DEVELOPMENT OF PHENOBARBITAL-SENSITIVE
CONTROL MECHANISMS FOR URIDINE DIPHOSPHATE
GLUCURONYLTRANSFERASE ACTIVITY
IN CHICK EMBRYO LIVER

BRIAN BURCHELL, GEOFFREY J. DUTTON, and
ANDREW M. NEMETH

From the Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland and
the Department of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT
Uridine diphosphate (UDP) glucuronyltransferase activity in chick liver rises at hatching
from near zero to adult levels. This rise will occur prematurely in embryo liver during
organ culture. Increase in enzyme activity during organ culture differs with embryo age:
in liver from 11-day old embryos it ceases at adult values; in liver from 5-day old embryos
it continues to much higher-than-adult levels. Phenobarbital added to culture medium
accelerates these rises in enzyme activity and elevates the plateau reached in 11-day embryo
liver to that observed in 5-day embryo liver. Kinetic analysis of the changes in enzyme
activity induced by phenobarbital during culture suggests that the regulatory mechanisms
for enzyme activity are different in 5- and 11-day embryo liver and that these differences
reflect developmental changes occurring in ovo.

INTRODUCTION
Uridine diphosphate (UDP) glucuronyltransferase activity is absent or very low in chick embryo liver
and rises to adult levels within 1–2 days after hatching (Dutton and Ko, 1966). This rise occurs
prematurely in embryo liver during cell or organ culture and is probably initiated by removal of
tissue from the embryonic environment rather than by culture conditions (Ko et al., 1967;
Skea and Nemeth, 1969)

The increase in UDP-glucuronyltransferase activity during organ culture differs with embryo age: in liver from 11-day old embryos it ceases at approximately adult values; in liver from 5-day old embryos it continues to much higher-than-adult levels, suggesting that the regulatory mechanisms for UDP-glucuronyltransferase activity are not yet fully developed (Skea and Nemeth, 1969). To test this possibility we have studied the effects of phenobarbital on the appearance of enzyme during culture. Phenobarbital administered to adult chickens increases liver UDP-glucuronyltransferase activity (G. J. Dutton and I. H. Stevenson, 1969, unpublished observations). We found that phenobarbital added to culture medium stimulates development of enzyme activity in both 5- and 11-day embryo liver, but differently as to amount and time-course of enzyme appearance (Dutton et al., 1970; Burchell et al., 1971). Thus, we suggest that the regulatory mechanisms for enzyme activity are different at these two stages, and that the differences reflect developmental changes occurring in ovo.
METHODS

Organ Culture

Fertile eggs (White Leghorns) were obtained from the Shaw Hatchery (West Chester, Pa.). Five whole livers or liver segments totalling approximately 5 mg from 5- and 11-day chick embryos, respectively, were placed on Millipore filter rafts (25 µ thick, 0.45 µ pore size) (Millipore Corp., Bedford, Mass.) supported by nylon screen (Nitetex, 656 µ grid) over the center well of an organ culture dish (Falcon Plastics, Div. of Bioquest, Oxnard, Calif.) containing 1.2 ml of Eagle’s medium with phenol red, 2 mM L-glutamine, 100 units penicillin, 0.25 µg Fungizone (E. R. Squibb & Sons, New York), and 100 µg streptomycin/ml of medium (Grand Island Biological Co., Grand Island, N. Y.). Tissue was incubated in CO₂-air (1:19 v/v) at 37.5°C, atmospheric pressure, and 100% humidity, and medium was replaced every 3 days (Skea and Nemeth, 1969).

