (●) and killed at the times shown. One group of animals was not fed (▲). Nuclei were isolated and RNA polymerase was assayed as described in the Materials and Methods section. Results are expressed as means $\pm$ S.E.M., with the numbers of animals given in parentheses.

decrease in activity after starvation for 18 h but that starvation for a longer period has no further effect.

Table 2. *Short-term response of Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei to feeding with the complete amino acid mixture*

Animals that had been starved for 18 h were fed with the complete amino acid mixture and killed 5 or 10 min later. Nuclei were isolated and RNA polymerase was assayed as described in Fig. 1. Individual results are shown together with those obtained from the 18 h-starved group and after 15 min with the T+-diet-fed group from Fig. 1 (given as means $\pm$ S.E.M.).

	RNA polymerase activity (μ U/mg of DNA)
18 h-starved rats	53.2 $\pm$ 1.2
Fed rats, after 5 min	69.0, 61.5, 65.1, 77.0, 51.9, 54.1
Fed rats, after 10 min	72.6, 89.3, 79.0, 61.9, 79.0, 85.9
Fed rats, after 15 min	73.7 $\pm$ 2.9

Effect of administered amino acid mixtures on DNA-dependent RNA polymerase. Animals that had been starved for 18 h were used in this study because their stomachs were empty and the activity of the DNA-dependent RNA polymerase appeared to be stable. They were fed with the complete amino acid mixture (T+ diet) or with the tryptophan-deficient mixture (T- diet) and killed at 15, 30, 45, 60 or 120 min later. The results are shown in Fig. 1. Clearly, the response of RNA polymerase to the complete amino acid mixture is rapid and almost linear over the first hour after the feeding, resulting in a doubling of activity, whereas the T- diet produces a much less vigorous response about 45 min after the feeding. Statistical analysis of these results showed that those obtained after 15, 30 and 45 min with the T--diet-fed group were not significantly different from each other or from those obtained with the 18 h-starved group, but that those obtained after 15 min and subsequently with the T+-diet-fed group were significantly ($P < 0.001$) different from those obtained with the 18 h-starved animals. Finally, the results obtained after 2 h with the T+-diet-fed and the T--diet-fed groups were both significantly different from those obtained with the colony fed group ($0.05 > P > 0.02$).

The response to the T+ diet was so rapid that shorter time-intervals after the feeding were examined. Table 2 shows the response of the nuclear RNA polymerase of individual animals 0, 5, 10 and 15 min after the feeding with the T+ diet. There is a significant difference between the results obtained with the 18 h-starved group and those obtained after 5 min with the T+-diet-fed group ($0.02 > P > 0.01$), and it is clear that some animals have already responded to the T+ diet within 5 min.

The increased activity of RNA polymerase occurring after the feeding with the T+ diet could be due to the synthesis of new RNA polymerase.

Table 3. Response of Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei to feeding with the complete amino acid mixture after pretreatment with cycloheximide or puromycin

Puromycin dihydrochloride was dissolved, immediately before injection, in 1 ml of sterile 0.15M-NaCl, pH adjusted to 7.5 with M-KOH, and administered by intraperitoneal injection 45min before the feeding in a dose of 100mg/kg body wt. (Villa-Trevino, Farber, Staehelin, Wettstein & Noll, 1964). Cycloheximide was dissolved in 1 ml of sterile 0.15M-NaCl and similarly injected in a dose of 50mg/kg body wt. (Fallon, 1967) 30 min before the feeding. Control animals were injected with similar doses of puromycin or cycloheximide and killed after 60 or 45min respectively. Nuclei were isolated and RNA polymerase was assayed as described in the legend to Fig. 1. Puromycin or cycloheximide were shown to have no effect *in vitro* on the RNA polymerase system up to concentrations of 500 μ g/ml. Results are expressed as means $\pm$ S.E.M., with the numbers of animals given in parentheses. Results obtained with starved rats after 15min with the T+ -diet-fed group are included for comparison.

