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INDUCTION OF UDP-GLUCURONYL TRANSFERASE mRNA IN EMBRYONIC CHICK LIVERS BY PHENOBARBITAL

MICHAEL R. JACKSON, SUE M. E. KENNEDY, GUY LOWN and BRIAN BURCHELL
Department of Biochemistry, University of Dundee, Dundee, Scotland, U.K.

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Abstract—Administration of phenobarbital to chick embryos increased hepatic microsomal UDP-glucuroyltransferase activity some 25-fold. The large phenobarbital-induced increase of UDP-glucuronyltransferase activity was correlated to an equivalent increase of immunochemically measurable UDP-glucuronyltransferase protein.

Poly(A^+) RNA isolated from the livers of chick embryos treated with either phenobarbital or saline was translated *in vitro*. Immunochemical analysis of the translation products indicated that phenobarbital induced a 30-fold increase in UDP-GT mRNA.

Fractionation of hepatic poly(A^+) RNA from phenobarbital-treated chick embryos by sucrose density gradient centrifugation indicated that the size of the UDP-GT mRNA was 21S.

These data show that phenobarbital induction of chick embryo liver UDP-glucuronyltransferase activity correlates with a similar large increase in the level of translatable mRNA for this enzyme.

In mammals hepatic microsomal UDP-glucuronyltransferase(s) (UDPGT*) is a family of isoenzymes which catalyse conjugation of many xenobiotics and endogenous compounds with UDP glucuronic acid [1, 2]. This enzyme family is of major importance in the inactivation and safe elimination of potentially toxic compounds. Individual UDP-glucuronyltransferase activities which catalyse the glucuronidation of a limited group of substrates can be specifically induced in mammalian liver by certain compounds such as phenobarbital, clofibrate and 3-methylcholanthrene [3–5]. Investigations into the molecular basis of induction of UDP-glucuronyltransferase activities in mammalian systems are hampered by: (a) the existence of several different isoenzymes, (b) the relatively small induction of these enzymes and (c) the lack of monospecific antibodies.

The developing chick embryo is in many respects a more convenient model for the study of both developmental and drug or hormone-mediated control of UDP-glucuronyltransferase gene expression [6, 7].

The chicken liver, in contrast to mammalian liver, is not required to glucuronidate bilirubin, and testosterone (another endogenous substrate glucuronidated in mammals) is mainly sulphated [8]; thus two of the major constitutive forms of UDP-glucuronyltransferase identified in mammals are unlikely to be present in chick hepatic microsomes [5, 8]. Indeed, chick liver microsomes UDP-glucuronyltransferase activities towards these two substrates are absent or very low, and to date, glucuronide formation observed in these microsomes is confined mainly to a few phenolic substrates [8].

The activity of hepatic UDP-glucuronyltransferase towards these phenolic substrates is virtually absent from chick embryo liver until hatching, when it rises suddenly to adult values [9]. Precocious development *in ovo* of this enzyme can be brought about by injection of phenobarbital or infusion of certain 11 *B*-hydroxy corticosteroids into embryonated eggs, such that UDP-glucuronyltransferase activity is increased from virtually zero by some 50-fold by corticosterone acetate [10] and up to 200-fold by phenobarbital [11]. These observations led to the discovery of the regulation of UDP-glucuronyltransferase by glucocorticoids in developing mammalian liver [12]. The enormous increases of UDP-glucuronyltransferase activity mediated by phenobarbital in chick embryo liver *in ovo* can also be demonstrated in chick embryo liver cultures [8].

We have recently purified hepatic phenol UDP-glucuronyltransferase to near homogeneity from the livers of chick embryos pretreated with phenobarbital [8]. The activities towards three phenol substrates copurified, and their glucuronidation was apparently catalysed by a single enzyme protein. Analysis of the purified enzyme preparation by SDS-polyacrylamide gel electrophoresis showed it to contain a single protein, with an estimated subunit molecular weight of 53 kDa. Furthermore, treatment of chick embryo with phenobarbital resulted in the synthesis of large amounts of this UDP-glucuronyltransferase protein [8, 13].

The developing chick embryo is thus an excellent model to study the molecular mechanisms involved in the regulation of UDP-glucuronyltransferase by drugs and hormones because all of the currently available evidence suggests that a single major enzyme species is responsible for the majority of glucuronidation of phenols (thus avoiding the complication from heterogeneity) and, secondly, the activity of this enzyme can be induced dramatically by administration of phenobarbital.

