Synthesis of Hepatic Polyamines, Ribonucleic Acid and S-Adenosylmethionine in Normal and Oestrogen-Treated Chicks

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1. The hepatic synthesis and accumulation of polyamines, RNA and S-adenosylmethionine were studied in normal and oestrogen-treated immature male chicks. 2. Ornithine decarboxylase activity in chick liver and in whole chick embryo homogenate was preferentially located in the soluble supernatant fraction. 3. In general the activities of the enzymes involved in the synthesis of polyamines and S-adenosylmethionine decreased with increasing age. 4. Starvation markedly decreased the activities of ornithine decarboxylase and adenosylmethionine decarboxylase, but had no effect on the spermidine synthase activity. Re-feeding sharply stimulated the activities of both decarboxylases. The hepatic adenosylmethionine decarboxylase activity had an apparent half-life of 20–25 min. 5. Treatment with oestradiol benzoate resulted in a parallel increase in the accumulation of liver spermidine, spermine and RNA. These changes were preceded by a stimulation of the nucleolar RNA polymerase activity and by an increase in the adenosylmethionine decarboxylase activity. The L-methionine adenosyltransferase activity was stimulated more slowly. 6. These results indicate that the nutritional state has a marked influence on the synthesis of polyamines in the chick liver. Further, treatment with oestrogen causes a very rapid stimulation of nucleolar RNA synthesis followed by a stimulation of the synthesis and accumulation of polyamines. These changes are probably important for the stimulation of yolk protein synthesis in avian liver.

Increased polyamine synthesis and accumulation has been found to occur in a number of systems characterized by rapid tissue growth (Raina & Jänne, 1970). These observations, as well as a stabilization of RNA-containing particles by polyamines, a stimulating effect of polyamines on RNA and protein synthesis *in vitro*, a parallelism in the accumulation of polyamines and ribonucleic acids, and an early stimulation of polyamine synthesis in response to various growth and hormonal stimuli (for references see Bachrach, 1973) suggest that polyamines probably play an important role in the synthesis of macromolecules.

A convenient model for studying the molecular events in hormone action is provided by oestrogen-induced yolk protein synthesis in immature male chicks. Oestrogen treatment results in a marked increase in the liver weight and in the content of hepatic DNA, RNA and protein (Jost *et al.*, 1973). A single injection of oestradiol into male chicks induces, after a lag period of about 20 h, a rapid synthesis of phosvitin, an egg-yolk protein normally synthesized in laying hens (Mäenpää & Bernfield, 1969; Bergink *et al.*, 1973). After a second injection phosvitin synthesis is stimulated much more rapidly (Mäenpää & Bernfield, 1969).

The changes in the hepatic synthesis and accumulation of polyamines and RNA accompanying

the oestrogen-induced phosvitin synthesis were studied in the present work. To our knowledge, the synthesis of polyamines and that of S-adenosylmethionine, a substrate used for both polyamine synthesis and for biological methylations, have not been explored in this system previously. Some of the characteristics of the polyamine-synthesizing enzymes in chick liver and chick embryos were also studied in detail. Part of this work has been published as a congress report (Eloranta *et al.*, 1974).

Experimental

Chemicals

DL-[1-¹⁴C]Methionine (sp. radioactivity 8.69 mCi/mmol), DL-[1-¹⁴C]ornithine monohydrochloride (7.66 mCi/mmol), DL-[5-¹⁴C]ornithine monohydrochloride (8.19 mCi/mmol), [1,4-¹⁴C]putrescine dihydrochloride (11.27 mCi/mmol), [¹⁴C]spermidine trihydrochloride (4.5 mCi/mmol), and [U-¹⁴C] GTP (355 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A.). S-Adenosyl-L-[Me-¹⁴C]methionine (55 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Unlabelled putrescine, spermidine and spermine, as well as dithiothreitol, were supplied by Calbiochem, Los Angeles, Calif., U.S.A. Pyridoxal

