*J. Mol. Biol.* (1976) **100**, 115–126

# Co-ordinate Synthesis of Ribosomes and Elongation Factors in the Liver of Immature Chicks following a Metabolic Shift Up

R. Lane Smith†, Oswald Baca‡ and Julian Gordon

*Friedrich Miescher-Institut*
*P.O. Box 273*
*CH-4002 Basel, Switzerland*

*(Received 7 July 1975)*

Treatment of immature chicks with estradiol results in the induction of egg yolk protein synthesis in the liver (Heald & McLachlan, 1963,1965). There is a twofold increase in newly synthesized messenger RNA associated with polysomes within the first six hours and an increase in total liver weight, RNA, and protein within 48 hours (Jost *et al.*, 1973).

Conditions have been established for reliable and quantitative determination of elongation factor 1, elongation factor 2 and ribosomal RNA in the livers of immature chicks, in order to study the quantitative relationship between these components of the protein synthesizing machinery during the "shift-up" situation following estrogen administration. Factor activity of high salt extracts was determined from initial rates of polyphenylalanine synthesis in the presence of excess complementary factor. Direct quantitation of EF-2§ was achieved by incorporation of [$^{14}$C]ADP-ribose from [$^{14}$C]NAD in the presence of diphtheria toxin. The reaction conditions were shown to give specific transfer of [$^{14}$C]ADP-ribose to EF-2 by analysis on gels containing sodium dodecyl sulfate. The fractionated [$^{14}$C]ADPrEF-2 was used to quantitate EF-2. The amounts of 18 S and 28 S rRNA were used as a measure of the ribosome content of the treated liver. Post-nuclear supernatant fractions were prepared in the presence of diethyl pyrocarbonate and Triton X100 and fractionated on sodium dodecyl sulfate/sucrose gradients. These conditions were shown to give quantitative determination of 18 S and 28 S rRNA. Analyzed in the above manner, the levels of EF-1, EF-2 and ribosomes were found to increase co-ordinately following estrogen administration, although EF-1 activity was shown to be rate-limiting for polyphenylalanine synthesis. The stoichiometry of EF-2 : ribosome was maintained at unity. However, the ratio of 18 S rRNA : 28 S rRNA was found not to be unity, but 1·5 : 1.

## 1. Introduction

The question of co-ordinate control of the synthesis of elongation factors and ribosomes has been the subject of a few studies in prokaryotes (Gordon, 1970; Carpenter & Sells, 1973) and in eukaryotes (Gill & Dinius, 1973; Alexis *et al.*, 1974). In prokaryotes it was earlier shown that the elongation factor content of cells has a constant

† Present address: Stanford University, School of Medicine, Department of Pediatrics, Stanford, Calif. 94035, U.S.A.

‡ Present address: The University of Minnesota, Department of Biochemistry, 227 Millard Hall, Minneapolis, Minn. 55455, U.S.A.

§ Abbreviations used: EF-2, elongation factor 2; ADPrEF-2, ADP-ribosylated EF-2; EF-1, elongation factor 1.

relation to the ribosome content in a variety of steady-state growth conditions (Gordon, 1970). More recently, elongation factor Tu and G content has been shown to parallel the burst of ribosomal RNA synthesis during shift-up growth conditions (Carpenter & Sells, 1973). In eukaryotes, Gill & Dinius (1973) have shown that the EF-2† content of a variety of cells and tissues in steady state bore a constant relation to their ribosome content. It is the purpose of this paper to present information on the regulation of synthesis of elongation factors and ribosomes during a shift-up situation in a eukaryotic system.

The system selected was the induction of the egg yolk proteins in the liver of immature chicks following treatment with estradiol (Heald & McLachlan, 1963,1965). During primary estrogen stimulation, there is a twofold increase in newly synthesized mRNA associated with polysomes within the first six hours, and also an increase in total liver weight, RNA and protein within the first 48 hours (Jost *et al.*, 1973).

In other systems, the elaboration of specific proteins in response to steroid hormones has been shown to be accompanied by increased synthesis of ribosomal RNA (Luck & Hamilton, 1972) and increased rates of protein synthesis (Means *et al.*, 1971; Palmiter, 1972*a*,*b*). In addition, there is evidence that soluble factor activities are enhanced following treatment with hormone (Clemens & Tata, 1973; Whelly & Barker, 1974).

This paper reports that following estrogen treatment of immature chicks there is a parallel increase of elongation factor content with increased ribosomal RNA content. These data suggest a co-ordinate control of the synthesis of these components. In addition, as suggested in other systems (Willis & Starr, 1971; Girgis & Nicholls, 1972), the data also show that one of the elongation factors, EF-1, is in rate-limiting amounts when assayed *in vitro* using synthetic messenger.