	RNA polymerase activity (μ U/mg of DNA)
T+-diet-fed rats, after 15 min	73.3 $\pm$ 2.9 (8)
Puromycin-pretreated	50.0 $\pm$ 1.5 (3)
T+-diet-fed rats, after 15 min	
Cycloheximide-pretreated	44.1 $\pm$ 1.7 (4)
T+-diet-fed rats, after 15 min	
18 h-starved rats	53.2 $\pm$ 1.2 (9)
Puromycin-pretreated	57.4 $\pm$ 3.7 (4)
18 h-starved rats	
Cycloheximide-pretreated	49.9 $\pm$ 5.3 (3)
18 h-starved rats	

This explanation was found to account for the increased activity of DNA-dependent RNA polymerase observed in rat uterus after oestrogen administration (Noteboom & Gorski, 1963; Nicolette & Mueller, 1966) or in rat liver after partial hepatectomy (Tsukada & Lieberman, 1964). This possibility was therefore tested by pretreating animals with cycloheximide or puromycin, feeding with the T+ diet and killing the animals 15min later. The effect of these drugs on the response of the RNA polymerase to the T+ diet is shown in Table 3. Statistical analysis showed that there was no significant difference between the results obtained with the 18h-starved group, the drug-treated starved group or after 15min with the drug-treated T+-diet-fed group. Thus it could be concluded that puromycin or cycloheximide did not affect the DNA-dependent RNA polymerase in starved animals but that the response of the T+-diet-fed animals was abolished. This suggests that the newly synthesized RNA polymerase differs from

Fig. 2. Response of Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei to Mg^{2+} ions *in vitro*. The experiment was as described in Fig. 1. Animals that had been starved for 18 h were fed either with the T+ diet ($\circ$) or with the T- diet ($\bullet$) and killed 1 h later. Starved animals were used as controls ($\blacktriangle$).

Fig. 3. Response of Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei to pH *in vitro*. The experiment was as described in the legend to Fig. 1. Animals that had been starved for 18 h were fed either with the T+ diet ($\circ$) or with the T- diet ($\bullet$) and killed 1 h later. Starved animals were used as controls ($\blacktriangle$).

the enzyme present in the starved animal. This conclusion was also reached by Tsukada & Lieberman (1965) in their study of DNA-dependent RNA

polymerase formed after partial hepatectomy in the rat on the basis of puromycin and fluorophenylalanine inhibition of synthesis of RNA polymerase *de novo*.

Some of the characteristics of RNA polymerase from 18h-starved animals and after 1h from the T+-diet-fed and from the T--diet-fed animals were examined in the hope of finding some differences,

Fig. 4. Response of Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei to actinomycin D *in vitro*. The experiment was as described in the legend to Fig. 1. Animals that had been starved for 18h were fed either with the T+ diet (○) or with the T- diet (●) and killed 1h later. Starved animals were used as controls (▲). Results are expressed as percentage inhibition produced by a range of 0.1 μ g–1mg of actinomycin D/mg of DNA present in the enzyme assay mixtures, and each point represents the mean of four determinations. The range of actinomycin D used was ten times that used by Widnell & Tata (1966) because of the shortened incubation period (3min as compared with 15min) used, and unlike these authors no prior pre-incubation with actinomycin D was used.

other than sensitivity to puromycin or cycloheximide, between the enzyme of the fed and starved groups. Fig. 2 shows that the optimum Mg^{2+} concentration is slightly greater for the enzyme from the fed groups than for that from the starved group. Also, the enzyme obtained after 1h from the T+-diet-fed group differed from that of the other groups in the greater activation at superoptimum concentrations of Mg^{2+} . This difference is also seen in the pH optimum (Fig. 3) for the enzyme obtained after 1h from the T+-diet-fed group. The optimum Mg^{2+} concentrations and pH optima suggest that the enzyme obtained after 1h from the T+-diet-fed group may differ from that obtained from the other groups, although these differences may not be significant.

The results given in Fig. 4 suggest that the DNA-dependent RNA polymerase obtained after 1h from the T+-diet-fed group may be more sensitive to inhibition by actinomycin D than that from the 18h-starved group, although that obtained after 1h from the T--diet-fed group also appears to differ from that of the other two groups, especially at low actinomycin D concentration.