5'-phosphate and cycloheximide were from Sigma Chemical Co., St. Louis, Mo., U.S.A. Actinomycin D was a kind gift from Merck, Sharp and Dohme, Rathway, N.J., U.S.A. ATP was purchased from Boehringer, Mannheim, Germany, and oestradiol benzoate was from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A. [$1\text{-}^{14}\text{C}$]Ornithine was treated with hydrochloric acid (Hölttä & Raina, 1973), and [^{14}C]putrescine was purified on a Dowex 50 (H^+ form) column (Raina *et al.*, 1970), before use. Commercial [$5\text{-}^{14}\text{C}$]ornithine contained impurities which in electrophoresis moved in the putrescine area. Purification was performed by preparative paper electrophoresis followed by chromatography on a Dowex 50 (H^+ form) column.

Carboxy- ^{14}C -labelled and unlabelled *S*-adenosyl-L-methionine were synthesized essentially as described by Pegg & Williams-Ashman (1969). Decarboxylated adenosylmethionine was prepared from adenosylmethionine and purified as described earlier (Raina & Hannonen, 1971).

Animals

Newly hatched male Leghorn chicks were supplied locally; they were maintained under constant light, in brooders each containing 3–50 chicks, and fed *ad libitum* with a standard breeding diet (Hankkija, Turku, Finland). Unless otherwise stated, the chicks were starved for 12 h before death and always killed between 08:00 h and 10:00 h to minimize diurnal variation in enzyme activities.

Oestradiol treatment

Oestradiol benzoate was dissolved in sesame oil (10–25 mg/ml) and injected intramuscularly into the leg muscles (50 mg/kg body wt.). This dose induces an increase in plasma protein-bound alkalilabile phosphate, to a maximum of approx. 180 $\mu\text{g}/\text{ml}$ after 5 days (P. H. Mäenpää, unpublished work). Where indicated an equivalent dose of oestradiol was given 2 weeks after the first injection. At this point plasma phosphoprotein had returned to the baseline content (Jost *et al.*, 1973).

Isolation of nuclei and nucleoli

To study the subcellular distribution of ornithine decarboxylase activity in chick liver or in whole chick embryos, nuclei were prepared either by the method of Chauveau *et al.* (1956) or as follows. Livers or whole 11–12-day embryos were homogenized in 4 vol. of 2.3 M-sucrose (pH 7.0) containing 1 mM-mercaptoethanol, 0.1 mM-EDTA, 3 mM-MgCl₂, 1 mM-dithiothreitol and 0.1 mM-pyridoxal phosphate with two strokes in a Potter-Elvehjem homogenizer. The homogenates were filtered through four

layers of gauze, layered on 4 ml of 2.2 M-sucrose/1 mM-mercaptoethanol/1 mM-dithiothreitol/0.1 mM-pyridoxal phosphate solution (pH 7.0) with or without 3 mM-MgCl₂, and centrifuged for 60 min at 40000 g_{av} . (19000 rev./min) at 4°C in an MSE swing-out rotor (catalogue no. 59590). The nuclear pellets were suspended in 0.25 M-sucrose containing 0.1 mM-EDTA, 1 mM-mercaptoethanol, 1 mM-dithiothreitol and 0.1 mM-pyridoxal phosphate, and checked for purity under the microscope.

For the preparation of nucleoli, nuclei were isolated from the livers by the method of Blobel & Potter (1966). Nucleoli were prepared by the sonication method as described earlier (Raina & Jänne, 1970). The final nucleolar pellet was suspended in 1 ml of 0.34 M-sucrose containing 3 mM-MgCl₂ and 10 mM-mercaptoethanol and stored in small batches in liquid N₂. No contaminating nuclei or extranucleolar material was detected by electron microscopy.