In this paper we describe that phenobarbital treatment of chick embryo livers resulted in a large

* Abbreviations: UDPGT, UDP-glucuronyltransferase; SDS, sodium dodecyl sulphate; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

increase of UDP-glucuronyltransferase activity which correlated with an immunochemically quantifiable increase of a single UDP-glucuronyltransferase protein and with an increase of the corresponding translatable UDPGT mRNA.

A small part of this work has been presented to an international symposium [5].

MATERIALS AND METHODS

Materials

Lubrol 12A9 was obtained from ICI (Organics Division). Guanidine thiocyanate was from Fluorochem; Oligo(dT) Cellulose (T-3) was obtained from Collaborative Research Inc.; Radioactive materials, ^{35}[S]methionine, ^{125}Iodine and 1-(1-^{14}C)naphthol along with NCS tissue solubilizer were from Amersham International Plc.; Scintran scintillation fluid and sodium phenobarbital were from BDH Chemicals; RNAse-free sucrose was obtained from Sigma (London) Chemical Co. All other chemicals where available were analytical reagent grade. Formalin-fixed, *Staphylococcus aureus* ghosts were prepared as described by Kessler [14].

The protein standards used for SDS/polyacrylamide gel electrophoresis (bovine serum albumin, pyruvate kinase, fumarase and aldolase) were purchased from Boehringer (Mannheim).

Fertile eggs from a "synthetic" cross-bred female line (broilers) were from Ross Poultry Farm, Arbroath, Scotland, and were incubated in an egg incubator at 37–38° and 55–60% relative humidity. Embryo age was dated from the start of incubation.

Methods

Administration of phenobarbital. Phenobarbital (10 mg in 0.3 ml of 0.9% NaCl) was injected into the air space of eggs containing 16-day embryos, through a small hole which was then sealed with candle wax; control eggs received saline solution only. The eggs were incubated for a further 3 days before the livers were removed.

Antibodies specific for UDPGT. Antibodies were raised in Suffolk Cross Blackface sheep against purified rat hepatic testosterone UDPGT by a combination of intradermal and subcutaneous injection. IgG was partially purified from this antiserum by $(NH_4)_2SO_4$ fractionation and chromatography on DEAE cellulose. Further characterization of this antibody preparation has been previously described [8, 13].

Assay of UDP-glucuronyltransferase. UDPGT activity towards 1-naphthol was assayed by the method of Otani [15]. Protein concentrations were measured by the Biuret method [16] using BSA as a standard.

Immunotitration of solubilized microsomal UDPGT. Microsomes (20 mg protein/ml 0.25 M Sucrose) prepared from chick embryo livers were solubilized by addition of an equal volume of 2% (w/v) lubrol in 0.2 M potassium phosphate buffer pH 7.4 as previously described [8]. Various amounts of sheep anti-rat UDPGT IgG (anti-UDPGT IgG) were incubated with constant amounts of solubilized microsomes (1.6 mg protein), incubation mixtures contained solubilized microsomes (50 μl) and IgG in PBS (100 μl) made up to a total volume of 300 μl with PBS. The total IgG concentration in each incubation was kept constant by addition of pre-immune IgG. Incubations were at 20° for 30 min, when 2 μl of a 10% w/v suspension of formalin-fixed *S. aureus* ghosts was added and incubations continued for a further 30 min. The incubations were centrifuged at 10,000 *g* for 10 min and 30 μl aliquots of the supernatant were assayed for UDPGT activity towards 1-naphthol.

Radioiodination of partially purified UDPGT and anti-UDPGT IgG. A 25–60% $(NH_4)_2SO_4$ fraction of solubilized microsomes chick embryo liver microsomes was prepared as previously described [8]. Radioiodination of this fraction, the partially purified anti-UDPGT IgG and of molecular weight standards was performed by the Chloramine T method [17].

Immunoblot analysis of microsomal proteins with anti-UDPGT IgG. Microsomal proteins were separated by electrophoresis through an SDS polyacrylamide gel, and the proteins were then transferred electrophoretically to nitrocellulose paper. The blot was incubated in 1% BSA in PBS for 1 hr, the labelled antibody was then added and incubation continued for a further hour before washing in PBS (5 × 10 min). The blot was then exposed for autoradiography at −70° overnight.

RNA purification. Chick embryo livers were isolated and RNA was extracted and purified by the guanidine thiocyanate procedure described by Chirgwin *et al.* [18]. Polyadenylated RNA (PolyA$^+$) was purified from total RNA by two cycles of oligo (dT) cellulose chromatography [19].