Assay for UDP-Glucuronyltransferase Activity

Liver tissue from a culture dish was homogenized by sonication in 0.6 ml of a solution of sucrose, ethylenediaminetetraacetate (EDTA), Tris, and magnesium chloride at pH 7.4. Protein was measured in a 0.23 ml sample of the sonicate by a modification of Lowry’s method (Oyama and Eagle, 1956); 0.23 ml of sonicate was added to 0.07 ml of a solution of UDP-glucuronic acid, o-aminophenol, and ascorbic acid. Final concentrations in the 0.3 ml reaction mixture were 29 mM sucrose, 0.145 mM EDTA, 33 mM Tris, 10 mM magnesium chloride, 0.14 mM o-aminophenol, 0.76 mM ascorbic acid, and 1.4 mM UDP-glucuronic acid. UDP-glucuronic acid was omitted in the control blank. The reaction mixture was incubated 30 min at 37°C. Enzyme action was stopped by addition of 0.3 ml of a 1:1 (v/v) mixture of ice-cold 2 mM potassium phosphate and 40% (w/v) trichloroacetic acid (TCA) at pH 2.20. The precipitate was removed by centrifugation at 2000 g for 5 min. To 0.4 ml of the supernatant fluid, 0.05 ml of 0.1% (w/v) sodium nitrite, 0.5% (w/v) ammonium sulphamate, and 0.1% (w/v) N-(1-naphthyl)-ethylenediamine dihydrochloride were added sequentially at intervals of 2 min with shaking. The mixture was then incubated at 25°C for 90 min in the dark. The absorbance at 560 nm was measured in a Zeiss spectrophotometer (Carl Zeiss, Inc., New York) (Ko et al., 1967).

Radioactive Analysis

Incorporation of L-leucine-1³⁴C and uridine-5³H into tissue pools, and protein and RNA, respectively, were measured as follows: Liver tissue from a culture dish was homogenized by sonication in 0.5 ml ice-cold 0.1 M potassium phosphate at pH 7.4. A sample (0.1 ml) was removed and assayed for protein (Oyama and Eagle, 1956). Human serum albumin (1.25 mg) and RNA (300 µg) were added to the remaining mixture, followed by 0.5 ml 20% (w/v) TCA. After standing 2 hr on ice, the precipitate was collected on a Fiberglas filter disk (2.3 cm diameter) by vacuum suction, washed with 5.0 ml of 0.04 M uridine, 0.38 mM L-leucine in 5% (w/v) TCA, and then with 1.0 ml of 70% ethanol, air-dried, and placed in a nylone vial.

The filtered fluid was combined with the TCA wash and shaken with an equal volume of ether until the aqueous layer reached pH 5.0. Visible traces of ether were removed with a Pasteur pipette. Air was bubbled through the mixture and then 3.0 ml of the aqueous layer was pipetted into a nylone vial and evaporated to dryness at 60°C.

Scintillation fluid consisted of 5% (v/v) NCS solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.), 5 g 2,5-diphenyloxazole (POP), and 0.5 g dimethyl 17,4-bis[2-(5-phenyloxazolyl)] benzene (POPPOP) (Packard Instrument Co., Inc., Downers Grove, Ill.) per liter of toluene; 10 ml was added to all vials and incubated for 2 hr at 60°C, cooled to 10°C, and counted in an Intertechnique ABA SL40 scintillation counter (Intertechnique Instruments, Inc., Dover, N. J.) to a confidence greater than 99%, at greater than 40% efficiency for the ³H label and greater than 50% for the ³¹C label.

Microscopic Examination

Tissues were fixed with Formalin and embedded in paraffin by standard methods. Tissue sections were stained with hematoxylin and eosin.

RESULTS

Effect of Phenobarbital on Viability of Cultured Embryo Liver

To compare the specific activity of UDP-glucuronyltransferase in livers from chick embryos of different ages, cultured for various periods, requires that all tissues contain approximately the same proportion of parenchymal cells. We have found from microscopic study that 5- and 11-day chick embryo livers retain a predominantly (more than 90%, v/v) parenchymal cell composition during organ culture on rafts over Eagle’s medium (see Methods).

5-DAY EMBRYOS: Livers flatten and spread out within 2-3 hr of explantation on to rafts. Tissue protein and DNA decrease by approxi-
mately one-half over 6 days of culture (Fig. 1). Only some 10–15% (v/v) of the tissue appears necrotic, suggesting that dead cells disintegrate rapidly and are leached into the medium. Otherwise, the tissue is composed almost entirely of parenchymal cells. With phenobarbital added (5.5 mM) to the medium, the loss of protein and DNA is slowed during culture but the ratio of protein/DNA (50, w/w) is maintained at the initial value, suggesting that phenobarbital decreases cell death (Fig. 1).