## 2. Experimental Procedure

### (a) *Materials*

[$^{14}$C]phenylalanine (spec. act. 513 Ci/mol) and [$^{14}$C]NAD (spec. act. 253 Ci/mol) were purchased from the Radiochemical Centre, Amersham, England. Poly(U) was purchased from Miles. GTP, sodium dodecyl sulfate and Triton X100 were from Serva. 17 $\beta$-estradiol was from Ciba–Geigy Limited, Basel, Switzerland. *Escherichia coli* stripped tRNA was purchased from General Biochemicals. Diethyl pyrocarbonate and 2-deoxy-D-ribose were from Fluka. NCS was from Amersham/Searle. Diphtheria toxin was obtained either as a gift from Dr A. Kaziro (pure crystalline toxin) or from Schweizerisches Serum- und Impfinstitut, Bern, Switzerland (crude toxin).

### (b) *Methods*

(i) *Hormone injection*

17 $\beta$-estradiol was administered to white Leghorn chicks (200 g $\pm$ 20 g) which were raised locally and fed a standard diet. The dosage was 50 mg hormone/kg body weight and the hormone was dissolved in propylene glycol at a concn of 50 mg/ml. Controls were injected with propylene glycol alone. All chicks were the same age when killed.

(ii) *Preparation of tissue*

Chicks were killed by decapitation following 12 h food deprivation. The livers were excised immediately and placed in ice. The total weight of groups of 4 livers was determined. The livers were combined and minced thoroughly. Each sample was then divided

† See footnote on page 115.

into 2 equal amounts. One part was taken for determination of rRNA and the other part for determination of elongation factor content.

(iii) *Preparation of extracts for elongation factor determinations*

Extraction of elongation factors was carried out with the use of a high salt buffer. These conditions have been shown by Gill & Dinius (1973) to yield complete extraction of the factors from the particulate fractions in homogenates. The minced liver tissue (6g) was homogenized in a Braun Teflon/glass homogenizer with a motor-driven pestle at 1500 rpm and with 7 strokes. The homogenization buffer was 0·05 M-Tris·HCl (pH 7·4), 0·5 M-KCl, 0·004 M-$MgCl_2$, 0·0001 M-$Na_2EDTA$, 0·002 M-dithiothreitol and 0·25 M-sucrose. The volume of homogenization buffer to weight of liver was 10:1. The homogenate was centrifuged at 100,000 ***g*** for 5 h. The upper layer of lipid material was removed and the supernatant solution was collected. A sample of the supernatant solution was dialyzed overnight against 100 vols of a low salt buffer, 0·05 M-Tris·HCl (pH 7·4), 0·025 M-KCl, 0·0002 M-$Na_2EDTA$, 0·002 M-dithiothreitol and 10% glycerol. After dialysis the samples were clarified by centrifugation at 10,000 ***g*** for 10 min and assayed immediately.

(iv) *Quantitation of elongation factor content by synthesis of polyphenylalanine*

The activities of both elongation factors were determined by synthesis of poly(Phe) in a poly(U)-dependent system with complementation of the respective factors. The assay was carried out in 0·1-ml reaction mixtures containing 0·05 M-Tris·HCl (pH 7·4), 0·08 M-$NH_4Cl$, 0·01 M-$MgCl_2$, 0·01 M-dithiothreitol, 0·001 M-GTP, 100 $\mu$g poly(U), 40 $\mu$g *E. coli* [$^{14}$C]Phe-tRNA (14 pmol of [$^{14}$C]Phe), 40 $\mu$g chicken liver ribosomes and the required amount of EF-1 and EF-2. All reactions were carried out at 37°C. The reactions were started by addition of poly(U), GTP and [$^{14}$C]Phe-tRNA as a single mixture. Time points were taken and initial rates determined. The reaction was stopped by the addition of 2 ml 5% trichloroacetic acid and the mixture incubated at 90°C for 10 min. Samples were collected on Millipore filters and washed with 10 ml 5% trichloroacetic acid. After drying the filters, radioactivity was determined as reported earlier (Smith & Gordon, 1974). The efficiency for $^{14}$C was 80 %.

Chicken liver ribosomes were prepared as previously described (Smith & Gordon, 1974), except isokinetic gradients were used. In the absence of factors, the ribosomes alone support the synthesis of 0·006 units of poly(Phe). The units of EF-1 or EF-2 activity are defined as pmol of poly(Phe) synthesized/min of assay in the presence of an excess of the complementary factor. EF-1 or EF-2, when present in saturating amount (1·4 units/assay), contributed background values of 0·009 and 0·007 units, respectively, in the absence of the complementary factor. EF-1 was prepared from chicken liver, 100,000 ***g*** supernatant solution by precipitation with $(NH_4)_2SO_4$ and chromatography on Sephadex G200 and hydroxylapatite (McKeehan & Hardesty, 1969). EF-2 was prepared to near homogeneity in a modified procedure based on that of Honjo *et al.* (1971) (Smith & Gordon, manuscript in preparation). [$^{14}$C]Phe-tRNA was prepared by the method of Conway (1964).