Enzyme assays

After the animals had been killed, the livers were rapidly removed, washed in ice-cold 0.25 M-sucrose and homogenized with a Potter-Elvehjem homogenizer in 3–4 vol. of ice-cold 0.25 M-sucrose containing 1 mM-mercaptoethanol, 0.1 mM-EDTA, 0.1 mM-pyridoxal phosphate and 1 mM-dithiothreitol. Dithiothreitol and pyridoxal phosphate, added to the homogenization medium just before use, considerably increased the stability of ornithine decarboxylase in enzyme preparations. A portion of the 105000 g_{av} . supernatant fraction of the homogenate was desalted by passing it through a Sephadex G-25 column (1 cm $\times$ 15 cm) previously equilibrated with the homogenization medium. The activities of spermidine synthase and spermine synthase were determined in the Sephadex G-25-treated samples. The activities of ornithine decarboxylase, adenosylmethionine decarboxylase and methionine adenosyltransferase were determined as a routine in the 105000 g_{av} . supernatant fraction; although somewhat higher specific activities were obtained when the Sephadex G-25-treated samples were used as the source of the enzymes, the general pattern of changes was the same.

The assay conditions for ornithine decarboxylase activity (EC 4.1.1.17) were essentially as described by Jänne & Williams-Ashman (1971a), except that 10 mM-EDTA was used in the reaction mixture to decrease the otherwise high blank value. Owing to a low enzyme activity in livers of starved animals, [$1\text{-}^{14}\text{C}$]ornithine (0.05 mM) of high specific radioactivity (7.66 mCi/mmol) was used as the substrate. The reaction was linear with time and the amount of the enzyme protein in the range used in the present investigation. The stoichiometry between the

production of putrescine and CO_2 was determined as described earlier (Jänne & Raina, 1968). The assay mixture for adenosylmethionine decarboxylase (EC 4.1.1.50) contained, in a total volume of 0.5 ml, 0.1 M-potassium phosphate (pH 7.4), 2 mM-putrescine, 0.07 mM-[carboxy- ^{14}C]adenosyl-L-methionine, 2.5 mM-dithiothreitol, 10 mM-EDTA and 4–9 mg of enzyme protein. Incubations of both ornithine decarboxylase and adenosylmethionine decarboxylase were carried out in 10 ml glass tubes which were equipped with polypropylene centre wells attached to rubber stoppers (Kontes Glass Co., Vineland, N.J., U.S.A.). After incubation at 37°C for 30 min, the reaction was stopped by injecting 1 ml of 50% (w/v) trichloroacetic acid or 2 M-citric acid through the stopper. The released $^{14}\text{CO}_2$ was trapped in 0.2 ml of 1 M-Hyamine hydroxide by shaking the tubes at 37°C for 30 min, and counted for radioactivity as described earlier (Jänne & Raina, 1968). The blank values were determined by heating the enzyme at 80°C for 5 min before adding the radioactive reaction mixture and incubating as described above.

The assay conditions for spermidine synthase (EC 2.5.1.16) (Raina & Hannonen, 1971; Jänne & Williams-Ashman, 1971b) and spermine synthase (Raina & Hannonen, 1971), as well as the separation of the radioactive products (Raina & Hannonen, 1971; Jänne & Williams-Ashman, 1971b; Hannonen *et al.*, 1972b) have been described earlier in detail. The recovery of spermidine and spermine was determined by the ninhydrin method (Raina *et al.*, 1967).