**11-DAY EMBRYOS**: Segments of liver cultured without phenobarbital in the medium round off over 3–4 days due to encapsulation by proliferating spindle-shaped cells. Capsule is one to two cells thick after 2 days of culture, five and six cells thick after 4 days, and seven to eight cells thick (some two liver cell diameters) after 6 days. Parenchymal cells make up the rest of the tissue, except for a small amount (10%, v/v) of necrosis in the center of segments after 4 days of culture. Capsule cells do not contain UDP-glucuronyltransferase activity (Ko et al., 1967; Skea and Nemeth, 1969). With phenobarbital added to the medium, capsules do not grow more than three cells thick, and segments do not round off. Protein loss is negligible with or without phenobarbital in the medium (Fig. 3).

**Effect of Phenobarbital on UDP-Glucuronyltransferase Activity in Cultured Embryo Liver**

**5-DAY EMBRYOS**: Specific activity of UDP-glucuronyltransferase increases over 5 days of culture from zero to a plateau 5 times above the adult level (Fig. 1), as found by Skea and Nemeth (1969). When phenobarbital is added to medium at the start of culture, approximately the same elevated plateau is reached but within 3 days (Fig. 1). The optimum concentration of the drug for this stimulation is 5.5 mM, though cell death as indicated by loss of protein is kept to a minimum by lower concentrations (Fig. 2). The tissue fully responds to phenobarbital addition up to at least 1 day after the start of culture (Fig. 1).

**11-DAY EMBRYOS**: Specific activity of UDP-glucuronyltransferase increases over 5 days of culture from zero (or very low values) to a plateau at adult level (Fig. 3), as found previously (Skea and Nemeth, 1969). However, when phenobarbital is added to medium at the start of culture, activity rises in the same period to a plateau 5 times higher than the adult level (Fig. 3). The optimum concentration of phenobarbital for this effect is the same as for the stimulation of enzyme development in 5-day embryo liver (Fig. 2). Moreover, 11-day

---

**Figure 1** Effect of phenobarbital on the development of UDP-glucuronyltransferase activity in 5-day chick embryo liver during organ culture. Sodium phenobarbital (5.5 mM) was added to medium at the start of culture or after 1 day of culture. Phenobarbital (•), without phenobarbital (○). Points with vertical bars, which indicate the standard errors of the means, represent the averages of between five and ten culture dishes. Points without vertical bars represent the averages of at least three culture dishes. **Insert:** DNA points (▼) represent the averages of two culture dishes. The standard errors of the means for the protein points (○, •) were less than 15% of the mean values and so have been omitted. Enzyme activity is expressed as µg o-aminophenylglucuronide formed/mg tissue protein per 80 min.
Effect of various concentrations of phenobarbital on development of UDP-glucurononitransferase activity in 5- and 11-day chick embryo liver during organ culture. Sodium phenobarbital was added to medium at the start of culture; tissue was analyzed after 3 days of culture. Points represent the averages of two culture dishes. Enzyme activity is expressed as µg o-aminophenyl glucuronide formed/mg tissue protein per 90 min.

Embryo liver responds fully to phenobarbital addition up to at least 5 days after onset of culture (Fig. 3). Removal of phenobarbital results in the rapid fall of activity induced by the drug (Fig. 4).

**Effect of Phenobarbital on UDP-Glucuronyltransferase Activity in Sonicated Embryo Liver**

Although the rapid fall of UDP-glucuronyltransferase activity in 11-day embryo liver on withdrawal of phenobarbital from the medium (Fig. 4) indicated that the drug did not accumulate in the cultured tissue, it remained possible that when phenobarbital was present in the culture medium, it was taken over with the tissue into the enzyme assay mixture and there exerted an activating effect on the enzyme. Sonicates (see Methods) of fresh and cultured 11-day embryo liver were, therefore, exposed to different concentrations of phenobarbital for periods of 30 min to 12 hr at 0° and 37°C; in some cases washed microsomes were prepared from the sonicates. Further, sonicates of fresh embryo liver were mixed with sonicates of embryo liver previously cultured in the presence of phenobarbital, and enzyme activity was compared with that of unmixed controls. From these experiments, no evidence could be found which might indicate activation of UDP-glucuronyltransferase in sonicated tissue by phenobarbital or its metabolites, suggesting, as did the gradual rise of activity during culture, that a long-term process such as protein synthesis, rather than rapid enzyme activation, is required for development of enzyme activity.

