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Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase Activity in Rat Liver after Protein Restriction

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Rats were fed for 6 days on a diet containing either 3 or 20% high-quality protein. Nuclei were isolated from liver and DNA-dependent RNA polymerases (EC 2.7.7.6) extracted with 1 M-$(NH_4)_2SO_4$. The proteins were then precipitated with 3.5 M-$(NH_4)_2SO_4$ and after dialysis applied to a DEAE-Sephadex column. The column was developed with a gradient of $(NH_4)_2SO_4$. Polymerase I separated well from α-amanitin-sensitive polymerase II. The enzyme activities were compared between the two dietary groups. Rats that had received 3% protein showed a lower polymerase I activity per g wet wt. of liver, per mg of DNA and per mg of protein. Polymerase II was lower in activity per g wet wt. of liver and per mg of DNA, but was higher per mg of protein. Polyacrylamide-gel electrophoretograms showed a higher proportion of contaminating proteins in polymerase II fractions isolated from 20%-protein-fed rats. The data explain the lower activity obtained per mg of protein in these rats. It is concluded that a decrease in dietary protein content from 20 to 3% induces a fall in content and specific activity of RNA polymerase I and II in liver.

DNA-dependent RNA polymerases (EC 2.7.7.6) from eukaryotes have been separated into two major groups by ion-exchange chromatography (Roeder & Rutter, 1969). Polymerase I (or enzyme A) is localized in the nucleolus and appears responsible for the synthesis of precursor ribosomal RNA (Roeder & Rutter, 1972). Polymerase II (or enzyme B) is localized in the nucleoplasm and is recognized by its sensitivity to the toxin α-amanitin (Kedinger *et al.*, 1970; Lindell *et al.*, 1970). Polymerase II appears to be involved in the synthesis of heterogeneous nuclear RNA (Zylber & Penman, 1971). Transcription of tRNA and 5S RNA genes by a third enzyme designated polymerase III has recently been described (Weinmann & Roeder, 1974).

Von der Decken & Andersson (1972) showed that RNA polymerase activity as measured in whole nuclei of liver was significantly different between rats fed on a diet containing either 20 or 3% high-quality protein. The apparent alterations in activity could be ascribed either to differences in amount or activity of the enzymes themselves or in availability of DNA template to be transcribed.

In view of these possibilities the total and specific activities of isolated RNA polymerase I and II were determined. Evidence is presented here that the enzyme activities are closely related to the dietary protein intake, and therefore at least partially responsible for the differences in RNA synthesis observed previously (von der Decken & Andersson, 1972).

A preliminary report of this work has been published (Andersson & von der Decken, 1974).

Materials and Methods

Materials

Chemicals were of analytical grade wherever possible. The sources were given in detail previously (von der Decken, 1968, 1969). In addition, deoxyribonuclease I DN-Ep., pancreatic ribonuclease A (type 1-A), ribonuclease T_1, CTP, UTP and salmon DNA were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.; [5,6-^{3}H]uridine 5′-triphosphate (47 Ci/mmol) from The Radiochemical Centre, Amersham, Bucks., U.K.; dithiothreitol (Cleland's reagent) from Calbiochem AG, Lucerne, Switzerland; acrylamide and bisacrylamide from Serva Feinbiochemica, Heidelberg, Germany; DEAE-Sephadex A-25 from Pharmacia AB, Uppsala, Sweden; and glass-fibre filters (type A) from Gelman, Ann Arbor, Mich., U.S.A.

Animals

Male Sprague–Dawley rats 30 days of age (70–80 g) were caged individually under the conditions described (von der Decken & Omstedt, 1972). For 3 days the rats were fed on a diet containing 10% protein. Thereafter the rats were divided into two groups. For 6 days one group was fed on a 20% (von der Decken & Omstedt, 1972) the other a 3% protein-containing diet (Omstedt & von der Decken,

1974). The two groups are referred to as protein-fed and protein-restricted respectively. Egg albumin was used as the protein source.