Sucrose gradient fractionation of poly(A) RNA.* Poly(A$^+$) RNA was incubated at 70° for 5 min in 10 mM Tris–HCl (pH 7.4) 1 mM EDTA, (TE buffer), cooled on ice and applied to a linear 5–20% (w/v) sucrose gradient prepared with RNAse-free sucrose in TE buffer by freeze/thaw [20]. Gradients (16 ml) were centrifuged for 18 hr at 29,000 rpm at 4° in a swing-out rotor. Fractions (300 μl) were collected using an ISCO 640 fractionator; absorbance at 254 nm was continuously monitored during fractionation with an ISCO UA5 absorbance monitor. RNA was ethanol-precipitated from each fraction, in the presence of 15 μg *Escherichia coli* tRNA.

In vitro *translation of poly(A$^+$) RNA.* A rabbit reticulocyte lysate was prepared by the method of T. Hunt (personal communication) and poly(A$^+$) RNA translated in the presence of ^{35}[S]methionine for 90 min at 30°; the conditions were optimized with respect to concentration of K^+, Mg^{2+} and mRNA as described by Pelham and Jackson [21]. *In vitro* protein synthesis was then assayed by trichloroacetic acid (TCA) precipitation of a small aliquot (2 μl) of the translation mixture followed by liquid scintillation counting to assess the total incorporation of the ^{35}S label in the precipitate. Values obtained were corrected for background by subtracting the counts obtained from control translations where no mRNA was added. Further analysis of translation products was by electrophoresis on linear gradient 7–9% SDS polyacrylamide slab gels [22] followed by fluorography [23].

Immunoisolation of UDP-glucuronyltransferase. UDPGT was immunoprecipitated from *in vitro*

translation products, and from the ^{125}I-labelled $(NH_4)_2SO_4$ fraction of solubilized chick embryo microsomes using the anti-UDPGT IgG, essentially using incubation and washing procedure described by Fagan *et al.* [24]. Incubation mixtures in 0.5 ml of buffer contained 0.5% Triton X-100, 0.5% sodium deoxycholate (DOC), 50 mM Tris–HCl (pH 7.5), 150 mM KCl and 10 mM methionine. Pre-immune IgG (40 μg) was added and the mixture incubated on ice for 2 hr. A 100 μl volume of formalin-fixed *S. aureus* ghosts (10% w/v) was then added and incubation continued for a further 30 min on ice. The suspension was then centrifuged for 3 min at 10,000 *g*, purified carrier UDPGT (0.1 μg) and antibody (40 μg) were added to the supernatant and incubated for 2 hr on ice. *S. aureus* ghosts (100 μl) were again added and incubation continued on ice for 30 min. After centrifugation at 10,000 *g* for 3 min the pellets were washed at 4°, either stringently (four washes with 1% Triton X-100, 1% DOC, 0.5 M KCl and 0.1% SDS), or non-stringently (four washes of immunoprecipitation buffer, before a final wash in 10 mM Tris–HCl (pH 7.5) and 0.5% Nonidet P40). Isolated antigens were released from the *S. aureus* ghosts by heating at 100° for 5 min in sample buffer containing SDS and 2-mercaptoethanol and then analysed by SDS polyacrylamide gel electrophoresis and fluorography as described above.

Quantitation of ^{35}S-labelled immunoprecipitated UDPGT. The portion of the dried-down fluorographed gel, containing the appropriate stringently washed immunoprecipitated material, was excised and treated with NCS tissue solubilizer for 2 hr at 60°; following neutralization, the amount of radioactivity in the slice was determined by liquid scintillation counting in 5 ml of Scintran. Values obtained were corrected for background by subtraction of the radioactivity in the equivalent gel slice from the incubation with pre-immune IgG.

RESULTS AND DISCUSSION

Immunochemical quantitation of UDP-glucuronyltransferase in chick embryo liver microsomes

Previous reports from this laboratory have shown that chick embryo phenol UDP-glucuronyltransferase can be purified to near homogeneity by conventional methods or isolated by immunoprecipitation using anti-UDP-glucuronyltransferase antibodies. A protein of Mr 53 kDa was observed by examination of these two purified preparations by SDS-polyacrylamide gel electrophoresis, indicating that a single UDP-glucuronyltransferase was isolated by two different and unrelated methods. These earlier studies also suggested that a newly synthesized protein of the same molecular weight accumulated following treatment of liver cells with phenobarbital [8, 13]. However, identification and quantitation of the induced putative UDP-glucuronyltransferase was required to confirm the induction of hepatic UDP-glucuronyltransferase protein in response to phenobarbital. Thus, we have attempted to measure induced UDP-glucuronyltransferase protein immunochemically. Hepatic microsomes isolated from saline- or phenobarbital-treated chick embryos were assayed for UDP-glucuronyltransferase activity towards 1-naphthol in the presence of the detergent Lubrol to measure maximum levels of activity [8]. The results obtained showed that microsomal UDP-glucuronyltransferase activity was increased some 27-fold by pretreatment of the embryos with phenobarbital (Table 1).