Efforts to increase UDP-glucuronyltransferase activity in sonicates of fresh and cultured chick embryo liver with detergents, additional periods of sonication, or dialysis were also unsuccessful.

**Effect of Phenobarbital on Synthesis of Protein and RNA in Cultured Embryo Liver**

Incorporation of leucine-14C and uridine-3H into embryo liver was studied during culture to estimate tissue viability, to identify any general effects of phenobarbital, and to provide a background for relating protein and RNA synthesis to enzyme development. Livers from 5-day embryos were used in these and the subsequent studies. When leucine-14C was added to medium, radioactivity reached a concentration in the tissue pool within 5 min which did not change for at least 4 hr; protein specific radioactivity increased linearly for at least 2 hr. Therefore, the radioactivities of tissue pool and protein were measured 2 hr after the addition of leucine-14C to the medium. In this manner the leucine pool and the rate of leucine incorporation into protein were estimated at various times during culture and found not to change (Fig. 5).
When uridine-3H was added to medium, radioactivity both in the tissue pool and in RNA increased linearly for at least 2 hr. 2 hr after addition of uridine-1H to medium, older cultures showed higher pool radioactivities than did fresher cultures. Incorporation of uridine-1H into RNA, however, did not change with the age of culture (Fig. 5). This suggested that the precursor pool of uridine for RNA synthesis may be in the medium, which possesses constant concentration and specific activity of uridine in these experiments, and that most of the tissue pool of uridine does not enter tissue RNA.

Phenobarbital (5.5 mm) when added to the medium at the start of culture had no effect on the incorporation (measured as in Fig. 5) of leucine-14C and uridine-1H into tissue pools or, respectively, into protein and RNA during the first 3 culture days. Any general effects related to stimulation of enzyme development would probably have been evident by this time.

The pattern of incorporation of isotopically labeled uridine into ribosomal and transfer RNA appears similar at the start of culture and after 3 days. Cultures containing 5.5 mm phenobarbital were incubated with uridine-3H for 2 hr at the start of culture or with uridine-14C after 3 days of culture. Tissues were mixed and sonicated. RNA was isolated and then fractionated by agarose-polyacrylamide gel electrophoresis (Peacock and Dingman, 1968). 14C and 3H radioactivity peaks were observed at positions corresponding to 32S, 28S, 18S, and 4S (5S shoulder) RNA, molecular weights being determined by reference to Escherichia coli RNA. The relative magnitudes and positions of the 14C and 3H peaks were the same. The 32S peaks were 10\% higher than the 28S peaks, which were twice as high as the 18S peaks, which were 20\% higher than the 4S peaks.

**Relation of Enzyme Development to Protein and RNA Synthesis**

Livers from 5-day old embryos were cultured with phenobarbital. After 20 hr, various amounts of cycloheximide, an inhibitor of amino acid incorporation into protein, were placed in the medium for 4 hr. Specific enzyme activity (Fig. 6)
FIGURE 4 Effect of removing phenobarbital from the medium on the specific activity of UDP-glucuronyltransferase in 11-day chick embryo liver during organ culture. Sodium phenobarbital (5.5 mm) was added to medium at the start of culture and removed on days 1, 4, or 5 of culture. Phenobarbital (●), no phenobarbital (○). Points represent the averages of at least three culture dishes. The time (t1/2) required for enzyme activities to decrease half-way back to the control values after removing phenobarbital from the medium on day 4 or 5 of culture was approximately 65 hr (marked by arrows). Enzyme activity is expressed as µg o-aminophenylglucuronide formed/mg tissue protein per 30 min.

and rate of leucine incorporation into protein (Fig. 7 a) were measured at intervals over the next 48 hr. Total leucine incorporation into protein over the 20-68 hr culture period was calculated from the areas below the curves describing the rates of leucine incorporation into protein (Fig. 7 a). Its degree of inhibition by different doses of cycloheximide was found exactly proportional to the inhibition of enzyme development at 68 hr (Fig. 7 b). This proportionality suggests that cycloheximide inhibits development of enzyme activity by decreasing the rate of synthesis from amino acids, either of enzyme or of a protein directly regulating enzyme activity. Furthermore, the rate of enzyme synthesis in the control cultures is probably constant between 20 and 68 hr of culture.