Purification of RNA polymerase I and II

All preparative procedures were carried out at 4°C. Nuclei were isolated (Chesterton *et al.*, 1972) from 10g of liver pooled from three rats. The nuclei were purified further by centrifugation through 1.45M-sucrose in 3mM-$MgCl_2$ and 0.1mM-dithiothreitol ($5000g_{av.}$ for 20min). RNA polymerases were extracted from the nuclei essentially as described by Doenecke *et al.* (1972). The medium contained 0.067M-Tris–HCl, pH7.9, measured at 25°C, 10mM-$MgCl_2$, 0.25mM-EDTA (disodium salt), 0.1mM-dithiothreitol, 25% glycerol and various concentrations of $(NH_4)_2SO_4$ as indicated. The nuclei were homogenized in a Dounce homogenizer (pestle B) in the medium containing 1M-$(NH_4)_2SO_4$. The homogenate was stirred for 1h and the viscous solution centrifuged for 10min at $12000g_{av.}$. The supernatant was made 3.5M with respect to $(NH_4)_2SO_4$, stirred for 3h, and centrifuged for 45min at $165000g_{av.}$ (50 Ti rotor, Spinco model L2-65 B centrifuge). The pellet after resuspension was dialysed for 17h against the medium without $(NH_4)_2SO_4$. After centrifugation for 5min at $5000g$ the supernatant was applied to a column (21mm × 50mm) of DEAE-Sephadex A-25 previously equilibrated with medium containing 20mM-$(NH_4)_2SO_4$. The column was washed with 20mM-$(NH_4)_2SO_4$ in the medium and the enzymes were eluted with a linear gradient between 20mM- and 0.5M-$(NH_4)_2SO_4$ in the medium. Transmittance was recorded with a LKB UVcord type 4701 A at 254nm and at a light-path of 3mm. Fractions (1.3ml) were collected and 50μl samples assayed for enzyme activity.

Enzyme assay

The total volume of the incubation mixture was 130μl. It contained 0.32mM each of ATP, GTP, CTP, 0.15mM-[^{3}H]UTP (6.15μCi/ml), 3mM-phosphoenolpyruvate, 20μg of pyruvate kinase/ml, 2mM-$MnCl_2$, 7.5mM-$MgCl_2$, 0.2mM-EDTA (disodium salt), 0.08mM-dithiothreitol, 50mM-Tris–HCl, pH7.9 (measured at 25°C) and 20% glycerol. $(NH_4)_2SO_4$ (between 16 and 70mM) and native or denatured DNA (200μg/ml) were present as indicated in the Figures and Tables. After incubation for 20min at 35°C, 100μl samples were transferred to glass-fibre filters. These were transferred to individual 25ml E-flasks. Ice-cold 10% (w/v) trichloroacetic acid containing 1mM-AMP (10ml) was added. After 15min the solution was changed to 10ml of 5% trichloroacetic acid containing 0.05M-$Na_4P_2O_7$. After gentle vibration for 15min this step was repeated, followed by one extraction in 10ml of 5% trichloroacetic acid for 15min and then 3 × 10ml of ethanol for 5min each. The glass-fibre filters were dried and counted for radioactivity (von der Decken, 1968) in a liquid-scintillation spectrometer (Intertechnique, model SL 30) at 30% efficiency.

Polyacrylamide-gel electrophoresis

Electrophoresis was either in 5% acrylamide and 3M-urea at pH8.9 (Davis, 1964) or in 10% acrylamide containing 0.05% sodium dodecyl sulphate at pH7.0 (Weber & Osborne, 1969).

DNA

DNA was dissolved in 1.5mM-trisodium citrate–15mM-NaCl and used as native DNA. Denatured DNA was prepared by treating native DNA solution for 20min at 90°C followed by immediate cooling in a mixture of solid CO_2 and ethanol. The 'melting' profile of the native and denatured DNA was determined (Näslund *et al.*, 1974).

Analyses

Proteins were determined as described by Lowry *et al.* (1951) with bovine serum albumin as a standard. DNA was determined by the diphenylamine method (Giles & Myers, 1965) with the following modification. DNA-containing preparation (20–40μl) was pipetted on to filter-paper disks (20mm diam., Munktell 00HH). These were washed twice for 5min in ice-cold 2% $HClO_4$ and extracted individually in 2ml of 10% $HClO_4$ at 70°C for 20min. The DNA of the extract was determined by using salmon DNA as a standard.