Table 4 shows the nucleoside triphosphate requirements for the enzyme from the three groups. There is an appreciable formation of poly (G, A, U) and some formation of poly (G, A, C) and poly (G, U, C). The enzyme obtained after 1h from the T--diet-fed group differs from that from the other groups in catalysing greater proportional poly (A, C, G) and poly (G, U, C) formation, and enzyme obtained after 1h from the T+-diet-fed group catalyses less proportional poly (A, U, G) formation than that from the other groups.

The poly (G, A, C) formation is the same and the poly (G, A, U) formation is twice as great as that found by Widnell & Tata (1966). This may be a feature of the 3min incubation period used in the

Table 4. *Characteristics of the Mg^{2+} -activated DNA-dependent RNA polymerase*

Animals that had been starved for 18h were treated as shown and rat liver nuclei were isolated. RNA polymerase activity was assayed as described in the Materials and Methods section. Omissions from the assay mixture were made as shown.

Assay system	RNA polymerase activity (μ U/mg of DNA)		
	18h-starved rats	T+-diet-fed rats, after 1h	T--diet-fed rats, after 1h
Complete	53	120	98
Mg^{2+} omitted	1	2	1
ATP omitted	7	14	17
CTP omitted	12	17	20
UTP omitted	18	44	48
ATP and CTP omitted	4	5	4
ATP and UTP omitted	6	11	11
CTP and UTP omitted	2	2	2
ATP, CTP and UTP omitted	3	4	3

Fig. 5. Incorporation of $[5\text{-}^3\text{H}]$ orotic acid into rat liver nuclei *in vivo*. Animals that had been starved for 18 h were used for all experiments. $[5\text{-}^3\text{H}]$ Orotic acid ($200\text{ }\mu\text{Ci}$) was administered by intraperitoneal injection, and animals were killed at 5, 10 or 15 min after injection (top abscissa). One group of animals was not fed (▲) and two groups were fed with the T+ diet (○) or the T- diet (●) and killed at the times indicated (bottom abscissa). Nuclei were isolated, sampled and assayed as described in the Materials and Methods section. Each point represents duplicate determinations on one animal. Several series of experiments demonstrated that consistent relative results could be obtained in each set of experiments, but absolute uptake of $[5\text{-}^3\text{H}]$ orotic acid varied considerably and therefore the results of only one series of experiments are shown.

present study compared with the Widnell & Tata (1966) incubation time of 15 min. These authors were not able to determine poly (G, U, C) formation because AMP incorporation was used as a measure of polymerase activity.

Incorporation of $[^3\text{H}]$ orotic acid into rat liver nuclei *in vivo*. The activity of DNA-dependent RNA polymerase changes rapidly after the feeding with the complete diet, and it was decided to ascertain whether RNA synthesis, as judged by $[^3\text{H}]$ orotic acid uptake *in vivo*, similarly increases. Initial experiments established that linear uptakes of $[^3\text{H}]$ orotic acid occurred up to 20 min after intraperitoneal injection of the labelled precursor, and uptakes were then determined in the 18 h-starved animals, and directly after feeding and at 40 min after feeding in the T+-diet-fed and T--diet-fed animals. Fig. 5 shows that the basal uptake varies within the groups, but as this presumably reflects dilution of the labelled pyrimidine triphosphates it can be ignored. The gradients of the uptakes represent synthesis rates and these are similar in the starved rats and at 0–15 min in the T--diet-fed rats, but the synthesis rate at 0–15 min in the T+-diet-fed

group is about 1.5 times that in the other groups. At 40–55 min after the feeding the synthesis rate in the T+-diet-fed animals is 3 times that in the T--diet-fed group, which in turn is about 1.5 times that at 0–15 min in the T--diet-fed group. Thus $[^3\text{H}]$ orotic acid uptakes appear to confirm that RNA synthesis does follow the Mg^{2+} -activated DNA-dependent RNA polymerase activity after administration of the diets. These results are, however, inconclusive because the effects of the dietary treatments on the nucleoside triphosphate pools in the liver are not known.

