Precocious Development of UDP-Glucuronyltransferase Activity in Chick-Embryo Liver after Administration of Adrenocorticotrophic Hormone and of certain 11β -Hydroxy Corticosteroids

By JULIAN E. A. LEAKEY, GRAHAM J. WISHART and GEOFFREY J. DUTTON
Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

(Received 30 April 1976)

1. Precocious development of UDP-glucuronyltransferase (EC 2.4.1.17) and of glucuronidation by endogenous compounds of known chemical composition is reported for the first time. 2. This development occurs precociously in chick-embryo liver after administration to the egg of mammalian adrenocorticotrophic hormone, of Synacthen (a synthetic compound possessing adrenocorticotrophic activity), or of certain corticosteroids possessing a hydroxy or an oxo group at C-11. 3. Corticosterone-dependent transferase development parallels the rise of infused corticosterone in plasma, but does not require the presence of embryo pituitary *in ovo*, and is demonstrable in embryo liver explants *in vitro*. 4. Competence of embryo liver transferase to respond to corticosterone (or dexamethasone) begins over days 13-14, the time of competence to respond to grafted pituitary gland. 5. The transferase appearing after treatment with corticosterone or adrenocorticotrophic hormone, like that appearing after pituitary grafting or on natural development and unlike that from the untreated embryo, is markedly activated by membrane-perturbation procedures, suggesting it appears through induction, not activation. 6. Thyroxine and tri-iodothyronine accelerate transferase development after treatment with adrenocorticotrophic hormone or corticosteroid to the rate seen after pituitary grafting. 7. A wide range of other hormones and steroids did not obviously influence transferase development in this system. 8. We suggest that grafted pituitary gland evokes precocious transferase development in embryo liver through production of adrenocorticotrophic hormone and hence of the active corticosteroids; thyrotropin and thyroxine hasten the process. The role of this mechanism in the natural development of UDP-glucuronyltransferase is discussed.

The development of glucuronidation, a major route of conjugation of toxic endogenous and xenobiotic molecules, has been widely studied in young animals, and its induction brought about there by several drugs (for references, see Dutton & Burchell, 1976). The mechanism responsible for its natural induction perinatally has, however, remained obscure. The developing chick embryo is in many respects a more convenient model for tackling the basis of this problem than is the mammalian foetus (see Dutton *et al.*, 1976).

Glucuronidation by chick-embryo liver is virtually absent until hatching, when the activity of the rate-limiting enzyme UDP-glucuronyltransferase (EC 2.4.1.17) increases suddenly to adult values (Dutton & Ko, 1966; Fyffe & Dutton, 1975). Precocious development *in ovo* of this enzyme, and hence of glucuronidation, can be brought about by injection of phenobarbital from day 0 of incubation (Wishart & Dutton, 1975a) or by grafting the cephalic region of the anterior pituitary gland from a hatching or hatched bird on to the chorioallantoic membrane of an embryonated egg (Wishart & Dutton, 1975b).

The latter finding suggested that a factor released into the embryonic circulation from the grafted tissue was responsible for the observed surge in transferase activity, and circumstantial evidence (Wishart & Dutton, 1975b) pointed to adrenocorticotrophic hormone, thyrotrophic hormone or follicle-stimulating hormone as the factor(s) responsible.

We report here evidence that adrenocorticotrophic hormone and certain corticosteroids stimulate precocious development of UDP-glucuronyltransferase activity and glucuronidation in chick embryo liver, and may be principally responsible for the surge of this activity observed when pituitary glands from chick or mammal are grafted. Preliminary accounts of part of this work have been published (Leakey & Dutton, 1975, 1976).