These detergent-solubilized microsomes were also incubated with various quantities of anti-UDPGT IgG to determine how much antibody is required to specifically immunoprecipitate the UDP-glucuronyltransferase activity from these two preparations. The linear immunotitration curves shown in Fig. 1 allow an estimation of the amount of IgG required to immunoprecipitate the phenol UDP-glucuronyltransferase activity. This method of quantitation of UDP-glucuronyltransferase protein indicated that 26-fold more IgG is required to remove UDP-glucuronyltransferase activity from hepatic microsomes from phenobarbital-treated chick embryos than to remove the enzyme activity from the equivalent amount of hepatic microsomes from saline-treated chick embryos (Table 1).

The useful information from this experiment is the relative rather than actual amounts of IgG required in each case. These values allow an estimation of

Table 1. Induction of hepatic microsomal UDP-glucuronyltransferase in chick embryo liver.

Treatment	1-naphthol UDPGT activity		Immunoreactive UDPGT protein*	
	nmol/min/mg protein	Fold increase	μg	Fold increase
Saline	0.6	1	2.5	1
Phenobarbital	16.2	27	65	26

Phenobarbital (10 mg) or saline was administered to 16-day-old chick embryos and livers were removed three days later for preparation of microsomes. The microsomes (0.5 mg) were assayed for UDPGT activity in the presence of 0.5 mg of the detergent Lubrol[12].

* Data are expressed as the amount of IgG fraction required to immunoprecipitate 50% of the 1-naphthol UDP-glucuronyltransferase activity in a Lubrol-solubilized microsomal fraction (1.6 mg protein).

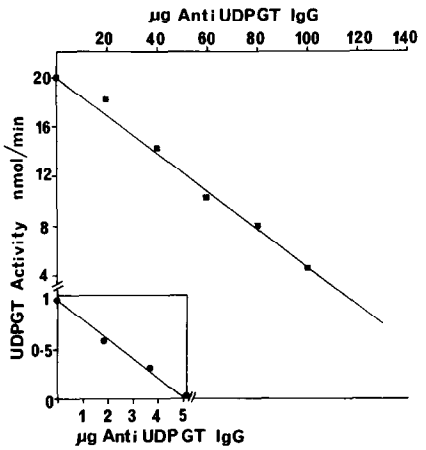

Fig. 1. Immunotitration of solubilized microsomal UDPGT activity from the livers of chick embryos with anti-UDPGT IgG. Solubilized microsomes (1.6 mg protein) prepared from the livers of 19-day-old chick embryo either treated with saline (●) or treated with phenobarbital (■) were incubated with various amounts of anti-UDPGT IgG (see Methods section). The immunoprecipitates were removed from the supernatant using *S. aureus* and centrifuged and the UDPGT activity remaining in the supernatant was assayed using 1-naphthol as substrate.

the relative amounts of UDPGT protein in the two samples.

The calculations do, however, depend on the anti-UDPGT IgG specifically immunoprecipitating UDP-glucuronyltransferase under the described incubation conditions. Analysis of the immunoprecipitates obtained under similar incubation conditions, but including ^{125}I-labelled microsomes, is hampered by the relatively high non-specific binding of the labelled proteins to the *S. aureus* membranes and detergent is required to reduce this to an acceptable level. In such an analysis (see Fig. 2A) a single protein of molecular weight 53 kDa (approximately the same size as the purified protein) is immunoprecipitated.

Furthermore, we have used ^{125}I-labelled anti UDPGT IgG in a western blot analysis of microsomal proteins from the two sources. The blot was incubated and washed in the absence of detergent and shows (Fig. 2B) that a single protein of molecular weight approximately 53 kDa is identified by the antibody (illustrating the specificity of the antibody under these conditions), and only in the microsomal proteins of the phenobarbital-treated animals. On this immunochemical evidence there is certainly a large induction of UDP glucuronyltransferase protein in the liver of developing chick embryo mediated by administration of phenobarbital, and we have estimated this induction to be approximately 26-fold.

Synthesis of UDP-glucuronyltransferase protein in vitro

Previous studies have indicated that hepatic UDP-glucuronyltransferase protein is synthesized *in ovo* in response to phenobarbital treatment [8]. We were interested to determine whether the induced levels of microsomal transferases were derived from an increase in the amount of the corresponding UDPGT mRNA. We have thus examined these levels by *in vitro* translation followed by selective immunoprecipitation from the translation products with anti-UDPGT IgG.