Analytical ultracentrifugation analyses were carried out in a MSE Centriscan 75 fitted with dual-beam u.v. optics.

Results

Characteristic properties of the enzymes

In preliminary experiments it was observed that a stepwise elution with $(NH_4)_2SO_4$ from DEAE-cellulose or DEAE-Sephadex columns resulted in a high contamination of the enzymes with ribonuclease. This obscured time- or enzyme-concentration-dependent increase in activity. On the other hand, elution by $(NH_4)_2SO_4$ gradient separated the nuclear extract into three main active components (cf. Fig. 2), two of which, polymerase I and II, will be compared here under the dietary conditions used. Separation into polymerase I and II was assured by sensitivity test to the toxin α-amanitin; enzyme I being insensitive whereas enzyme II was completely inhibited by the toxin (see Fig. 2).

Activity of enzyme I and II increased with time of incubation for at least 30min (Fig. 1*a*). In comparative studies 20min of incubation were used. The enzyme activities also increased with amount of protein added (Fig. 1*b*). The concentration of

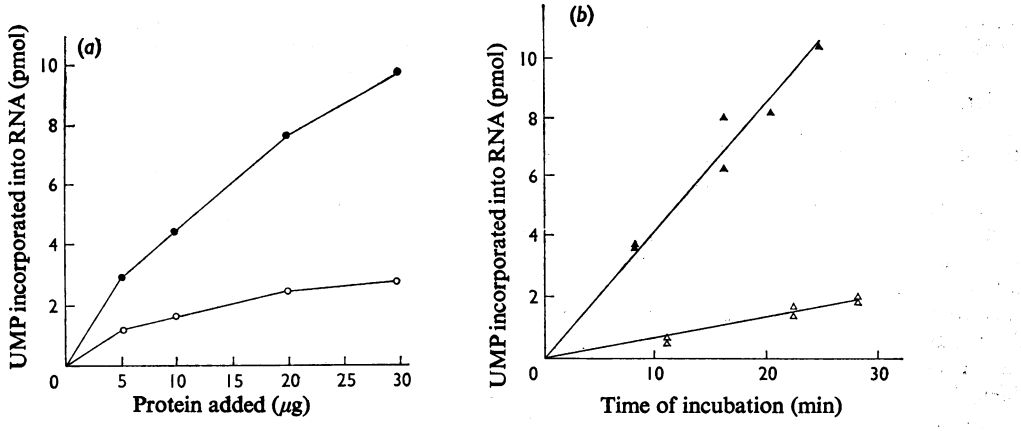

Fig. 1. *Time- and protein-dependent incorporation of [³H]UMP into RNA*

The incubation system contained in 130μl, 0.32mM each of ATP, GTP and CTP, 0.15mM-[³H]UTP, 3mM-phosphoenolpyruvate, 20μg of pyruvate kinase/ml, 2mM-$MnCl_2$, 7.5mM-$MgCl_2$, 0.2mM-EDTA (disodium salt), 0.08mM-dithiothreitol, 50mM-Tris–HCl buffer, pH7.9 measured at 25°C, and 20% glycerol. Incubations with polymerase I contained 30mM-$(NH_4)_2SO_4$ and 200μg of native DNA/ml, incubations with polymerase II 60mM-$(NH_4)_2SO_4$ and 200μg of denatured DNA/ml. (*a*) 13.7μg of polymerase I (○) or 10.9μg of polymerase II (●) were added. Incubation was at 35°C for the indicated times. (*b*) Increasing amounts of polymerase I (△) or polymerase II (▲) were added. Incubation was at 35°C for 20min. After incubation RNA was extracted (see the Materials and Methods section) and radioactivity measured.