Materials and Methods

Tropic hormones were obtained as described previously (Wishart & Dutton, 1975b). L-Thyroxine, 3,3',5-tri-iodo-L-thyronine (sodium salt), oxytocin, insulin, glucagon, adrenaline, noradrenaline, di-

butyryl cyclic AMP and all steroids were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Thyrotropic-hormone-releasing hormone and prolactin were from Calbiochem, Hereford, U.K. Synacthen, a polypeptide of amino acids linked as in the first 24 amino acids of natural mammalian adrenocorticotrophic hormone, and of which 1 mg is equivalent to 10 i.u. of the hormone, was from CIBA Laboratories, Horsham, Surrey, U.K. [4-¹⁴C]-Corticosterone was from The Radiochemical Centre, Amersham, Bucks., U.K. Embryonated eggs were obtained and incubated as described previously (Wishart & Dutton, 1975b). Hypophysectomy of 36 h embryos, organ culture and injection *in ovo* were as described by Betz (1965), Burchell *et al.* (1972) and Wishart & Dutton (1975a) respectively.

Continuous infusion of compounds into eggs was by the method of Leakey & Dutton (1975). In this procedure, a cheap commercial plastic fermentation lock (Boots, Nottingham, U.K.) is sealed into an opening above the air space; the dissolved compound is placed in the chamber of the lock and travels by capillarity along a filter-paper strip on to the exposed chorioallantoic membrane; controls are treated identically, with solvent only. 'Infusion for 4 days from day 13 to day 17' means infusion from 21:00h on day 13 until 21:00h on day 17 (96h). Egg incubations began at 21:00h on day 0. Talc pellets containing the compound under test were prepared by the method of Ungar & Van Pilsom (1966) by using a 25-ton press (Specac, Sidcup, Kent, U.K.), and placed on the exposed chorioallantoic membrane.

Radioactive steroid was used as follows. [4-¹⁴C]-Corticosterone solution (as received) was evaporated to dryness and re-dissolved in ethanol. Samples were added to unlabelled compound to give a final concentration of 0.04 μ mol of corticosterone/ml (0.05–0.10 μ Ci/ml) in Earle's balanced salt solution (Flow Laboratories, Irvine, Scotland, U.K.) containing final concentrations of 1% (v/v) ethanol and 0.2% bovine serum albumin (Sigma). Some 0.5 ml of this solution was infused into the egg per day for 4 days. (a) Plasma radioactivity or (b) plasma corticosterone was then determined on 0.5 ml of plasma from each embryo; (a) used the extraction method of Kalliecharan & Hall (1974); when the extract of plasma was added to the scintillation vial 96–99% of the radioactivity was found in the extract. In (b) the extract from (a) was concentrated *in vacuo* and chromatographed on silica gel (Bennett & Heftmann, 1962) in ethyl acetate/chloroform/water (9:1:1, by vol.). After scanning with a RTLS-1H thin-layer scanner (Panax Equipment, Redhill, Surrey, U.K.) by using the stock corticosterone solution as standard, the corticosterone-containing area was scraped off, extracted twice with methylene chloride and the extract added to scintillation vials; the rest of the plate's radioactive area was treated similarly. Samples in

vials were counted for radioactivity on a Unilux II scintillation counter (Nuclear-Chicago), with Aquasol (New England Nuclear Inc., Boston, MA, U.S.A.) as scintillant. Counts ranged from 200 to 1000 d.p.m., depending on steroid concentration; background was 30–50 d.p.m., depending on experiment. Counting was for 10 min. Counting efficiency was 50–60%, measured from channel ratios. Approximate concentration of added corticosterone was estimated from the specific radioactivity of a stock solution put through the same extraction and assay procedure, and from the fraction of total label (always >50%) travelling as corticosterone on chromatography; for these approximations, isotope dilution by endogenous plasma corticosterone (relatively much less than infused steroid in plasma) was ignored.

UDP-glucuronyltransferase activity towards *o*-aminophenol was assayed in 2-s sonicates or gently prepared homogenates by the procedure of Winsnes (1969). Sonication when required was performed on the homogenates with a Dawe Soniprobe 1130A (6 mm micro-tip) at step 2. Glucuronidation of *o*-aminophenol in slices was measured by the method of Dutton & Storey (1962). Assay duplicates when performed did not differ more than $\pm 5\%$; usually single samples from duplicate eggs were used, each assayed with and without added UDP-glucuronic acid. Reaction rates were linear. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Results are expressed as mean $\pm$ s.d. unless otherwise indicated.