Poly(A$^+$) RNA was isolated from livers of phenobarbital-treated (PBRNA) or saline-treated (control or CRNA) chick embryos. Identical amounts of poly(A$^+$) RNA isolated from each source were translated in reticulocyte lysates *in vitro* and both RNA preparations were shown to direct the same levels of protein translation, with only minor differences in the spectrum of translation products, (compare lanes a and b in Fig. 3A). The ^{35}(S)-labelled translation products were incubated with pre-immune IgG and then incubated with anti-UDPGT IgG to specifically immunoprecipitate newly synthesized UDP-glucuronyltransferase protein. The radioactive proteins in the immunoprecipitates were then examined by SDS-polyacrylamide gel electrophoresis and fluorography. Preliminary experiments of this type were confused by the presence of two non-specifically isolated proteins in the immunoprecipitates. The conditions used in the immunoprecipitations were thus varied to find detergent concentrations which would allow the antibody to specifically immunoisolate the *in vitro*-translated UDPGT, and thus allow us to measure the levels of this protein. It was found necessary to include relatively high levels of detergent in the immunoprecipitate wash buffer (compare Fig. 3A and 3B). In Fig. 3A two polypeptides can be seen to be immunoisolated from the translation products of PBRNA and not CRNA with anti-UDPGT IgG. However, only the 53 kDa protein is

Table 2. UDP-glucuronyltransferase mRNA levels in livers from saline-treated and phenobarbital-treated chick embryos.

Treatment	Radioactivity of the 53 kDa UDP glucuronyltransferase, protein (cpm)			
	Expt 1	Fold increase	Expt 2	Fold increase
Saline	65	1	21	1
Phenobarbital	1750	27	730	35

Phenobarbital (10 mg) or saline was administered to 16-day-old chick embryos. The livers were removed three days later for preparation of poly(A$^+$) mRNA. The poly(A$^+$) RNA was translated *in vitro* in a reticulocyte lysate. The amount of TCA precipitable radioactivity in each immunoprecipitation reaction was: *Expt 1*, 2.7×10^6 cpm; *Expt 2*, 1.4×10^6 cpm.