(v) *Quantitation of EF-2 by ADP-ribosylation*

EF-2 was measured by ADP-ribosylation in the presence of diphtheria toxin using reaction conditions as described by Gill & Dinius (1973). Each (0·1-ml) reaction mixture contained 0·2 M-histamine·HCl (pH 7·8), 2 to 5 $\mu$l of the extract prepared as above and excess diphtheria toxin either pure or as an $(NH_4)_2SO_4$ fraction prepared according to Gill & Dinius (1973). After 5 min pre-incubation at 37°C, the reaction was initiated by addition of [$^{14}$C]NAD. The radioactively labeled protein was then precipitated with 5% trichloroacetic acid. The precipitate was centrifuged, dissolved in 50 $\mu$l 0·1% sodium dodecyl sulfate, 0·1 M-dithiothreitol, 0·04 M-Tris·OH (just enough to neutralize the residual trichloroacetic acid) (Howard *et al.*, 1975) and fractionated with a discontinuous sodium dodecyl sulfate/acrylamide gel system (Neville, 1971). After running the bromphenol blue dye 9 cm from the top of the gels, they were fractionated into 1-mm slices with a Gilson Automatic Aliquogel fractionator. Each slice, homogenized in 0·9 ml water, was digested overnight at 30°C with 0·5 ml NCS in capped vials. The radioactivity of the gel

was then determined using 15 ml of Bray's scintillation mixture (Bray, 1960) and counting in a Nuclear Chicago Isocap 300. The counting efficiency was determined by a parallel analysis in a combustion system (Oxymat: Intertechnique). Both systems yield identical (75%) efficiencies. Counts corresponding to [$^{14}$C]ADPrEF-2 were integrated.

(vi) *Preparation of post-nuclear extracts for ribosomal RNA determinations*

Post-nuclear supernatant fractions were prepared as described by Venkatesan & Steele (1972) with the use of a combination of Triton X100 to free the nuclei of rough endoplasmic reticulum and diethyl pyrocarbonate to prevent ribonuclease activity. The minced tissue was homogenized as described above, but with 2·5 vols 0·3 M-Tris·HCl (pH 7·4), 0·025 M-KCl, 0·005 M-$MgCl_2$ and 0·25 M-sucrose. The buffer was made 0·5% (v/v) with respect to diethyl pyrocarbonate immediately prior to use. After homogenization, 2·5 vols of the same buffer was added followed by the addition of 5 vols of the above buffer, but containing 2% Triton X100. The homogenate was then centrifuged immediately at 3000 rpm in the Sorvall SS34 rotor for 5 min. The resulting post-nuclear supernatant liquid was removed and stored frozen in liquid $N_2$ prior to analysis of the rRNA content on sucrose gradients.

(vii) *Quantitation of ribosomal RNA*

Direct quantitation of rRNA in the post-nuclear supernatant solutions was by spectrophotometric determination at 260 nm following fractionation of the RNA on sodium dodecyl sulfate/sucrose gradients. The gradient buffer was 0·02 M-sodium acetate, 0·005 M-$Na_2EDTA$ and 0·5% sodium dodecyl sulfate (final pH 4·9). Convex exponential sucrose gradients were prepared from buffered sucrose (5% to 20%; w/v) with an 8-ml constant mixing volume for six 4-ml gradients. The gradient sample contained 5 to 10 $\mu$l post-nuclear supernatant solution made to 100 $\mu$l in 0·5% sodium dodecyl sulfate. Gradients were centrifuged for 2·5 h at 16°C and at 56,000 rpm in the Beckman SW56 rotor and were analyzed with the system of Noll (1969) on a Varian 3400 spectrophotometer. The rRNA content was determined by integration of the areas under the respective peaks of 18 S and 28 S RNA from the $A_{260}$ profile. Quantitation was based on RNA molecular weights of $0{\cdot}70 \times 10^6$ and $1{\cdot}58 \times 10^6$ for 18 S and 28 S, respectively (Loening, 1968) and specific absorbances as determined below.

(viii) *Determination of specific absorbancy of RNA*

To determine the specific absorbancy for the 18 S and 28 S rRNA approximately 600 $A_{260}$ units of RNA were fractionated into 18 S and 28 S fractions by sucrose gradients scaled up (Beckman SW27 rotor) from the analytical gradients described above. This material was collected separately, precipitated with ethanol, washed with ethanol, ethanol/ether and then exhaustively dried and weighed. A sample of the material was dissolved in water for spectrophotometric analyses. The total P was determined by the analytical department of Ciba-Geigy Limited. The specific absorbancy was calculated from the measured P content and the nucleotide molecular weights and base composition data from Baca *et al.* (1973).

## 3. Results

Before analysis of the possible co-ordinate control of the synthesis of EF-1 and EF-2 and ribosomes, it is necessary to assess critically the analytical procedures used.