Table 1. *Requirement of DNA template for the incorporation of precursor [³H]UTP into RNA*

Incubation of the control was as described in Fig. 1 for 20min at 35°C. Additions or omissions were as indicated. Deoxyribonuclease was added at a concentration of 50μg/ml and actinomycin D at 40μg/ml. The concentration of enzymes present were as follows. Protein-fed rats, polymerase I 18μg of protein; polymerase II 8.6μg of protein. Protein-restricted rats, polymerase I 14μg of protein; polymerase II 5.1μg of protein. Values are the mean of duplicate incubations.

	UMP incorporated into RNA (pmol/mg of protein)			
	Protein-fed		Protein-restricted	
Experimental conditions	Polymerase I	Polymerase II	Polymerase I	Polymerase II
Control	179.2	908.4	113.7	890.7
Control without DNA	13.1	13.2	6.1	9.9
Control plus deoxyribonuclease	14.0	78.1	6.8	114.0
Control plus actinomycin D	28.9	73.6	15.8	8.6

enzymes used in the dietary experiments was below 20μg of protein.

The size of the DNA to be transcribed has been reported to be important (Flint *et al.*, 1974). The salmon DNA used contained two major components with $s^0_{20,w}$ values of 13 and 49. These correspond to molecular weights of 1.8×10^6 and 8.8×10^7 respectively (Studier, 1965). The size was sufficient to assure good transcription efficiency.

The dependence of enzyme activity on DNA template is summarized in Table 1. Omission of the template decreased the activity of polymerase I and II from the two dietary groups by 95–99%. The presence of deoxyribonuclease during incubation decreased incorporation of precursor [³H]UTP into RNA by 90%. Actinomycin D blocks deoxyguanine and therewith template activity of DNA. In the presence of the antibiotic 85–90% inhibition of the incorporation of precursor [³H]UTP into RNA was observed.

The results on the dependence of the incorporation of precursor [³H]UTP into RNA on all four nucleoside triphosphates are summarized in Table 2. Omission of the three non-radioactive nucleoside triphosphates decreased the incorporation by more than 90%. The absence of ATP or GTP decreased the incorporation by 90–95%. The requirement for CTP was less pronounced. In its absence, polymerase I

activity was decreased by 70–75% and polymerase II by 60–67%.

The nature of product synthesized during incubation was characterized by treatment with alkali or ribonuclease (Table 3). Exposure for 10min at 70°C in 1M-NaOH hydrolysed the major part of the radioactive products synthesized by polymerase I or II into non-acid-precipitable components. Digestion with pancreatic ribonuclease A for 10min at 35°C transferred 72% of the acid-precipitable components synthesized by polymerase I into a soluble form. However, no effect by ribonuclease was obtained on the product synthesized by polymerase II. Whereas pancreatic ribonuclease cleaves at pyrimidine nucleotides, T_1 ribonuclease cleaves at guanylate residues. But none of the enzymes had any effect although the time of treatment was prolonged to 30min at 35°C.

Enzyme activities of protein-fed and protein-restricted rats

Nuclei were prepared from 10g of liver. The liver was pooled from three rats/dietary group. As shown in Fig. 2 the elution pattern of the two nuclear extracts were similar with respect to distribution of the enzymes but were markedly different in transmission spectrum at 254nm. The absorbance of the nuclear extracts from 10g of liver from protein-fed rats was higher (Fig. 2*a*) than that from 10g of liver from protein-restricted rats (Fig. 2*b*).

Evaluation of the results was made by calculating polymerase activities per g wet wt. of liver, per mg of protein and per absorbance unit of 1.0 at 254nm in 1ml of solution with light-path of 3mm (Table 4).

(*a*) *Polymerase I activity.* As measured per g wet wt. of liver, activity of protein-restricted rats was significantly lower as compared with protein-fed rats. Significant differences were also obtained of activity per mg of protein. When calculated per absorbance unit, enzyme activity of protein-restricted rats was similar to that of protein-fed rats.

(*b*) *Polymerase II activity.* Activity per g wet wt. of liver was significantly lower in protein-restricted compared with protein-fed rats. On the other hand, activity per mg of protein and that per absorbance unit were higher in preparations from protein-restricted rats.