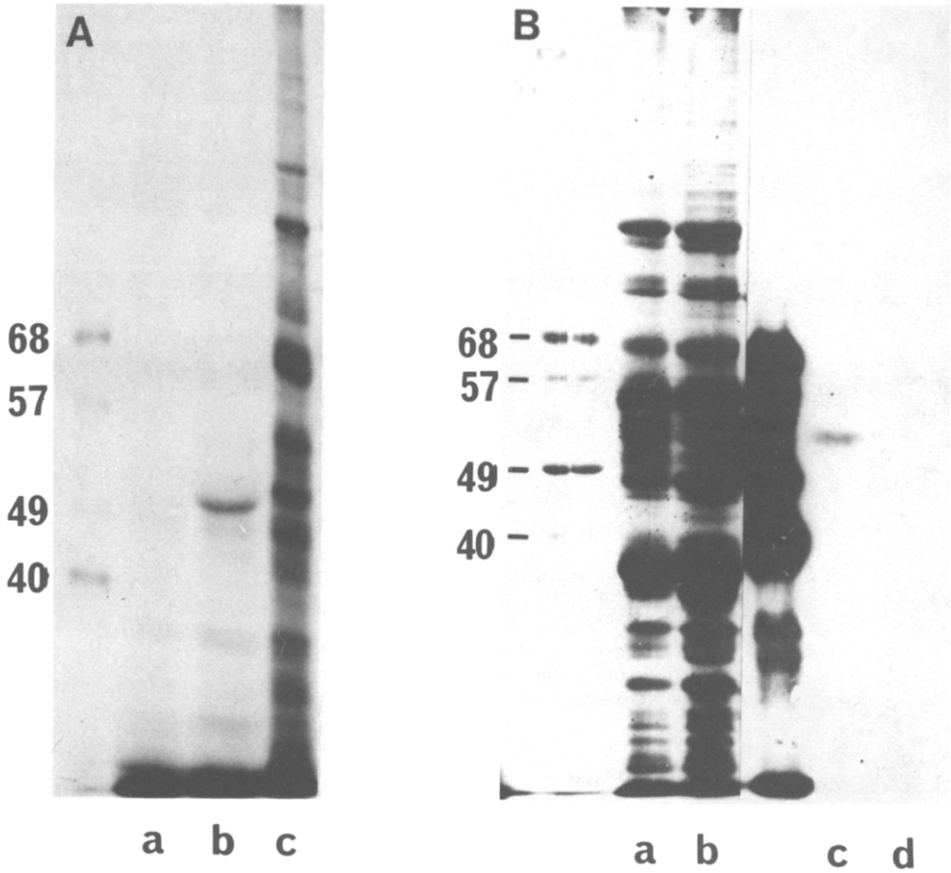

Fig. 2. Immunochemical isolation of UDPGT from chick embryo liver microsomes. (A) Analysis by SDS-polyacrylamide gel electrophoresis and fluorography of ^{125}I labelled $(NH_4)_2SO_4$, 25–60% fraction of solubilized chick embryo liver microsomes (lane c), iodinated proteins immunoisolated from this microsomal fraction with anti UDPGT IgG (lane b) or sheep pre-immune IgG (lane a) as described in Methods, where immunoprecipitates are stringently washed. The numbers indicate Mr $\times 10^{-3}$ of marker proteins. (B) Western blot analysis of chick embryo microsomal proteins with anti UDPGT IgG. Lanes a and b show a Coomassie blue-stained SDS-polyacrylamide gel analysis of microsomal proteins prepared from the livers of chick embryos administered either phenobarbital or saline respectively. Lanes c and d are an autoradiogram of a blot of an identical gel probed with radioiodinated anti-UDPGT IgG and aligned with the aid of labelled standard proteins.

immunoisolated under the higher stringency washing (Fig. 3A and 4C), and further, it is approximately the same size as the purified UDP-glucuronyltransferase, the protein identified by immunoprecipitation from iodinated solubilized microsomes (Fig. 2A) and by immunoblot analysis (Fig. 2B). The mobility of the immunochemically identified protein is unlikely to be disturbed by the large subunit of IgG only when using the latter of these techniques; estimates of the molecular weight of the immunoprecipitated protein is likely to be slightly distorted (as both proteins are of approximately the same size). Despite this it seems unlikely that the synthesis of this UDP-glucuronyltransferase occurs via a distinctly larger precursor polypeptide.

Quantitation of the induction of UDP-glucuronyltransferase mRNA by phenobarbital

Quantitation of the levels of UDPGT mRNA in PBRNA and CRNA was achieved by determining the radioactivity of the specifically immunoprecipitated 53 kDa protein (see Methods), and expressing these values as a percentage of the total radioactivity (TCA precipitatable) present in the corresponding immunoprecipitation incubation. The results (Table 2) show UDPGT mRNA to be of much greater abundance in PBRNA where it represents approximately 0.06% of the mRNA compared to the levels in CRNA where it represents only 0.002% of the translatable mRNA. These figures show that the levels of translatable UDPGT in the control animals are relatively low; however, the immunoprecipitates obtained with anti-UDPGT IgG always contained significantly more radioactivity at the 53 kDa position than with preimmune IgG. Using this analysis we have calculated that a 31-fold induction of translatable hepatic UDPGT mRNA occurs in chick embryos on administration of phenobarbital.

Partial purification and sizing of UDP-glucuronyltransferase mRNA

PBRNA was further fractionated by sucrose density centrifugation to obtain an enriched population