Results

Administration of hormone

The inconclusive results after injection of a hormone, as distinct from a xenobiotic such as phenobarbital, into the egg have been described (Wishart & Dutton, 1975b; Leakey & Dutton, 1975, 1976). Development of a simple infusion method (Leakey & Dutton, 1975; see the Materials and Methods section) ensured reasonably reproducible findings, and allowed some measure of the amount of hormone in plasma under conditions of enzymic response. Unless otherwise stated, results in the present paper were obtained with the infusion technique. It should be noted that more of the compound is administered than reaches the plasma, much of it being sequestered in yolk; e.g. concentration of infused corticosterone there could reach 8 times that in plasma.

Tropic hormones

As previous work (Wishart & Dutton, 1975b) had suggested that the active factor produced by the pituitary graft might be adrenocorticotrophic, thyrotrophic

Table 1. *Effect of tropic hormones on embryo/liver UDP-glucuronyltransferase*

Hormones were dissolved in sterile balanced salt solution and infused (see the Materials and Methods section) on to chorio-allantoic membrane of egg from days 13–17, at 0.5 ml/24 h. Assays are as in the Materials and Methods section. The number of embryos are in parentheses. Where s.d. values would not be informative, range and arithmetical mean is given. For brevity, results are grouped according to amounts administered; they were dose-dependent except where marked (*). Typical enzyme activity (nmol of *o*-aminophenyl glucuronide/h per mg of protein) of embryo livers in pituitary-grafted eggs was 20–150, and in hatched birds 1 week or older, 30–50.

Hormone	Total amount administered	<i>o</i> -Aminophenyl glucuronide formed (nmol/h per mg of protein)
Adrenocorticotrophic hormone (pig)	2–30 i.u. 45–150 i.u. 200 i.u.	1.4 ± 1.0 (4) 17.7 ± 2.8 (7) 47.5 ± 14.7 (4)
Follicle-stimulating hormone (pig)	30–100 i.u.	<5.0* (5)
Growth hormone (pig)	1.0–8.0 i.u.	<5.0* (4)
Thyrotropic hormone (ox)	10–60 i.u.	1.2–10.8, 4.5* (7) (range and mean)
Thyrotropic hormone releasing hormone (synthetic)	1.2–5.5 µmol	<6.0* (6)
Prolactin (human, assay standard)	18–50 ng	0.6–3.0* (3) (range)
Prolactin (sheep, crude preparation)	1.2–2.5 mg	0.5–2.4, 1.6* (4) (range and mean)
Synacthen	3.3–5.0 mg	8.1–56.5, 27.8* (5) (range and mean)
None (solvent infusion)	0–2 ml	<6.0* (10)

or follicle-stimulating hormone, these and other tropic hormones were administered to the embryo. Table 1 indicates that only the adrenocorticotrophic hormone preparation evoked precocious development of UDP-glucuronyltransferase in host embryo liver, and it raised the enzyme activity to that noted in embryos exposed to pituitary grafts. An impurity in the hormone preparation is not likely to be responsible, for similar stimulation was given by Synacthen, the artificially synthesized active moiety of adrenocorticotrophic hormone. Thyrotropic hormone, prolactin and follicle-stimulating hormone were too expensive or insoluble for use at higher dosage, and before investigating the effect of other hormones it was decided to follow the role of adrenocorticotrophic hormone by administering corticosteroids.

Steroid hormones

A variety of steroids was administered, chiefly by continuous infusion. Table 2 shows that precocious development of transferase occurred with those naturally occurring or synthetic steroid hormones possessing a hydroxy group at C-11 (corticosterone, cortisol, aldosterone) or, less effectively, an oxo group there (cortisone), provided that no hydroxyl group exists at C-3 (tetrahydrocortisol, tetracortisol). Steroid specificity was not followed further at this time.