For the assay of methionine adenosyltransferase (EC 2.5.1.6) activity a slight modification of the method of McKenzie & Gholson (1973) was used. The incubation mixture contained, in a total volume of 0.25 ml, 0.15 M-Tris/HCl buffer (pH 7.6), 0.15 M-MgCl₂, 0.25 M-KCl, 5 mM-dithiothreitol, 10 mM-ATP, 2 mM-L-[1- ^{14}C]methionine (0.5 $\mu\text{Ci}/\mu\text{mol}$) and 0.7–1.2 mg of the enzyme protein. Under these conditions the system was essentially saturated with respect to methionine and ATP. The reaction was linear with time up to 20 min and with the amount of enzyme protein up to 2.5 mg. The incubation was performed in plastic tubes at 37°C for 15 min, and stopped by adding 50 μl of 10% (w/v) HClO_4 . Analysis of the radioactive adenosylmethionine was then performed as described by McKenzie & Gholson (1973). Blanks were made similarly except that ATP was not included in the incubation mixture. To establish the degradation rate of enzymically synthesized adenosylmethionine, two standard tubes containing 15 nCi of [*Me*- ^{14}C]adenosylmethionine, but no ATP, were used; the labelled adenosylmethionine was added to one tube before and to the other after the incubation. The mean of these standards was used to calculate the recoveries.

The RNA polymerase (EC 2.7.7.6) assays were

performed in 0.1 ml reaction mixtures essentially as described by Grummt & Lindigkeit (1973). The reaction mixture contained 0.1 M-Tris/HCl (pH 8.5), 5 mM-MgCl₂, 10 mM-dithiothreitol, 6 mM-NaF, 0.1 M-(NH₄)₂SO₄, 0.3 mM-ATP, 0.3 mM-CTP, 0.3 mM-UTP, 0.009 mM-[^{14}C]GTP and 5–20 μl of nucleolar suspension containing 0.2–1.5 μg of DNA. Incubations were performed at 37°C for 15 min and terminated by adding an equal volume of ice-cold 10% (w/v) trichloroacetic acid containing 40 mM-NaH₂PO₄ and 25 mM-Na₄P₂O₇. The precipitates were collected on glass-fibre filters (Whatman GF/A), washed with 5% (w/v) trichloroacetic acid, dissolved in 0.5 ml of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, Ill., U.S.A.) and counted for radioactivity in 5 ml of toluene-based scintillant [4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in 1 litre of toluene].

The enzyme activities did not appreciably change for at least 1 week when stored at –80°C. However, repeated freezing and thawing resulted in significant losses in the activities of ornithine decarboxylase and adenosylmethionine decarboxylase and methionine adenosyltransferase.

Assay of nucleic acids, polyamines and protein

Portions of homogenates were immediately treated with cold 10% (w/v) trichloroacetic acid. Polyamines were extracted into alkaline butanol, separated by electrophoresis (Raina, 1963) and analysed by the ninhydrin method (Raina *et al.*, 1967).

Nucleic acids were extracted into hot trichloroacetic acid, and DNA was then determined by the diphenylamine reaction and RNA by the orcinol method both as described by Ashwell (1957).

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Intracellular location of ornithine decarboxylase activity

It has been reported that as much as half of the ornithine decarboxylase activity of the whole chick-embryo homogenate resides in the nuclear fraction (Snyder *et al.*, 1970). It was therefore desirable to do additional studies to confirm this point. Analysis of the ornithine decarboxylase activity of the nuclear and soluble supernatant fractions (see the Experimental section) prepared from the livers of 1–9-week-old male chicks revealed that in every case the nuclear fraction contained less than 2% of the ornithine decarboxylase activity of the supernatant fraction. Mitochondrial and microsomal fractions also produced radioactive CO_2 from [1- ^{14}C]ornithine, but there was no stoichiometric formation of putrescine from [5- ^{14}C]ornithine.

Table 1. *Ornithine decarboxylase activity in the nuclear fraction of homogenates of whole chick embryos*

Nuclear fractions were prepared as described in the Experimental section. A refers to nuclei obtained by the method of Chauveau *et al.* (1956); preparation B was obtained by centrifugation through a 2.2M-sucrose 'cushion' containing 3 mM-MgCl₂. The number of embryos in a pooled sample is given in parenthesis. Calculation of the recovery of nuclei is based on the DNA content of the homogenate. The nuclear ornithine decarboxylase activity is also expressed as a percentage of the activity found in the soluble supernatant fraction obtained by the conventional method described in the Experimental section.