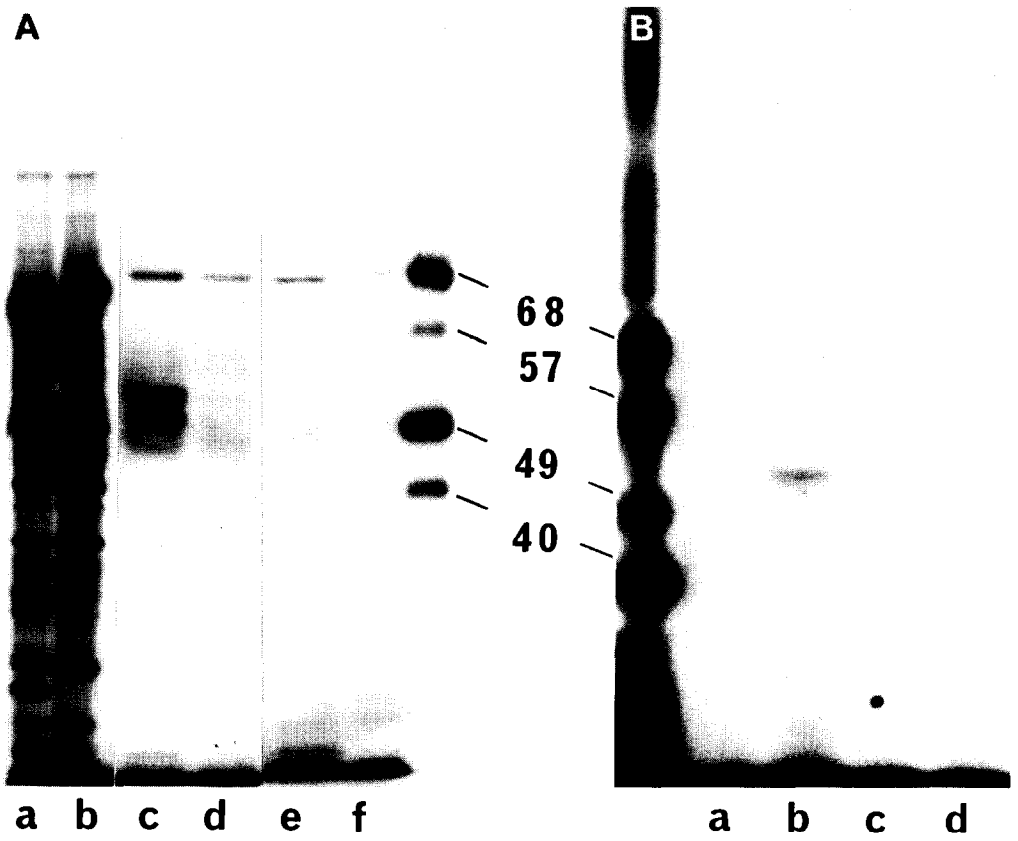

Fig. 3. Analysis of the *in vitro* translation products of chick embryo liver poly(A^+) RNA by SDS polyacrylamide gel electrophoresis. (A) PBRNA and CRNA was translated *in vitro*, and the translation products analysed by SDS-polyacrylamide gel electrophoresis and fluorography either directly (tracks a and b) or after incubation with anti-UDPGT IgG (tracks c and d) or pre-immune IgG (tracks e and f). In each case the translation products derived from PBRNA are shown before those from CRNA. (B) The *in vitro* translation products of CRNA and PBRNA were incubated with pre-immune IgG followed by anti-UDPGT IgG as above. The immunoprecipitates were then washed stringently (see Methods) before being analysed by SDS-polyacrylamide gel electrophoresis and fluorography. The immunoprecipitates illustrated were those obtained on incubation of the translation products of CRNA and PBRNA with either anti-UDPGT IgG (lanes a and b respectively) or pre-immune IgG (in lanes c and d respectively).

of UDPGT mRNA which could be used to prepare a chick liver cDNA library. Sized fractions of the poly(A^+) RNA (Fig. 4A) were translated *in vitro*, and the radioactive translation products analysed by SDS polyacrylamide gel electrophoresis and fluorography (Fig. 4B). The results show the spectrum of radioactive translation products which are synthesized by the various mRNA fractions. These different translation mixtures were incubated with anti-UDPGT IgG to immunoisolate the newly synthesized UDPGT proteins and thereby identify the RNA fractions which contain UDPGT mRNA. Figure 4C shows that UDPGT proteins are synthesized to the greatest extent in fractions h and i, indicating that the message coding for this enzyme is approximately 21S in size.

Regulation of UDP-glucuronyltransferase by phenobarbital

Numerous studies have established that phenobarbital induces the synthesis of a variety of enzymes associated with drug metabolism. Administration of phenobarbital has been shown to increase the amount of translationally active mRNA for cytochrome P-450 [25–27] NADPH-cytochrome P-450 reductase [28], epoxide hydrolase [29, 30] and glutathione-*S*-transferase [31, 32] in rat liver cells. The molecular mechanism responsible for these increases is, however, unknown [33]. Induction of the synthesis of both UDPGT protein and of translationally active UDPGT mRNA by phenobarbital has been shown in rats [5] and mice [34].

The results presented here show that phenobarbital induces a 26-fold increase in UDP-glucuronyltransferase activity which can be correlated to a 26-fold increase of UDPGT protein level and a 31-fold increase in translatable UDPGT mRNA. This increased level of UDPGT mRNA may be due to increased rate of its transcription, decreased rate of its degradation or a combination of both events. The exact mechanisms responsible for phenobarbital induction of UDPGT mRNA cannot be determined