Experiments with [4-¹⁴C]corticosterone yielded some information on plasma concentrations achieved by the infusion technique. The minimum amount of corticosterone needed for reproducible response in transferase activity after infusion for 4 days (days

13–17) was 0.08 µmol. On using [4-¹⁴C]corticosterone under these conditions, the label was found in plasma within 24 h. Within 4 days, plasma corticosterone had reached 300 pmol/ml, 5 times that reported (Kalliecharan & Hall, 1974) in untreated embryos of that age. The rise in amount of labelled corticosterone in plasma was always associated with precocious development of the transferase, which lagged 1.5–2 days behind increase in plasma ¹⁴C (Fig. 1).

A single injection of corticosterone acetate (in the same solvent) into the air space also evoked transferase activity, but the method appeared less sensitive than infusion. Injection of 0.8–1.0 µmol was needed before embryo liver homogenates, assayed 4 days later, could form 10–12 nmol of glucuronide/h per mg of protein; at the maximum non-lethal dosage of 2 µmol, the liver formed 30–50 nmol/h per mg of protein. However, 0.08 or 1 µmol of corticosterone after 4 days' infusion allowed formation of 30–70 or 150 nmol of glucuronide/h per mg of protein respectively.

In contrast, dexamethasone, believed to possess a long biological half-life (Bunim *et al.*, 1958) was highly effective by injection, 0.04 µmol raising transferase activity to 27 of the above units within 3 days.

Corticosterone and cortisol, when administered in talc pellets (see the Materials and Methods section) also precociously evoked the transferase, though not so efficiently as when infused.

Corticosterone and overall glucuronidation

UDP-glucose dehydrogenase is present at near adult activity in chick embryo liver (Fyffe & Dutton,

Table 2. *Effect of steroids on embryo liver UDP-glucuronyltransferase*

Steroids were dissolved in ethanol and added to balanced salt solution with bovine serum albumin to give final concentrations as shown in 1% (v/v) ethanol and 0.2% albumin. This solution was infused and enzyme assayed as described for Table 1. Results are expressed as for Table 1, except that within the groupings given, dose-dependence was not significant. * Indicates that at higher concentrations 2% (v/v) ethanol was present. The following steroids, in amounts noted, did not raise enzyme activity above the control range: deoxycorticosterone acetate (21-hydroxy-4-pregnene-3,20-dione acetate), 1.0–4.0 μ mol (6); tetrahydrocortisol (3 α ,17 α ,21-trihydroxy-5 β -pregnane-11,20-dione), 0.4–2.0 μ mol (9); tetrahydrocortisol (3 α ,11 β ,17 α ,21-tetrahydroxy-5 β -pregnan-20-one), 0.9–2.0 μ mol (6); pregnenolone (3 β -hydroxy-5-pregnen-20-one), 1.0–2.0 μ mol (5); progesterone (4-pregnene-3,20-dione), 0.6–4.0 μ mol* (8); testosterone acetate (17 β -hydroxy-4-androsten-3-one acetate), 0.9–2.0 μ mol (4); oestriol [1,3,5(10)-oestratriene-13,16 α ,17 β -triol], 0.9–4.0 μ mol* (7).

Steroid	Amount administered (μ mol)	<i>o</i> -Aminophenyl glucuronide formed (nmol/h per mg of protein)
Corticosterone acetate	0.02	3.0, 2.0
(11 β ,21-dihydroxy-4-pregnene-3,20-dione acetate)	0.08–0.2	43.7 $\pm$ 22.0 (6)
	0.6–1.0	78.6 $\pm$ 47.8 (8)
Corticosterone	0.8–1.0	44.6 $\pm$ 8.6 (4)
Cortisol acetate	0.02	1.7, 1.0
(11 β ,17,21-trihydroxy-4-pregnene-3,20-dione acetate)	0.1	8.4, 7.0
	0.2	24.1 $\pm$ 10.2 (4)
	1.0	71.0 $\pm$ 42.3 (5)
Aldosterone	0.8	22.2, 23.3
(11 β ,21-dihydroxy-3,20-dioxo-4-pregnen-18-al)		
Cortisone acetate	1.0	0.9, 4.2, 4.9
(17 α ,21-dihydroxy-4-pregnene-3,11,20-trione acetate)	2.0	19.3, 18.7, 131.3