### (a) *Measurement of ribosomal RNA*

The aim was to achieve the simplest and yet most direct and quantitative procedure for determining the absolute amounts of rRNA in chick liver. A procedure has been published (Venkatesan & Steele, 1972) which circumvents the two major difficulties in ribosome quantitation: (a) RNAase degradation and (b) losses due to sedimentation of the rough microsomes with nuclei. Ribonuclease activity was

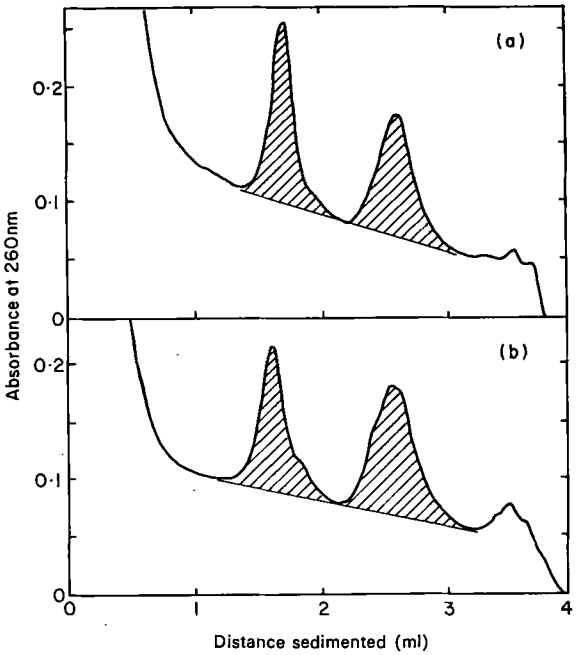


FIG. 1. Comparison of the sodium dodecyl sulfate/sucrose gradient profiles of (a) a post-nuclear supernatant fraction prepared and analyzed as described in Experimental Procedures and (b) the total homogenate from which it was derived. Equivalent volumes were applied to each gradient.

prevented by addition of diethyl pyrocarbonate and the membranes were freed from the nuclei by limited treatment with Triton X100.

We have taken the post-nuclear supernatant fraction from this procedure, treated it with sodium dodecyl sulfate and analyzed it directly on an analytical sodium dodecyl sulfate/sucrose gradient. The 18 S and 28 S rRNA were then quantitated directly from the gradient. A representative gradient is shown in Figure 1(a).

TABLE 1

*Recovery of ribosomal RNA on sucrose gradients*

| Fraction analyzed | Total RNA applied | RNA recovered as 18 S | RNA recovered as 28 S |
|---|---|---|---|
| Homogenate | 17·4 | 3·95 | 5·27 |
| Total RNA from homogenate | | 3·46 | 4·66 |
| Post-nuclear supernatant | 16·2 | 4·22 | 5·18 |
| Total RNA from post-nuclear supernatant | | 3·36 | 3·66 |

An homogenate and a post-nuclear supernatant were prepared as described in Experimental Procedures as for rRNA analysis. Total RNA was extracted from samples of both by phenol/sodium dodecyl sulfate, and then homogenate, post-nuclear supernatant fraction and the total RNA derived from them were analyzed for their content of 18 S and 28 S RNA as described in Experimental Procedures. Total RNA applied and RNA recovered as 18 S and 28 S were all expressed as $A_{260}$ units/ml of original fraction.

We have extensively evaluated the recovery of rRNA under these conditions, and the evaluation is given in Figure 1 and Table 1. Even when the total homogenate was applied to a sucrose gradient containing sodium dodecyl sulfate, the profile obtained was indistinguishable from that obtained with the above standard procedure, where the post-nuclear supernatant fraction was used (compare Fig. 1(a) and (b)). Table 1 shows the integrated data from Figure 1. The Table also includes data integrated from gradients where the material was first deproteinized by extraction with phenol. The integrated 18 S and 28 S rRNA values were reduced 20% by the extraction. This difference is fully accounted for by the loss during extraction with phenol, and is not due to the removal of interfering substances from the gradient. This is shown by the comparison of the phenol procedure and the Fleck & Munro (1962) method for total RNA analysis (Fig. 2). Thus, the sodium dodecyl sulfate/sucrose gradient procedure yields data which is a simple direct quantitation of the ribosome content of the original material. The combined 18 S and 28 S rRNA values only came to 50% of the total RNA applied to the gradients (Table 1).

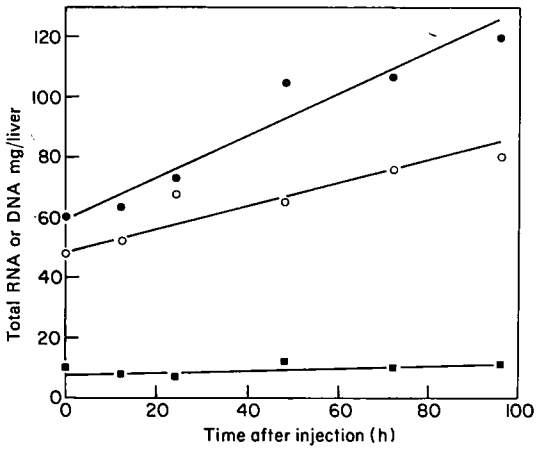


FIG. 2. Total RNA and DNA determinations on livers at different times following injection of estradiol. The total RNA was determined either by the procedure of Fleck & Munro (1962) (—●—●—) or as material absorbing at 260 nm following extraction with phenol (—○—○—) as described by Baca & Paretsky (1974). Total DNA (—■—■—) was determined by the method of Burton (1956). These determinations were on homogenates prepared as described in preparation for determination of rRNA in Experimental Procedures.