Table 2. *Effect of nucleoside triphosphates on the incorporation of precursor [^{3}H]UTP into RNA*

The incubation system was that described in Fig. 1 but the nucleoside triphosphates were added as indicated at the concentrations given in Fig. 1. The time of incubation was for 20min at 35°C. The concentration of enzymes present were as follows. Protein-fed rats, polymerase I 19μg of protein; polymerase II 11μg of protein. Protein-restricted rats, polymerase I 16μg of protein; polymerase II 4.5μg of protein. Values are the mean of duplicate incubations.

	UMP incorporated into RNA (pmol/mg of protein)			
	Protein-fed		Protein-restricted	
Addition of nucleoside triphosphates	Polymerase I	Polymerase II	Polymerase I	Polymerase II
[^{3}H]UTP plus ATP, GTP, CTP	176.4	1013.5	119.5	953.1
[^{3}H]UTP plus ATP, GTP	48.7	393.2	34.2	326.9
[^{3}H]UTP plus ATP, CTP	15.4	48.1	9.1	71.5
[^{3}H]UTP plus GTP, CTP	13.8	65.9	11.2	55.3
[^{3}H]UTP alone	22.9	11.1	10.3	19.5

Table 3. *Treatment with alkali or ribonuclease of the radioactive acid-precipitable RNA obtained after incubation*

Incubation was as described in Fig. 1 for 20min at 35°C. The concentration of enzymes present were as follows. Protein-fed rats; polymerase I 18μg of protein; polymerase II 7.4μg of protein. Protein-restricted rats; polymerase I 14μg of protein; polymerase II 4.8μg of protein. Treatment after incubation was as indicated. Incubation with 1M-NaOH was for 10min at 70°C. Thereafter the samples were neutralized with HCl and processed in the usual manner (see the Materials and Method section). Incubation with pancreatic ribonuclease A (150μg/ml) was for 10min at 35°C. Values are the mean of duplicate incubations.

	UMP incorporated into RNA (pmol/mg of protein)			
	Protein-fed		Protein-restricted	
Treatment after incubation	Polymerase I	Polymerase II	Polymerase I	Polymerase II
None	179.2	925.3	113.7	840.1
Alkali	50.7	8.3	29.1	9.2
Pancreatic ribonuclease A	27.6	930.3	8.0	838.8

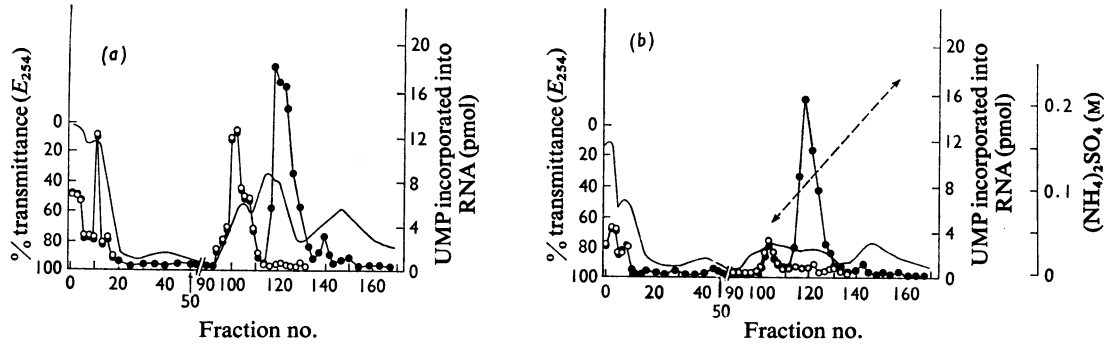

Fig. 2. *Column chromatography on DEAE-Sephadex A-25 of nuclear extracts obtained from protein-fed (a) and protein-restricted (b) rats*

A column (21 mm × 50 mm) of DEAE-Sephadex A-25 was used, and was developed with a gradient of 0.02–0.5 M-$(NH_4)_2SO_4$ in the medium and fractions (1.3 ml) were collected. The nuclear extracts were prepared from 10 g wet wt. of liver as described in the Materials and Methods section. A 50 μl sample from every second fraction was incubated for 20 min as described in Fig. 1. In a separate experiment 0.7 μg of α-amanitin/ml was added. Radioactivity in the absence (●) and in the (○) presence of α-amanitin, transmittance (——) and concentration of $(NH_4)_2SO_4$ (----) were measured.