Effect of administered amino acid mixtures on plasma and liver tryptophan concentrations. The results described above were obtained with two diets that differed only in the presence or absence of tryptophan, and it was decided to measure the concentrations of this amino acid in plasma and liver after 18 h starvation and at 15, 30, 45, 60 and 120 min after the feeding with the diets. Fig. 6 shows the expected rapid rise in plasma tryptophan concentration after the feeding with the T+ diet and the relatively stable plasma concentration after administration of the T- diet. Fig. 7 shows the liver concentrations of free tryptophan in both dietary groups. Predictably those in the animals fed with T+ diet rise rapidly to a maximum, at 1 h, which is three times the starvation value and which returns to the starvation value within 2 h. The T- diet produces unexpected results: initially the tryptophan concentration does not change, but within 30–45 min after the feeding it rapidly rises to a maximum that is twice the starvation level, and it is still elevated 2 h after the feeding. This increase in liver tryptophan concentration in the T--diet-fed animals could be due to protein degradation in the liver cell, as Gan & Jeffay (1967) have shown that about 50% of the rat liver's intracellular amino acid pool of lysine is derived from liver protein breakdown and the present work suggests that the intracellular pool of tryptophan has been supplemented by (increased?) protein catabolism in the T--diet-fed animal.

DISCUSSION

If the Mg^{2+} -activated DNA-dependent RNA polymerase of rat liver nuclei synthesizes rRNA, as suggested by Widnell & Tata (1966), then the present study suggests that the starved animal makes rRNA at about 80% of the rate in the colony fed animals. This decreased rate is not quite as low as the value of 65% found by Hirsch & Hiatt (1966) for the turnover of liver ribosomes in starved rats, but the animals that they used were at the end of their growth phase and therefore less adaptive to dietary changes, whereas the animals used in the present study were in the middle of their linear

Fig. 6. Response of rat plasma tryptophan concentration to feeding with the complete and tryptophan-free amino acid mixtures. Animals that had been starved for 18 h were killed either without being fed (▲) or after being fed with the T+ diet (○) or the T- diet (●). The plasma tryptophan concentration was determined as described in the Materials and Methods section. Results are expressed as means $\pm$ S.E.M., with the numbers of animals given in parentheses.

Fig. 7. Response of rat liver free tryptophan concentration to feeding with the complete and tryptophan-free amino acid mixtures. Animals that had been starved for 18 h were killed either without being fed (▲) or after being fed with the T+ diet (○) or the T- diet (●). The liver tryptophan concentration was determined as described in the Materials and Methods section. Results are expressed as means $\pm$ S.E.M., with the numbers of animals in parentheses.

growth phase. Hirsch & Hiatt (1966) also found that the fractional catabolic rate of rat liver rRNA was 23% in the starved animal compared with 14% in the fed animal. These findings give a quanti-

tative basis to the observations by Kosterlitz (1947) and Bernhard, Haguénau, Gautier & Oberling (1952) that starvation decreases the cytoplasmic content of RNA of rat liver and that the site of loss is in the endoplasmic reticulum.

The decrease in the total liver content of RNA on starvation suggests that re-feeding should result in an increase in liver RNA synthesis. Stenram (1956a) did in fact observe a rapid increase in the size of rat liver nucleoli within 6 h after the feeding of a high-protein diet, Kosterlitz (1947) found an increase in the total RNA content of rat liver and Bernhard *et al.* (1952) observed a rapid regeneration of endoplasmic reticulum of liver cytoplasm. The present work indicates that the increased RNA synthesis in liver consequent on re-feeding is accompanied by a very rapid response of the Mg^{2+} -activated DNA-dependent RNA polymerase. This response (T+ diet; Fig. 1) is also relatively long-lasting, still being present 2 h after administration of the complete amino acid mixture. The increased activity of the RNA polymerase is due, at least in part, to synthesis of a new enzyme that, because of its rapid synthesis, is sensitive to short periods of exposure to puromycin or cycloheximide, unlike the enzyme from starved animals, which is not affected by these agents (Table 3).