Figure 2 also shows the DNA determinations on a series of homogenates and post-nuclear supernatant fractions. These post-nuclear supernatant fractions contain about 10% of the total cellular DNA (data not shown). From the absorbancy, this amount of DNA could not contribute more than a 5% error to the rRNA measured by absorbancy on the gradients.

One other possible source of error is the treatment with diethyl pyrocarbonate. It has been reported that treatment with diethyl pyrocarbonate modifies the spectral properties of RNA (Solymosy *et al.*, 1971). We have verified the specific absorbancy for the 18 S and 28 S rRNA separately as described in Experimental Procedures. Our determinations yielded a value of 27·5 $A_{260}$ units/mg of RNA for 18 S RNA and

28·8 $A_{260}$ units/mg of RNA for the 28 S RNA. In addition, the spectrum was not significantly different from RNA extracted without diethyl pyrocarbonate treatment (data not shown).

(b) *Analysis of EF-1 and EF-2 polyphenylalanine activity*

As one measure of elongation factor content, activity values for the two factors were determined using a poly(U)-dependent protein synthesizing system. While the assay cannot inherently yield absolute quantities, we felt it would be a valuable complement to the other method for measuring relative amounts. In addition, the assay, when used with crude systems, is susceptible to various inhibitory activities. However, the measurement of the initial rates and verification of the linearity with respect to the factor which is limiting, minimizes the difficulties. Figure 3(a) shows the response to varying amounts of factor extract over an eight minute period. At

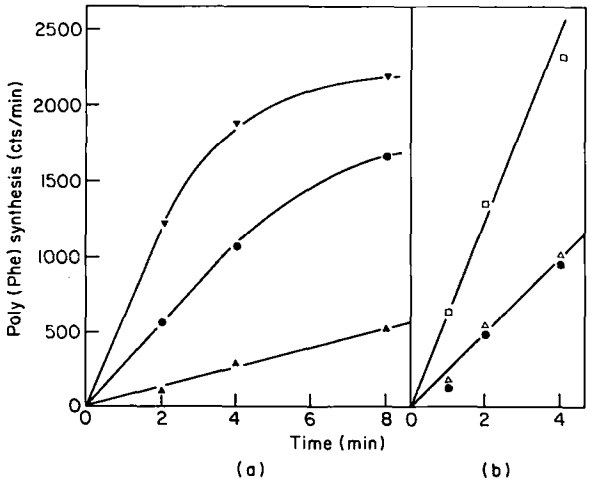


FIG. 3. Time-course for poly(Phe) assays. In (a), either 4 $\mu$l (—▲—▲—), 8 $\mu$l (—●—●—) or 16 $\mu$l (—▲—▲—) of the factor extract prepared as described in Experimental Procedures, were added to a reaction mixture, and 20-$\mu$l samples taken at the times indicated. For EF-1 and EF-2 assay. In (b), the 8 $\mu$l of factor extract was assayed alone (—●—●—) or in the presence of saturating amounts of EF-1 (—□—□—) or EF-2 (—△—△—).

higher amounts of factor extract added, there was a linear response. The lowest amount, however, gave a non-linear response. It has been suggested that this is due to dilution of factors on the ribosomes (Collins *et al.*, 1972). Similar curves were obtained for each of the extracts from the different stages of treatment with estrogen. Figure 3(b) shows the typical response obtained with a factor extract at a concentration which was linear with respect to amount, and assayed with the addition of either EF-1 or EF-2. The response was linear with respect to time and no lag was detectable. The relative amount of factor was determined from the slopes of the linear portion of similar curves obtained for each extract. It is evident from Figure 3(b) that addition of EF-2 gave no significant increase in poly(Phe) synthesis, whereas addition of EF-1 resulted in a significant stimulation. Thus EF-1 is the rate-limiting component under these assay conditions. The data obtained for factor specific activity after different times of treatment with estrogen are summarized in Figure 4.

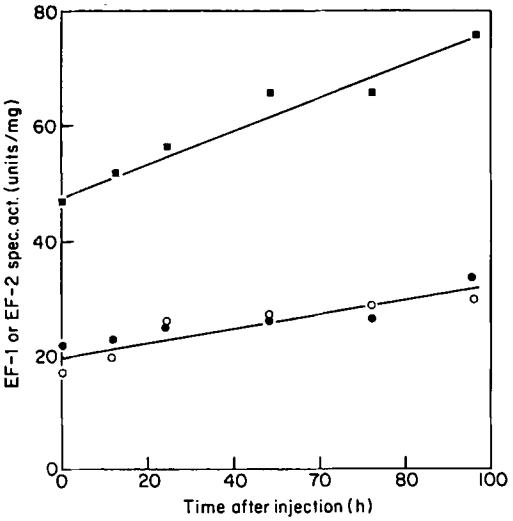


FIG. 4. Spec. act. measurements of elongation factor content from livers at various times following injection of estrogen. The spec. act. were expressed as units/mg of factor extract protein as described in Experimental Procedures, with initial rates determined over 4-min time courses, for factor extract alone (--○—○—) or with the addition of saturating amounts of EF-1 (—■—■—) or EF-2 (—●--●—)

Further confirmation of the validity of the assay system came from a comparison of the activities in the poly(Phe) system with the ADP-ribosylation of EF-2. If there are no significant interfering activities with either assay system as a measure of EF-2, then they will bear a constant ratio to each other. This was found to be the case (see below). Further support for this comes from the observation that there is a constant ratio of these activities found throughout purification of EF-2 (Smith & Gordon, manuscript in preparation).