Development of enzyme activity and leucine-14C incorporation into protein were blocked by cycloheximide also in 5-day embryo livers cultured without phenobarbital in the medium, and

FIGURE 5 Rates of incorporation of leucine-14C and uridine-3H, respectively, into protein and RNA of 5-day chick embryo liver during organ culture. Leucine-14C (0.42 µCi/ml) and uridine-3H (0.42 µCi/ml) were added to medium after various periods of culture, and the 14C and 3H radioactivities were measured 2 hr later in the TCA-soluble (○, pools) and insoluble (●, protein and RNA) fractions of the tissue and expressed per mg tissue protein. Points represent the averages of four culture dishes. dpm = disintegrations per minute.

FIGURE 6 Effect of various concentrations (0.1, 0.5, and 2.0 µg/ml medium) of cycloheximide on development of UDP-glucuronyltransferase activity in 5-day chick embryo during organ culture. Sodium phenobarbital (5.5 mm) was added to medium at the start of culture. Cycloheximide was added to medium at 20 hr of culture for 4 hr. Points represent the averages of four culture dishes. Insert: There were no significant differences in the protein content of the differently treated cultures and, therefore, the values were averaged. Enzyme activity is expressed as µg o-aminophenylglucuronide formed/mg tissue protein per 30 min.
Figure 7  (a). Effect of various concentrations (0.1, 0.5, and 2.0 µg/ml medium) of cycloheximide on the rate of incorporation of leucine-14C into protein of 5-day chick embryo liver during organ culture. Sodium phenobarbital (5.5 mm) was added to medium at the start of culture. Cycloheximide was added to medium at 20 hr of culture for 4 hr. Leucine-14C (0.42 µCi/ml) was added to medium after various periods of culture, and the radioactivities in tissue pool and protein were measured an hour later. Points (O) indicating specific radioactivity of tissue protein represent the averages of two culture dishes. Radioactivity in tissue pools of control and cycloheximide-treated cultures were averaged (•) because they did not appear to be significantly different: individual values usually fell within 25% of the mean; the standard errors of the means were approximately 5% of the mean values. (b). Proportionality of inhibition by various concentrations of cycloheximide (0.1, 0.5, and 2.0 µg/ml medium) of enzyme development at 68 hr of culture (Fig. 6), and of total leucine-14C incorporation into protein between 20 and 68 hr of culture, calculated from the areas under the curves describing the rates of leucine-14C incorporation into protein (Fig. 7a).

in 11-day embryo liver cultured with phenobarbital. Cycloheximide (2 µg/ml medium) during 4 hr of culture had no effect on uridine-3H uptake into tissue pool or incorporation into RNA.

Development of enzyme activity did not appear to depend immediately on RNA synthesis, for it was unaffected over 1 day of culture either by 5-fluorouridine (4 µg/ml medium added at the start of culture) or by actinomycin D (2 µg/ml medium added at the start of culture and removed after 4 hr by replacing medium). These experiments cannot be considered conclusive, however, because actinomycin D inhibited uridine incorporation into RNA only approximately 80%, and 5-fluorouridine may not alter the function of the relevant molecules of RNA.

Related Observations

Action of Other Drugs: Sodium diethylbarbiturate and 3,4-benzpyrene were as effective as sodium phenobarbital in increasing the specific activity of UDP-glucuronyltransferase in 11-day chick embryo liver during organ culture. Sodium barbiturate was less effective and Triton X-100, a detergent, was ineffective (0.1%, w/v) or inhibitory (1%, w/v).

p-Nitrophenol as Acceptor: The specific activity of UDP-glucuronyltransferase measured with p-nitrophenol as glucuronyl acceptor (Storey, 1965) increases in 11-day embryo liver during organ culture along with the increase in activity towards o-aminophenol (see Methods), and this increase is enhanced by phenobarbital in the same manner as the activity towards o-aminophenol. Parallel development of activity towards both substrates suggests that both might be glucuronidated by the same enzyme.

Effect of Phenobarbital on Other Enzymes: The specific activities of serine hydroxymethylase (Ulevitch, 1971) and glucose 6-phosphatase (King, 1932) in chick embryo liver (5- and 11-day), respectively, remained unchanged or declined during organ culture, and sodium phenobarbital in the medium (5.5 mm) had no effect. These enzymes are not known to be induced in adult chickens by the drug. The specific activity of ribonuclease (Louis-Ferdinand and Fuller, 1970) in chick embryo liver is maintained.
in organ culture, but falls 50% in the presence of 5.5 mM phenobarbital, supporting observations made in vivo (Seifert and Remmer, 1971).