Age of embryos (days)	Nuclear preparation	Recovery of nuclei (%)	Ornithine decarboxylase activity (pmol of ¹⁴ CO ₂ /g wet wt. of liver)		Nuclei supernatant (%)
			Supernatant	Nuclei	
11 (6)	A	44	13560	1390	10.3
	B	19	13560	1080	8.0
11 (10)	A	55.7	17250	2310	13.4
	B	30.1	17250	400	2.3
12 (4)	A	65	12820	660	5.1
	B	47.5	12820	220	1.7

Fig. 1. Changes with age in the activities of liver polyamine-synthesizing enzymes and methionine adenosyl-transferase in male chicks

The activities of ornithine decarboxylase (○), S-adenosyl-L-methionine decarboxylase (●), methionine adenosyl-transferase (■) and spermidine synthase (▲) were assayed as described in the Experimental section. Each point represents the mean of two or three pooled samples, each obtained from two livers. —, Changes in fed chicks; ----, changes in animals starved for 12h.

Nuclei were prepared from 11–12-day-old chick embryos. Ornithine decarboxylase activity in sonicated nuclear preparations varied between 1.7 and 13.4% of the activity found in the supernatant fraction (Table 1). In general, the enzyme activity was considerably higher in preparations made by the method of Chauveau *et al.* (1956). However, the nuclear pellets obtained by this procedure were copious and contained macroscopically visible impurities, e.g. eye pigments. In addition, haematoxylin–eosin staining of smear preparations revealed the presence of whole cells and cell aggregates, e.g. nucleated erythrocytes. The addition of a 2.2M-sucrose 'cushion' under the homogenate resulted in a considerably cleaner nuclear pellet, which still contained some erythrocytes. Similar results with regard to the distribution of ornithine decarboxylase activity were obtained when MgCl₂ was omitted from the homogenization medium or if nuclear suspension instead of sonicate was used as the enzyme. In conclusion, we could not confirm the finding of Snyder *et al.* (1970) that the ornithine decarboxylase activity in whole chick-embryo homogenates is preferentially located in the nuclear fraction.

Effect of age and nutritional state

As shown in Fig. 1, in fed animals the specific activities of liver adenosylmethionine decarboxylase, spermidine synthase and methionine adenosyl-transferase decreased with increasing age, whereas the ornithine decarboxylase activity increased up to the age of 6 weeks and then rapidly declined. The high ornithine decarboxylase activity coincided with the rapid growth period. Starvation for 12h markedly decreased liver ornithine decarboxylase and adenosylmethionine decarboxylase activities,

Fig. 2. Effect of starvation and re-feeding on liver ornithine and S-adenosyl-L-methionine decarboxylase activities in normal and cycloheximide- or actinomycin D-treated male chicks

After 12h starvation the animals aged 4 weeks had free access to food. Before re-feeding, the groups indicated were treated intraperitoneally with 10mg of cycloheximide/kg (black columns) or 2.5mg of actinomycin D/kg (columns shaded with vertical bars) in 0.9% NaCl. Each column represents the mean of three pooled samples, each obtained from two livers. The bars indicate S.E.M. For assay conditions see the Experimental section.

but had no effect on the spermidine synthase and methionine adenosyltransferase activities. These findings agree well with the slow turnover rates of the last two enzymes (see below). Starvation for 12h did not significantly change the total spermidine and spermine contents of the liver (results not shown). The concentration of spermidine decreased from 3 to 11 weeks of age, whereas spermine remained fairly constant. This resulted in a significant decrease with age in the ratio of spermidine to spermine (results not shown), as has also been demonstrated in the rat (Jänne *et al.*, 1964).