### (c) *Analysis of EF-2 ADP-ribosylation*

Gill & Dinius (1973) have extensively analyzed possible sources of error in the determination of EF-2 by ADP-ribosylation in the presence of diphtheria toxin. They showed that the addition of histamine to the reaction selectively inhibits the formation of poly(ADP-ribose) by an enzyme of nuclear origin, and the histamine inhibition of the ADP-ribosylation of EF-2 can be overcome by the use of high levels of NAD and toxin. We have confirmed this and, in addition, we have slightly modified the sample preparation procedure to maximize the yield of EF-2 as measured by the poly(Phe) assay. This involved the addition of a sulfhydryl protection agent to the homogenization buffer and the elimination of the Norite adsorption step to remove endogenous NAD which would otherwise give isotope dilution. The Norite (Norite A, acid-washed, from Serva) appeared to remove some of the EF-2. For this we substituted dialysis and the [$^{14}$C]NAD was present in amounts where no isotope dilution was detectable even with undialyzed extract. Under our conditions a background of hot trichloroacetic acid-insoluble material was detectable in the absence of diphtheria toxin. This background was highly variable, but even when maximally present, was not apparent on gel electrophoresis in the presence of sodium dodecyl sulfate

presumably due to extreme heterogeneity. This is shown in Figure 5. In separate experiments (not shown) the stained band with pure EF-2 and the radioactivity of the pure [$^{14}$C]ADPrEF-2 all had the same mobility as the labeled crude material shown in Figure 5. The analysis by sodium dodecyl sulfate gels shows that no other protein in the S100 fraction, apart from EF-2, acts as a substrate for the diphtheria toxin ADP-ribosylation reaction under these conditions.

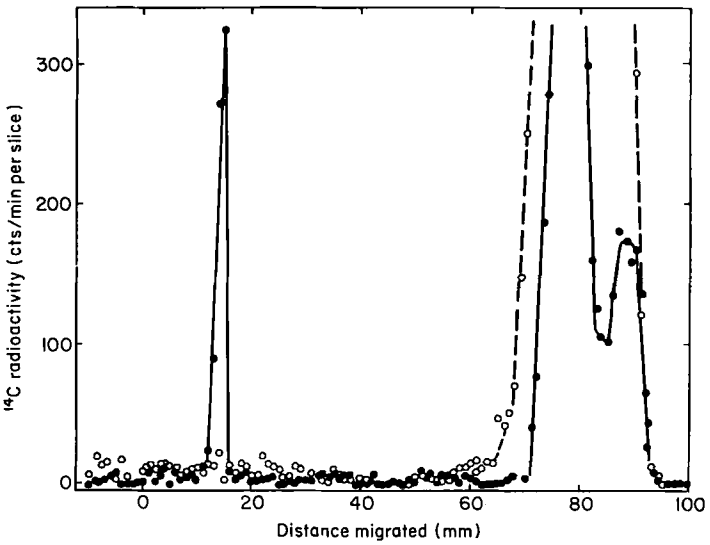


FIG. 5. Sodium dodecyl sulfate/polyacrylamide electrophoresis of [$^{14}$C]ADPrEF-2. The factor extract was incubated and analyzed as described in Experimental Procedures (—●—●—). In a control sample, diphtheria toxin was omitted (—○—○—).

### (d) *Response of ribosomal RNA, EF-1 and EF-2 to treatment with estrogen*

The analytical procedures described above were all applied to extracts obtained from chicks which had been treated with estrogen for varying times. In order to facilitate direct comparison of the relative increases in all parameters, they are expressed in total units per liver, and then as a fold increase in the units per liver over the non-estrogen-treated controls. These results are summarized in Figure 6. It can be seen that there is approximately a twofold net increase in EF-1, EF-2 and rRNA per liver. These increases are not significantly different from each other kinetically or quantitatively and there is no significant difference between the findings from the poly(Phe) and ADP-ribosylation assays.

Since the ADP-ribosylation assay for EF-2 and the determinations of ribosomal RNA yield absolute units, this affords an opportunity to determine the molar ratio in which they are present as well as the co-ordinacy of their synthesis with respect to time. The relevant data from Figure 6 was recalculated in those terms and is plotted in Figure 7. It can be seen that there is no significant change in the molar ratio following treatment with estrogen and that the molar ratio of EF-2 to ribosomal RNA is maintained at a value close to unity.