**Change in culture conditions:** Monolayer cell cultures were prepared from 11-day embryo liver by the method of Skea and Nemeth (1969). Increase in the specific activity of UDP-glucuronyltransferase measured with either o-aminophenol or p-nitrophenol as acceptor (Skea and Nemeth, 1969) was enhanced in these monolayer cell cultures by phenobarbital in the same manner as in organ cultures (Fig. 3). The effect of phenobarbital on enzyme development in organ cultures, therefore, does not depend on tissue architecture.

Phenobarbital stimulates the development of UDP-glucuronyltransferase activity in 11-day chick embryo liver during organ culture to the same high level whether organ cultures are maintained at high oxygen levels (on rafts under O₂:CO₂, 19:1, v/v) or at low levels (immersed in liquid medium as described by Ko et al., 1966), as well as at the atmospheric concentration of oxygen used in the present work. If tissue is immersed in a deep tube of medium where oxygen levels are very low, development of the enzyme is negligible even in the presence of phenobarbital.

A medium richer in amino acids and glucose than Eagle’s, such as F12 (Grand Island Biological Co.), did not improve protein retention or enzyme development in 5-day embryo liver during organ culture. Addition of chick or chick embryo serum to medium was also without noticeable effect. Horse or calf serum could be toxic.

**Effect of phenobarbital on cultures of hatched chick liver:** The specific activity of UDP-glucuronyltransferase (o-aminophenol as acceptor) in 7-day old hatched chick liver falls during organ culture approximately 50% within 2–3 days, but is increased above starting levels if phenobarbital is present in the medium. The level of UDP-glucuronyltransferase activity in 7-day old hatched chick liver was between 5 and 10 µg o-aminophenylglucuronide formed/mg protein per 30 min.

**Discussion**

Phenobarbital enhances the induction of UDP-glucuronyltransferase activity in cultured chick embryo liver. The above experiments (Figs. 6 and 7) with cycloheximide have suggested that changes in UDP-glucuronyltransferase activity during culture are related to changes in the concentration of enzyme, or of a protein directly regulating its activity. Thus, we have adopted a simple formulation (Berlin and Schimke, 1965) to describe changes in enzyme activity as a function of rates of enzyme synthesis and degradation which have made it possible to interpret, in these terms, the action of phenobarbital.

At a steady-state concentration of enzyme, rates of synthesis and degradation are equal.

$$S = Pk$$

$$P = S/k$$

P is enzyme concentration expressed in moles × mg protein⁻¹, S is the zero order rate constant for synthesis expressed in moles × mg protein⁻¹ × min⁻¹, and k the first order rate constant of degradation expressed in min⁻¹. Berlin and Schimke (1965) have pointed out that the time required to go from one steady-state concentration of enzyme to another (P → P₀) is a function of kᵢ, and independent of Sᵢ.

$$t_{\frac{1}{2}} = 1/k_i$$

Approximately the same steady state of UDP-glucuronyltransferase activity is reached in 5-day embryo liver with and without phenobarbital in the medium (Fig. 1). However, only 3 days of culture time (t₁/₂ = 3½) is required with phenobarbital, in contrast to 5 days (t₁/₂ = 5½) without phenobarbital. Therefore, 1/k₀ = S₀, 1/k = S, and ½ k = kᵢ. Because P = P₀, ½ S = Sᵢ. Thus, S and k are both increased by a factor of 1.7 (½) by phenobarbital. Such a balanced increase suggests that regulation of S and k are coupled in the 5-day embryo liver cell and that phenobarbital acts at a single locus to control both processes. This analysis is an approximation because the lag in development of enzyme activity in 5-day embryo liver (Figs. 1 and 6) indicates that rate constants are changing gradually (Berlin and Schimke, 1965). The period of gradual change appears to be brief, however, because constant rate of protein synthesis during culture (Fig. 5), and proportionality of protein synthesis and enzyme development between 20 and 68 hr of culture (Figs. 6 and 7), suggests that rate of enzyme synthesis becomes constant not later than 20 hr after the start of culture.