Table 4. *DNA-dependent RNA polymerase activity in liver of rats fed on* 20 *or* 3% *protein*

A sample of every second fraction obtained after DEAE-Sephadex chromatography was incubated as described in Fig. 1 for 20 min. The total radioactivity of polymerase I and II was calculated and computed to give activity per g wet wt. of liver; activity per 1.0 absorbance unit in 1 ml at 254 nm with light-path of 3 mm; and activity per mg of protein after protein determination as described by Lowry *et al.* (1951). The results are expressed as pmol of UMP incorporated into RNA. The results are the mean of five independent experiments per dietary group ± S.E.M. n.s., Not significant.

	UMP incorporated into RNA (pmol) per		
	g wet wt. of liver	1.0 E_{254} unit·ml·0.3 cm^{-1}	mg of protein
Polymerase I:			
20% protein diet	126.7 ± 6.9	289.4 ± 15.8	232.0 ± 12.7
3% protein diet	32.2 ± 5.1	288.8 ± 45.4	112.5 ± 17.7
Statistical significance	$P<0.001$	n.s.	$P<0.001$
Polymerase II:			
20% protein diet	391.3 ± 38.1	973.4 ± 94.8	724.3 ± 70.5
3% protein diet	216.2 ± 49.3	2717.5 ± 620.0	1058.8 ± 241.6
Statistical significance	$P<0.05$	$P<0.05$	n.s.

Table 5. *Effect of dietary protein intake on DNA content of liver*

The nuclear preparations were analysed for DNA and the content per g wet wt. of liver was calculated. The protein concentration of the same nuclear preparation was determined and the DNA/protein ratio was estimated. The results are the mean of six independent experiments/dietary group ± S.E.M. The results shown in Table 4 on activity/g wet wt. were used to obtain pmol of UMP incorporated into RNA/mg of DNA.

Diet	DNA (mg content/g wet wt. of liver)	DNA/protein ratio of nuclear preparation	UMP incorporated into RNA (pmol/mg of DNA)	
			Polymerase I	Polymerase II
20% protein	1.336 ± 0.072	0.0406 ± 0.0020	94.85 ± 5.18	292.9 ± 28.5
3% protein	1.597 ± 0.052	0.0903 ± 0.0077	20.16 ± 3.17	135.4 ± 30.9
Statistical significance	$P<0.02$	$P<0.001$	$P<0.001$	$P<0.01$

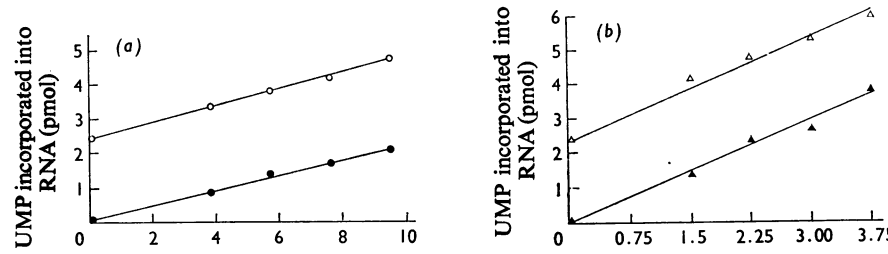

Fig. 3. *Effect of RNA polymerases from protein-fed rats on the activity of that of preparations from protein-restricted rats*

Incubation was for 20min as described in Fig. 1. Increasing concentrations as indicated of RNA polymerase from protein-fed rats were incubated alone or added to a constant amount of RNA polymerase from protein-restricted rats. (*a*) RNA polymerase I. ●, Protein-fed rats; ○, protein-fed rats together with 19μg of protein of RNA polymerase from protein-restricted rats. (*b*) RNA polymerase II. ▲, Protein-fed rats; △, protein-fed rats together with 3.1μg of protein of RNA polymerase from protein-restricted rats. Values are means of two independent experiments each one with duplicate incubations.