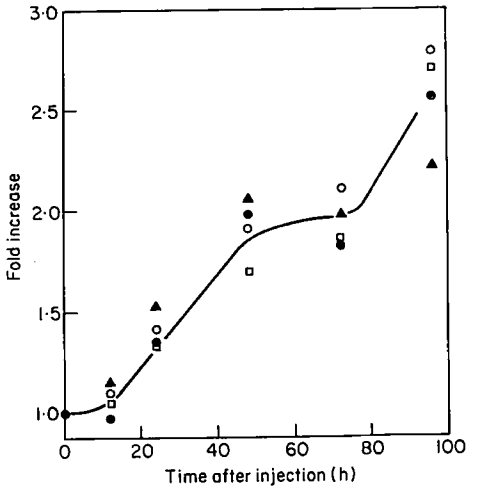


FIG. 6. Net increase in various parameters from livers at various times following injection of estradiol. Values for rRNA (—▲—▲—), ADPrEF-2 (—●—●—), poly(Phe) (EF-1 —□—□—; EF-2 —○—○—) were determined as described in Experimental Procedures, and expressed as fold of increase in total amount per liver.

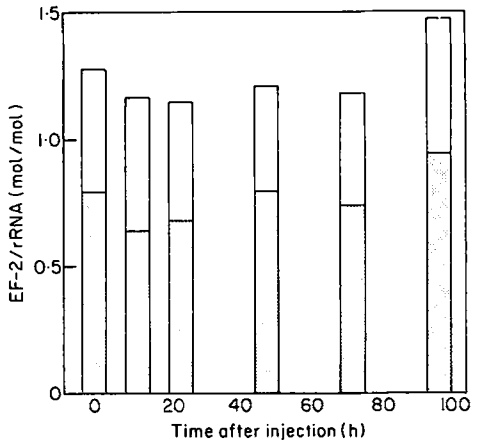


FIG. 7. The stoichiometry of EF-2: rRNA at various times following injection of estradiol. The ratios of pmol ADPrEF-2 to pmol either 28 S (open) or 18 S (hatched) rRNA were calculated from the same experiment as in Fig. 6.

## 4. Discussion

The purpose of this investigation was to quantitate the elongation factor content of the immature chick liver under conditions analogous to a shift up in bacteria. Following a single administration of estradiol, the synthesis of the egg yolk proteins phosvitin (Jost *et al.*, 1973) and lipovitellin (Jost *et al.*, 1975) is stimulated and egg yolk proteins become a major product. In addition, there is a sizeable stimulation of synthesis of a variety of macromolecular species, reflected by increases in total RNA, protein and liver mass (Jost *et al.*, 1973). It is often assumed in the literature

(e.g. Gill & Dinius, 1973) that total RNA is a reflection of ribosomal RNA and that rRNA represents 80% of the total. We have investigated this assumption here and found that, at least for this system, this value is somewhat high (Table 1), but that the increase in total RNA observed following treatment with estrogen reflects partly a net synthesis of rRNA.

In the measurements of EF-2 levels with diphtheria toxin, we have also tested a widely made assumption, namely that EF-2 is the only protein which is ADP-ribosylated in the presence of diphtheria toxin (Pappenheimer & Gill, 1973). It has never been rigorously proven that the toxin is absolutely specific and that no other protein acts as an acceptor for ADP-ribose. We have shown here that the only protein in a crude extract which functions as an acceptor in the presence of the toxin has the electrophoretic mobility of EF-2 on gel electrophoresis in the presence of sodium dodecyl sulfate (Fig. 3). The assumption is therefore valid.

We have adopted the methodology, described in this report, which avoids the assumptions that are often implicitly made, and found that the ratio of EF-2 to ribosomes is 1:1. It has been observed that a constant and approximately unit ratio of EF-2:ribosomes is preserved in a variety of tissues and organs (Gill & Dinius, 1973) and under dietary protein restriction (Alexis *et al.*, 1974). With more rigorous methodology, we find the constant unit ratio is preserved during the step up from untreated chick liver to that which has been stimulated for the massive production of egg yolk proteins.

We have also made parallel measurements of EF-1 and EF-2 by activity in the poly(U) system, and found that both increase co-ordinately. The absence of absolute measurements of EF-1 prevents any conclusions about absolute cellular concentrations of EF-1, but the co-ordinacy of synthesis suggests that the cell is regulated in such a way that the amounts of elongation factors are never rate-limiting. Although EF-1 is rate-limiting in the *in vitro* assays (Table 3, Willis & Starr, 1971; Girgis & Nicholls, 1972), this may not necessarily reflect the kinetic situation *in vivo*. The increased specific activities following treatment with estrogen (Fig. 4) are therefore a response to an increased demand for overall protein synthetic capacity and do not reflect any special regulatory role, as is sometimes implied (see review, Pain & Clemens, 1973). However, the phenomenon does imply an as yet not understood coupling of the transcription of rRNA genes with the transcription and/or translation of mRNA for the synthesis of elongation factors.