The very pronounced effect that the nutritional state of the animal had on the liver ornithine decarboxylase activity is shown in Fig. 2. Overnight starvation decreased the activity of the enzyme to almost zero. Re-feeding sharply increased the enzyme

Fig. 3. Effect of oestradiol on the polyamine and nucleic acid content of chick livers

The animals aged 2 weeks were injected with 50mg of oestradiol benzoate/kg in sesame oil intramuscularly into the legs 0–144h before death. Control animals received the solvent 12h before being killed. The food was removed 12h before death. The symbols Δ , $\blacktriangle$, $\square$ and $\blacksquare$ indicate the contents of spermine, spermidine, RNA-P and DNA-P respectively. Each point represents the mean $\pm$ S.E.M. of four or five livers. Where not shown the S.E.M. bars fall within the symbols. The average liver weight at 0, 12, 24, 48, 96 and 144h after oestradiol was 2.95, 3.26, 3.34, 3.94, 3.61, 4.56 and 3.84g respectively. For assay methods see the Experimental section.

activity. This rise was prevented by cycloheximide and actinomycin D, suggesting that enzyme synthesis *de novo* is needed for the increase. The activity of adenosylmethionine decarboxylase decreased to a smaller extent during the 12h starvation, but re-feeding resulted in an even more rapid rise in the activity compared with ornithine decarboxylase. Also, this rise was abolished by inhibitors of protein and RNA synthesis.

Effect of oestradiol

A single dose of oestradiol caused a significant increase in the liver weight and in the total content of hepatic RNA, spermidine and spermine (Fig. 3). The spermidine content per liver began to increase 12h after the oestradiol treatment and reached its maximum, 230% of the control value, at 96h. These changes were closely paralleled by the accumulation of RNA, which reached its maximum value, 187% of the control, at the same time-point. The spermine content of the total liver was not elevated until 24h and then increased in parallel with spermidine (Fig. 3). The ratio of spermidine to spermine increased from 0.52 to 0.70 during the first 48h

Fig. 4. Effect of oestradiol on the activities of *S*-adenosyl-L-methionine decarboxylase and methionine adenosyltransferase

Assay conditions for *S*-adenosyl-L-methionine decarboxylase (●) and methionine adenosyltransferase (■) were as described in the Experimental section. Each point represents the mean $\pm$ S.E.M. of four or five livers. For methionine adenosyltransferase the S.E.M. bars fall within the symbols. For other details see the legend for Fig. 3.

and then decreased towards the control value. The content of DNA per liver also showed some increase (30 and 17% at 96 and 144h respectively), but this was considerably less than that observed in RNA and polyamines.

As shown above (Fig. 2), the nutritional state of the animal had a marked effect on the activities of ornithine decarboxylase and adenosylmethionine decarboxylases. Oestradiol did not significantly change the activities of these enzymes in fed animals (results not shown). However, in animals starved for 12h, oestradiol treatment significantly increased the adenosylmethionine decarboxylase activity 12h after the injection. The elevated activity returned back to normal during the next 4 days (Fig. 4). In contrast, the activity of methionine adenosyltransferase, the enzyme producing substrate for biological transmethylation reactions and polyamine synthesis, increased slowly. It was significantly elevated at 24h, and reached a maximum (about 140% of the control values) 4 days after the hormone treatment.

The changes in the activities of ornithine decarboxylase and adenosylmethionine decarboxylase in response to oestrogen treatment were studied in greater detail. In 1–2-week-old chicks the liver ornithine decarboxylase activity decreased to almost undetectable values during starvation for 12h (Fig. 1). Oestrogen treatment caused only a small and variable increase in this activity (not shown).

Fig. 5. Changes in the activity of liver *S*-adenosyl-L-methionine decarboxylase after primary and secondary stimulation by oestradiol

Half of the groups received two injections of oestradiol benzoate (50 mg/kg), the first at the age of 10 days and the second at the age of 23 days (secondary stimulation, ----). The other groups were given only one injection at 23 days (primary stimulation, —). All control animals received the oil vehicle 4h before death and half of the groups (secondary stimulation) also at the age of 10 days. The animals were starved for 12h before death. Each point represents the mean $\pm$ S.E.M. of four or five livers. Where not shown the S.E.M. bar falls within the symbol. For assay conditions see the Experimental section.