The stimulated enzyme therefore appears to differ from the basal enzyme only by its rapid synthesis and destruction, and this suggests that it acts as a regulator of rRNA synthesis. The requirement for such a regulator is obvious. After the intake of a complete amino acid mixture the liver cell amino acid content will rise and there will be a stimulation of protein and ribosome function. As the increased liver cell amino acid content is likely to be present for a few hours only (Christensen, 1964) some means of 'switching off' the increased rRNA synthesis is required, and the short-lived enzyme would appear to fulfil this requirement. This is not to suggest that other mechanisms of control are not operative under these circumstances, but nonetheless the rapidity of the response of the RNA polymerase does suggest such a function.

Several authors have reported increases in Mg^{2+} -activated DNA-dependent RNA polymerase in rat liver nuclei after various procedures. Thus within 12 h of partial hepatectomy the RNA polymerase activity doubled (Tsukada & Lieberman, 1964), within 12 h of growth-hormone administration RNA polymerase activity had doubled (Pegg & Korner, 1965) and 45 h after tri-iodothyronine injection RNA polymerase activity had nearly doubled (Tata & Widnell, 1966). It is clear that the nutritional response of the rat liver RNA polymerase is extremely rapid when compared with these results. The present work also implies that the activity of liver RNA polymerase must oscillate during the

Fig. 8. Relationship between liver tryptophan content and RNA polymerase in 18 h-starved animals (▲) and in animals fed with the T+ diet (○). The results are derived from Figs. 1 and 7 and the RNA polymerase activities and tryptophan concentrations have been randomly paired to give 40 sets of results.

Fig. 9. Relationship between liver tryptophan content and RNA polymerase in 18 h-starved animals (▲) and in animals fed with the T- diet (●). The results are derived from Figs. 1 and 7 and the RNA polymerase activities and tryptophan concentrations have been randomly paired to give 37 sets of results. The results obtained after 2 h for the T--diet-fed group have been excluded from this graph for the reasons given in the text.

day, being greater for a short time after meals containing protein. A corollary is that care should be taken in interpreting increases in RNA polymerase activity after experimental procedures until the effects of food ingestion alone have been determined.

That there is an early lack of response of RNA polymerase activity to feeding with the amino acid mixture lacking tryptophan implies that tryptophan is required for the response of the enzyme, because at the time when the liver tryptophan concentration rises to a value that in the T+-diet-fed animal produces a response of the RNA polymerase (Figs. 1 and 7) the RNA polymerase activity begins to increase sharply. This observation suggests a relationship between the liver tryptophan concen-

tration (acting merely as a 'marker' for the general amino acid content) and the DNA-dependent RNA polymerase activity; this is shown in Figs. 8 and 9. It should be noted from Figs. 1 and 7 that after 2 h in the T--diet-fed group the liver tryptophan concentration is at variance with the RNA polymerase activity, and it is likely that the pools of amino acids other than tryptophan have decreased by the increased degradation that occurs when imbalanced amino acid mixtures are given (Deshpande, Harper & Elvehjem, 1958). Accordingly the determinations of liver tryptophan concentration and RNA polymerase activity after 2 h in the T--diet-fed group were excluded from Fig. 9. There is a strong probability that the response of RNA polymerase to the liver tryptophan concentration (fortuitously acting as a measure of amino acid content of the liver) is the same in both the T+-diet-fed and T--diet-fed animals because the correlations in both groups belong to the same populations (i.e. are not significantly different from each other).

The present results show that at 2 h after feeding with the T- diet the liver tryptophan concentration is appreciably raised above the starvation value. Wunner *et al.* (1966) observed that at this time the dimer proportion of the polyribosomes was still increasing, although this may not be a valid measure of polyribosome disaggregation. Allen, Raines & Regen (1969) observed a 50% diminution in the extent of charging of tRNA_{Trp} after feeding with a T- diet, but they do not state at what time after the feeding these results were obtained. The findings of these authors suggest that the amino acid concentrations affecting RNA polymerase are in a different intracellular pool from the amino acids affecting polyribosome stability and the extents of charging of tRNA. Functional heterogeneity of intracellular amino acid pools has been proposed by Kipnis, Reiss & Helmreich (1961) in their study of protein synthesis in rat diaphragm and rabbit and guinea-pig lymph-node cells, and has received support from the work of Hider, Fern & London (1969) in their investigation of the intracellular amino acids in rat muscle.

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