It is normally assumed that the origin of 18 S and 28 S rRNA from a common large transcription product implies that the two species are present in equimolar amounts in the cell. It can be seen from Figure 7 that there is apparently a 1·5-fold excess of 18 S rRNA throughout the period of treatment with estrogen. This finding may possibly be accounted for by different rates of turnover of the two species, which have hitherto been defined as metabolically stable. A shorter half-life has been reported for 28 S rRNA in resting 3T3 cells (Abelson *et al.*, 1974; Kolodny, 1975). Nothing is known about the metabolic stability of rRNA in an organ, which may in fact resemble contact-inhibited cells. It is also not clear whether the excess 18 S rRNA represents authentic small subunit RNA or whether it represents a pool of the large subunit RNA in the process of being degraded. Nevertheless, the conclusion remains that if one considers the average between the 18 S and the 28 S contents as the true ribosome content, then the average ratio of EF-2:ribosomes is 1:1 throughout the drastic metabolic change due to estrogenization.

We are extremely grateful to Dr Frank Solomon for helpful suggestions. We thank Drs Y. Kaziro and Y. Nishizuka for the gift of purified diphtheria toxin, Dr G. Howard for critical reading of the manuscript and Mr G. Parzyjagla and Mr H. P. Ramjoué for their excellent technical assistance.

## REFERENCES

Abelson, H. T., Johnson, L. F., Penman, S. & Green, H. (1974). *Cell*, **1**, 161–165.
Alexis, S. D., Young, V. R. & Gill, D. M. (1974). *Biochem. J.* **142**, 185–188.
Baca, O. G. & Paretsky, D. (1974). *Infect. Immunity*, **9**, 939–945.
Baca, O. G., Hersh, R. T. & Paretsky, D. (1973). *J. Bacteriol.* **116**, 441–446.
Bray, G. A. (1960). *Anal. Biochem.* **1**, 279–280.
Burton, K. (1956). *Biochem. J.* **62**, 315–323.
Carpenter, G. & Sells, B. H. (1973). *FEBS Letters*, **35**, 31–35.
Clemens, M. J. & Tata, J. R. (1973). *Eur. J. Biochem.* **33**, 71–80.
Collins, J. F., Moon, H. M. & Maxwell, E. S. (1972). *Biochemistry*, **11**, 4187–4194.
Conway, T. W. (1964). *Proc. Nat. Acad. Sci., U.S.A.* **51**, 1216–1220.
Fleck, A. & Munro, H. N. (1962). *Biochim. Biophys. Acta*, **55**, 571–583.
Gill, D. M. & Dinius, L. L. (1973). *J. Biol. Chem.* **248**, 654–658.
Girgis, G. R. & Nicholls, D. M. (1972). *Biochim. Biophys. Acta*, **269**, 465–476.
Gordon, J. (1970). *Biochemistry*, **9**, 912–917.
Heald, P. S. & McLachlan, P. M. (1963). *Biochem. J.* **87**, 571–576.
Heald, P. S. & McLachlan, P. M. (1965). *Biochem. J.* **94**, 32–39.
Honjo, T., Nishizuka, Y., Kato, I. & Hayaishi, O. (1971). *J. Biol. Chem.* **246**, 4251–4260.
Howard, G. A., Smith, R. L. & Gordon, J. (1975). *Anal. Biochem.* **67**, 110–114.
Jost, J. P., Keller, R. & Dierks-Ventling, C. (1973). *J. Biol. Chem.* **248**, 5262–5266.
Jost, J. P., Pehling, G. & Baca, O. G. (1975). *Biochem. Biophys. Res. Commun.* **62**, 957–965.
Kolodny, G. M (1975). *Exp. Cell Res.* **91**, 101–106.
Loening, U. E. (1968). *J. Mol. Biol.* **38**, 355–365.
Luck, D. N. & Hamilton, T. H. (1972). *Proc. Nat. Acad. Sci., U.S.A.* **69**, 157–161.
McKeehan, W. L. & Hardesty, B. (1969). *J. Biol. Chem.* **244**, 4330–4339.
Means, A. R., Abrass, I. B. & O'Malley, B. W. (1971). *Biochemistry*, **10**, 1561–1569.
Neville, D. M. Jr (1971). *J. Biol. Chem.* **246**, 6328–6334.
Noll, H. (1969). In *Techniques in Protein Biosynthesis* (Sargent, J. & Campbell, P. W., eds), vol. 2, pp. 101–179, Academic Press, London.
Pain, V. M. & Clemens, M. J. (1973). *FEBS Letters*, **32**, 205–212.
Palmiter, R. D. (1972*a*). *J. Biol. Chem.* **247**, 6450–6461.
Palmiter, R. D. (1972*b*). *J. Biol. Chem.* **247**, 6770–6780.
Pappenheimer, A. M. Jr & Gill, D. M. (1973). *Science*, **182**, 353–358.
Smith, R. L. & Gordon, J. (1974). *FEBS Letters*, **43**, 223–226.
Solymosy, F., Hüvös, P., Gulyás, A., Kapovits, I., Gaál, Ö., Bagi, G. & Farkas, G. L. (1971). *Biochim. Biophys. Acta*, **238**, 406–416.
Venkatesan, N. & Steele, W. J. (1972). *Biochim. Biophys. Acta*, **287**, 526–537.
Whelly, S. M. & Barker, K. L. (1974). *Biochemistry*, **13**, 341–346.
Willis, D. B. & Starr, J. L. (1971). *J. Biol. Chem.* **246**, 2828–2834.