The difference in transmission pattern between nuclear extracts of the two groups of rats suggested a lower cellular protein content in the liver of protein-restricted animals. Consequently, the number of cells per g wet wt. and thus the DNA content would be higher in protein-restricted as compared with protein-fed rats. As shown in Table 5 there were statistically significant differences in the DNA content/g wet wt. of liver between the two groups of rats. Further, the DNA/protein ratio of the nuclear preparations from protein-restricted rats was more than twice that of protein-fed rats. The results on DNA content (Table 5) and polymerase activity/g wet wt. (Table 4) were computed to give enzyme activity/mg of DNA (Table 5). Activity of both polymerase I and II showed pronounced differences between the dietary rats, the enzymes of protein-restricted animals being significantly lower in activity than those of protein-fed rats.

To ensure that a lower enzyme activity was not due to the presence of inhibitors in the preparations, the additive activity in the preparations obtained from the two dietary groups was measured. Increasing concentrations of polymerase I or II obtained from protein-fed rats were added to a constant amount of the corresponding enzyme from protein-restricted animals (Fig. 3). With both enzymes there was an additive incorporation of precursor [^{3}H]UTP into RNA. Similar results of an additive effect were obtained by reversing the system by using a constant amount of enzyme from protein-fed rats and increasing concentrations of enzyme from protein-restricted animals (results not shown in the Figure).

Polyacrylamide-gel electrophoresis

The results presented in Table 5 together with the transmission pattern (Fig. 2) made it likely that the separated enzymes of protein-fed rats were contaminated by other proteins. The fractions applied to the gels corresponded to the same amount of wet weight of liver for the two dietary groups. Electrophoresis in polyacrylamide gel at pH8.9 in the absence of sodium dodecyl sulphate showed a broad band of protein (Fig. 4*a*). As seen from the densitometer records the protein content was lower with preparations from protein-restricted rats.

In the presence of sodium dodecyl sulphate the enzymes were resolved into distinct bands (Fig. 4*b*). In the case of polymerase I a similar pattern was obtained with preparations from protein-fed and protein-restricted animals. But as estimated by Amido Black staining the amount of protein present was less after protein restriction.

Polymerase II of protein-fed rats contained a high proportion of proteins migrating ahead in the anodic direction. The corresponding bands were weak or absent in preparations from protein-restricted rats. It may be concluded that these proteins were responsible for the high absorbance observed with preparations from protein-fed rats and seen in the region of polymerase II. The distribution of the remaining bands were similar for the two groups of rats with the amount of protein present being lower after protein restriction.

Discussion

The procedure used in the present study to solubilize RNA polymerase included treatment of nuclei with $(NH_4)_2SO_4$ and separation of the enzymes by ion-exchange chromatography. By this method three fractions were obtained; polymerase I and II and an α-amanitin-insensitive fraction which was not adsorbed on DEAE-Sephadex. A similar pattern

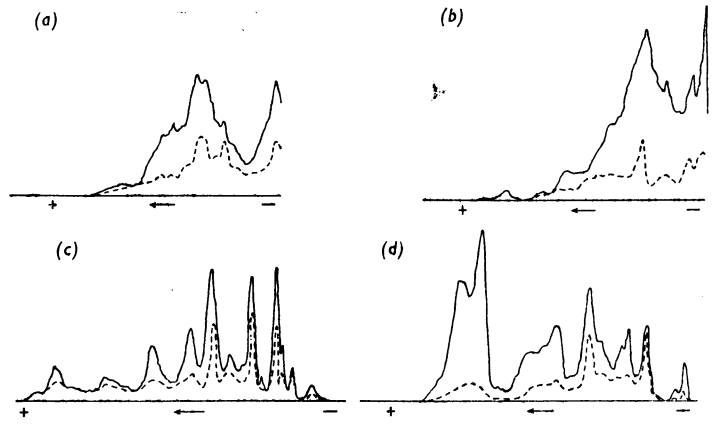

Fig. 4. *Polyacrylamide-gel electrophoretogram of polymerase I- and II-containing fractions*