In segments of 11-day embryo liver a steady state of UDP-glucuronyltransferase activity is reached in approximately 5 days of culture both with and
without phenobarbital in the medium, but the plateau value is 6 times higher with phenobarbital (P') than without (P) the drug (Fig. 3).

\[ k = k' \]

\[ 6P = P' \]

\[ 6S = S' \]

Thus, phenobarbital increases S in 11-day embryo liver but is without effect on k. This conclusion is also supported by comparing the half times for \( 0 \rightarrow P' \) and \( P' \rightarrow P \), the latter observed on removing phenobarbital from the medium (Fig. 4). The half time of the fall from \( P' \) to \( P \) (no phenobarbital) equals the half time of the rise (with phenobarbital) from \( 0 \) to \( P' \), and so again it is shown that \( k = k' \) and that \( P' > P \) because \( S' > S \). The mechanisms by which phenobarbital affects the synthesis and degradation of UDP-glucuronyltransferase do not appear to change during culture but remain characteristic of embryo age (Figs. 1 and 3). Thus, these mechanisms are probably present in embryo liver at the onset of culture and change in vivo between day 5 and 11.

Previous work suggests that the premature development of UDP-glucuronyltransferase activity in cultured chick embryo liver is initiated by removing the tissue from an inhibitory embryonic environment (Skea and Nemeth, 1969). Increase in enzyme activity could depend on a parallel increase (or activation) of a component directly involved in the synthesis of enzyme, or on a process completed during the initial hours of culture. We have tentatively adopted the latter view because the rate of enzyme synthesis in cultured 5-day liver appears to remain constant during the most rapid increase of enzyme activity (20–68 hr of culture, Figs. 6 and 7), which would suggest that a continuing increase in a rate-limiting component involved in enzyme synthesis does not occur.

UDP-glucuronyltransferase activity is located in endoplasmic reticulum. The amounts of rough endoplasmic reticulum increase in 5- and 11-day embryo liver during organ culture (Benzo and Nemeth, 1971; F. Rheingold and B. Burchell, 1972, unpublished observations). Phenobarbital added to medium (3.5 mm) augments these increases, and in 11-day embryo liver also markedly reduces the number of attached ribosomes (F. Rheingold and B. Burchell, 1972, unpublished observations). That phenobarbital influences enzyme development differently in 5- and 11-day embryo liver during culture may be related to this ribosome-stripping effect.

We wish to thank Mr. W. Meldrum for skilled technical help.

This work was partly supported by grants from the U. S. Public Health Service (HD 00373) to Dr. Nemeth, from the Medical Research Council of Great Britain to Dr. Dutton, and from the Science Research Council of Great Britain to Dr. Burchell.

Received for publication 3 February 1972, and in revised form 17 July 1972.

REFERENCES

Benzo, C., and A. M. Nemeth. 1971. J. Cell Biol. 48:235.

Berlin, C. M., and R. T. Schimke. 1965. Mol. Pharmacol. 1:149.

Burchell, B., G. J. Dutton, and A. M. Nemeth. 1971. Biochem. J. 125:23P.

Dutton, G. J., K. B. Hanson, and B. Burchell. 1970. Biochem. J. 120:15P.

Dutton, G. J., and V. Ko. 1966. Biochem. J. 99:550.

King, E. J. 1932. Biochem. J. 26:292.

Ko, V., G. J. Dutton, and A. M. Nemeth. 1967. Biochem. J. 104:991.

Lous-Ferdinand, R. T., and G. C. Fuller. 1970. Biochem. Biophys. Res. Commun. 38:811.

Oyama, V. L., and H. Eagle. 1956. Proc. Soc. Exp. Biol. Med. 91:305.

Peacock, A. C., and C. W. Dingman. 1968. Biochemistry. 7:668.

Seifert, J., and H. Remmer. 1971. Biochem. Pharmacol. 20:553.

Skea, B. R., and A. M. Nemeth. 1969. Proc. Natl. Acad. Sci. U. S. A. 67:795.

Storey, I. D. E. 1965. Biochem. J. 95:201.

Ulevitch, R. J. 1971. Ph.D. Dissertation. University of Pennsylvania, Philadelphia.