After 2 weeks of age, a single injection of oestradiol increased the activity of liver ornithine decarboxylase up to twice the control value when measured at 4h. However, because of a large variation between individual animals these results are not shown here. The activity of adenosylmethionine decarboxylase increased rapidly from 2–4h after the hormone injection (Fig. 5). The increase in activity was even more rapid during a secondary stimulation with oestradiol. The apparent half-life of the hepatic adenosylmethionine decarboxylase activity (20–25min) did not differ in fed, starved and starved–oestradiol-treated chicks after cycloheximide treatment (results not shown).

The activities of spermidine synthase, spermine synthase and methionine adenosyltransferase remained essentially unchanged for at least 90min in the livers of cycloheximide-treated animals (Fig. 6). Spermidine synthase and spermine synthase activities did not significantly change within 24h after the oestradiol treatment (results not shown). These differences in the apparent turnover rates of adenosylmethionine decarboxylase and spermidine synthase and spermine synthase, and the changes

Fig. 6. Effect of the administration of cycloheximide on the activities of liver polyamine-synthesizing enzymes and methionine adenosyltransferase in fed chicks

The animals used were 5 weeks old. The points indicating the activity of S-adenosyl-L-methionine decarboxylase (●) or methionine adenosyltransferase (■) represent the mean of six animals. Spermidine synthase (▲) and spermine synthase (△) were determined from two pooled samples, each obtained from three livers. Cycloheximide (10mg/kg) was given intraperitoneally in 0.5ml of 0.9% (w/v) NaCl.

in these enzyme activities after oestradiol treatment, suggest that separate proteins catalyse the decarboxylation of adenosylmethionine and the propylamine transfer (see also Hannonen *et al.*, 1972b).

The earliest change in hepatic enzyme activities after a single dose of oestradiol was the stimulation of the nucleolar RNA polymerase. It was significantly elevated as early as 30min after the administration of the hormone and remained at this elevated value for at least 3 days (Fig. 7a). After the secondary stimulation, however, the RNA polymerase activity did not begin to increase until 2h after the injection, but reached values almost 3 times the control ones in 24h (Fig. 7b). Injection of the vehicle alone does not increase the polymerase I activity at 96h (Van den Berg *et al.*, 1974).

Discussion

The present results show that oestradiol treatment of immature male chicks resulted in a rapid stimulation in the synthesis and accumulation of hepatic polyamines, which paralleled the changes in the ribonucleic acid. Oestrogen treatment also stimulated the activity of liver L-methionine adenosyltransferase. Our results further demonstrate that the activities of hepatic ornithine decarboxylase and

Fig. 7. RNA polymerase activity in isolated nucleoli before and after injection of oestradiol

(a) represents primary stimulation of 2-week-old chicks with 10mg of oestradiol benzoate/kg (given intramuscularly into the legs in 0.5ml of sesame oil). (b) represents secondary stimulation 2 weeks after the primary one. Untreated animals served as controls. Each point indicates the mean $\pm$ S.E.M. of four to six preparations, each derived from two livers. For assay conditions see the Experimental section.

adenosylmethionine decarboxylase are to a large extent controlled by the nutritional state of the animal.