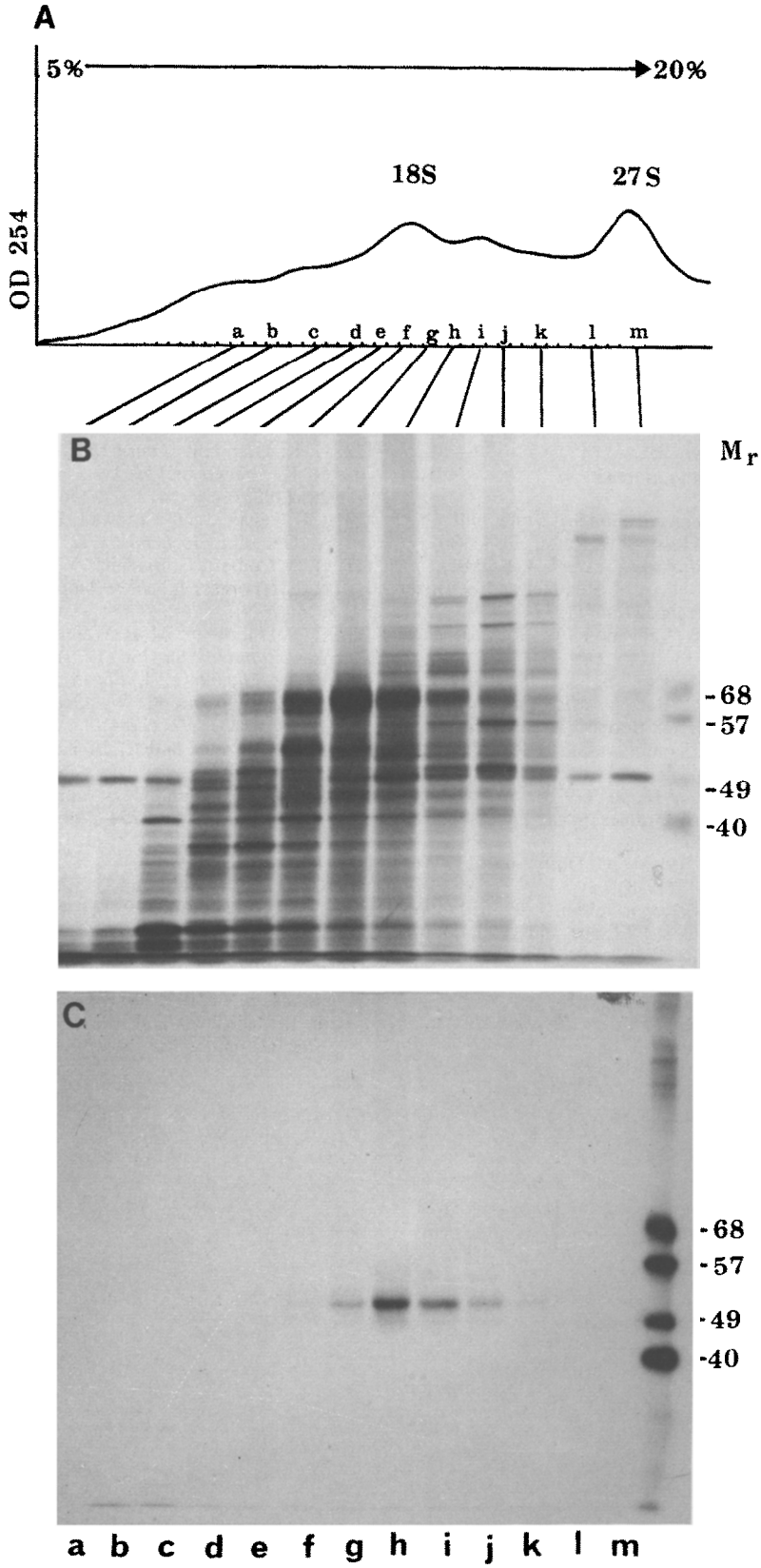

Fig. 4. Sizing of chick embryo UDPGT mRNA by sucrose gradient fractionation. PBRNA (200 μg) was loaded onto a 16 ml 5–20% sucrose gradient after centrifugation. This was fractionated with continuous monitoring of the optical density at 254 nm (Fig. 4A), rRNA peaks are at 18S and 27S were used as markers. Figure 4b shows analysis by SDS-polyacrylamide gel electrophoresis, and fluorography of the *in vitro* translation products of the RNA in the fractions indicated. Figure 4c shows a similar analysis of the immunoprecipitates obtained on incubation of the indicated translation products with anti-UDPGT IgG, with stringent washing as described in Methods. The numbers on the left of the fluorograms indicate Mr $\times 10^{-3}$ of marker proteins.

without additional specific cDNA probes to measure rates of transcription and degradation of UDPGT mRNA. However, it should be possible to study these events much more easily in chick embryos than in mammalian species, as the system is simplified by the presence of one major species of UDP-GT mRNA which is greatly induced by phenobarbital. Thus, this system should be a good model in which to try to determine the mechanism of action of phenobarbital during induction of UDP-glucuronyltransferase.

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