The active fractions obtained after DEAE-Sephadex chromatography were used for electrophoresis. The volume applied (100 μl) corresponded to the same amount of wet wt. of liver for the two dietary groups. After electrophoresis the gels were stained with Amido Black and the protein pattern was recorded on a microdensitometer. ——, Protein-fed rats; ----, protein-restricted rats. For (*a*) and (*b*) electrophoresis was at pH 8.9 in the absence of sodium dodecyl sulphate but with 3 M-urea present (Davis, 1964). For (*c*) and (*d*) electrophoresis was at pH 7 in the presence of 0.05% sodium dodecyl sulphate (Weber & Osborne, 1969). (*a*) and (*c*) polymerase I; (*b*) and (*d*) polymerase II.

has been recorded with nuclear extracts from mouse liver (Versteegh & Warner, 1973).

A number of criteria were used to show that true DNA-dependent RNA polymerase activity was being assayed; dependence of activity on DNA template and nucleoside triphosphates: digestion of the product with alkali and with ribonuclease. Although sensitive to alkali digestion the product synthesized by polymerase II was protected from hydrolysis by ribonuclease. Probably a RNA–protein or RNA–DNA complex was formed during incubation making the RNA resistant to ribonuclease.

The purpose of this investigation was to compare RNA polymerase I and II activity between dietary groups. It has been shown (von der Decken & Omstedt, 1974) that liver weight changes with protein intake, being lower on protein restriction. Irrespective of liver size, the enzymes were isolated from the same amount of wet weight. With liver weight and enzyme activity/g wet wt. being lower in protein-restricted rats, total polymerase activity was considerably decreased. The lower activity was shown not to be due to the presence of inhibitors in the preparations. DNA determination revealed a higher number of cells/g wet wt. of liver (but not per total liver) on protein restriction. Taking into consideration the lower enzyme activity/g wet wt. and the higher number of cells, it follows that activity/cell was markedly decreased in protein-restricted rats. The differences in wet weight observed were ascribed to the higher protein content in the cells of protein-fed animals.

The proportion of proteins other than polymerases present in the active fractions was elucidated by polyacrylamide-gel electrophoresis. In the absence of sodium dodecyl sulphate the polymerases did not resolve into distinct bands. Densitometer tracings of the gels suggested approximately a higher amount of enzyme and contaminating protein in polymerase II fractions of protein-fed rats. In the presence of sodium dodecyl sulphate polymerase I and II separated into several subunits. Separation into subunits and molecular-weight determinations have been described for rat liver, calf thymus and HeLa-cell enzymes (Weaver *et al.*, 1971; Kedinger & Chambon, 1972; Sugden & Keller, 1973). It appeared that polymerase II from protein-fed rats contained a high proportion of proteins other than the enzyme. From the transmission pattern it was apparent that polymerase II was more affected by overlapping proteins than polymerase I. These results, together with the polyacrylamide-gel electrophoretograms, explained the difficulties encountered when enzyme activity was expressed per mg of protein or per absorbance unit. On the other hand, the most objective information on polymerase activity under various dietary conditions was obtained when enzyme activity was estimated, per mg of DNA or per g wet wt. of tissue.

The present results clearly showed that both types

of RNA polymerase were subject to changes in activity on nutritional influence. The effect of diet on rat liver RNA polymerases has been discussed (Henderson, 1972). There are obvious discrepancies in the literature as to the effect of complete starvation, protein starvation and protein feeding on nuclear RNA synthesis. But feeding time and feeding conditions were not the same in most of the experiments and direct comparisons are therefore difficult.

Separation of multiple forms of RNA polymerase from nuclei allowed for more detailed information on the effect of feeding on nuclear function (Spencer & Henderson, 1971). Overnight starvation decreased the main bands of activity. In line with these results are the results presented here and obtained after protein restriction for 6 days. When proteins were the limiting component in the diet an overall decrease in enzyme activity was observed. The outcome of this investigation is certainly consistent with previous results on nuclear and nucleolar RNA synthesis (von der Decken & Andersson, 1972), and emphasizes the pronounced susceptibility of RNA polymerase I and II to nutritional factors.

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