The regulation of the polyamine-synthesizing enzyme activities in the livers of immature male chicks showed some special features. The large and rapid fluctuation of ornithine decarboxylase activity, which depended on the nutritional state of the animal, was most interesting. To what extent this is reflected in the cellular putrescine pool is not known at the present time. However, the content of hepatic spermidine and spermine decreased less than 10% during the 12h starvation. The large variation in the ornithine decarboxylase activity between the individual animals is probably due to differences in the food intake and in the ability to 'store' food in the stomach. Therefore in these young animals it was difficult to evaluate the role of diurnal rhythm in the regulation of ornithine decarboxylase activity. Rapid changes in hepatic ornithine decarboxylase activity in response to starvation and feeding (Schrock *et al.*, 1970; Fausto, 1971; Domschke & Söling, 1973; T. O. Eloranta & A. M. Raina,

unpublished work), and the dependence of the stimulation of the enzyme activity on RNA and protein synthesis in response to feeding (Fausto, 1971), have been demonstrated also in the rat. The latter finding suggests that at least in this case protein synthesis *de novo* is needed for the stimulation of the ornithine decarboxylase activity. Because of the variation between individual animals we could not accurately determine the turnover rate of the ornithine decarboxylase activity. However, in cycloheximide-treated animals it seemed to decline more rapidly than the adenosylmethionine decarboxylase activity. The apparent half-life for the latter enzyme was 20–25 min. This is less than that reported by Hannonen *et al.* (1972a) for the rat liver enzyme (35 min). Changes in the liver adenosylmethionine decarboxylase activity in response to starvation and feeding have also been observed in the rat (Domschke & Söling, 1973). On the other hand, little has been known about the effect of the nutritional state on the activity of hepatic spermidine synthase.

The report by Snyder *et al.* (1970) showing that about 50% of the total ornithine decarboxylase activity of the chick-embryo homogenate resides in the nuclear fraction has raised some discussion about the role of nuclei as a site for polyamine synthesis (see Tabor & Tabor, 1972). The present results demonstrate that the original method of Chauveau *et al.* (1956) is not suitable for the preparation of nuclei from this particular source. Our results suggest that, as in other sources, in chick embryos also the ornithine decarboxylase activity is largely if not exclusively located in the cytosol fraction.

Surprisingly little is known about the relationship between polyamine and adenosylmethionine synthesis, whereas a number of papers have described changes in the activities of polyamine-synthesizing enzyme in response to various hormonal and growth stimuli (see Bachrach, 1973). The slow and relatively small increase in the methionine adenosyltransferase activity after oestradiol treatment suggests that the activity of this enzyme is not rate-limiting during the early accumulation of hepatic spermidine after the hormone treatment.

Oestradiol treatment markedly increased the accumulation of liver polyamines and RNA in immature male chicks. The accumulation of RNA has been observed by other workers (Jost *et al.*, 1973; Van den Berg *et al.*, 1974). The stimulation of the adenosylmethionine decarboxylase activity was observed at 4 h after the administration of the hormone. This was preceded by a rise in the activity of nucleolar RNA polymerase. In some other target tissues the effects of oestrogens on macromolecular synthesis have been well documented [for a review, see O'Malley & Means (1974)]. These studies have

indicated that at least in rat and calf uterus and in chick oviduct the activities of RNA polymerases I and II are increased after oestradiol administration *in vivo* and also *in vitro*. In chick oviduct, a concomitant increase in polymerases I and II was first demonstrable at about 6 h after oestrogen administration (see O'Malley & Means, 1974). Our results indicate that in chick liver the activity of polymerase I was maximally stimulated already at 30 min after the administration of oestradiol. This is somewhat similar to the stimulation of polymerase I observed in rat uteri at 1 h after the administration of the hormone (see O'Malley & Means, 1974).

An early stimulation of the methylation of uterine tRNA and rRNA has also been observed after the administration of oestradiol to immature rats (Munns & Katzman, 1971).

Taken together, our results demonstrate that in immature male chicks a single injection of oestradiol results in a rapid stimulation of hepatic nucleolar RNA synthesis followed by a stimulation in the synthesis and accumulation of polyamines. These changes are probably important for the synthesis of specific yolk proteins (e.g. phosvitin) induced by the hormone treatment.

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