1975). It was therefore not surprising that corticosterone infusion (0.4 μ mol over 4 days) increased formation of *o*-aminophenyl glucuronide by embryo liver slices from 0.8 $\pm$ 0.5 (4) to 8.1 $\pm$ 1.6 (4) nmol/h per mg of protein (results expressed as in Table 1). Hatched-chick liver activities (4 days old) were 3.8 $\pm$ 1.3 (4) nmol/h per mg of protein.

Thyroxine and tri-iodothyronine

The role of thyrotropic hormone was studied further by examining the effect of infused thyroxine or tri-iodothyronine. At high doses (Table 3) application of thyroxine occasionally raised transferase activity to the lower 'positive' values noted with corticosterone. As such response required an almost lethal dose of thyroxine, it may have incurred a stress reaction, with consequent liberation of corticosteroids. The role of thyroxine by itself was thus difficult to determine. Its synergistic effect with corticosterone was therefore investigated.

Synergism of corticosterone and thyroxine

Concentrations of thyroxine that killed the embryo within 4 days (see Table 3), failed to raise transferase activity within 2 days (Table 4); when applied with

corticosterone they speeded the embryo's response to the steroid so that activities previously attained after 4 days with a given concentration of the steroid alone (as in Table 2 or Fig. 1) were now reached within 2 days (Table 4). Similar results were found with tri-iodothyronine. This faster rate of precocious transferase development equalled that seen with pituitary grafts, suggesting their effect is exerted through both adrenocorticotrophic and thyrotropic hormones.

Competence to respond to corticosteroids

Wishart & Dutton (1975*b*) noted that response to a grafted pituitary gland occurred only on or after days 13–14. Response to corticosterone also occurs on or after days 13–14. When applied in each case 4 days previously (Table 5), corticosterone clearly has raised transferase activity by day 16, but not by day 14; by day 15 it is beginning to raise activity. As Fig. 1 suggests a delay of 1–2 days before induction is detectable, response is likely to develop over days 13–14. Dexamethasone behaved like corticosterone in evoking response only after days 13–14, and unlike other drugs such as phenobarbital or allylisopropylacetamide (Wishart & Dutton, 1975*a*), towards which the embryo displays no obvious age-limit of competence.

Fig. 1. Corticosterone infusion and UDP-glucuronyltransferase activity

[4-¹⁴C]Corticosterone was infused into eggs from 21:00h on day 13; plasma radioactivity (Δ) and liver transferase activity (▲) were assayed in the one embryo then and at 24h intervals until 21:00h on day 17. Two embryos were used each day and results indicate mean and range. Methods are as in the text.

Site of action of corticosterone

Response of transferase to corticosterone appeared independent of host pituitary gland, for it occurred to the same extent in hypophysectomized embryos, sham-operated controls and normal embryos. Corticosterone appeared to act directly on the liver cell. This was shown by cultivating explants from 15-day-embryo liver for 3 days in the presence of 15 μM-corticosterone and 1 μM-thyroxine. A small, but reproducible, activity above controls exposed only to 1 μM-thyroxine was noted. Because of background activity from the spontaneous induction of transferase always noted in embryonic liver explants (Ko *et al.*, 1967; Skea & Nemeth, 1969), larger steroid-dependent inductions in culture might not be evident (see Leakey & Dutton, 1976).

Table 3. Effect of thyroid hormones on embryo liver UDP-glucuronyltransferase

For methods of application, assay and expression of results, see Table 2. Results marked (*) are placed in order of increasing dose.

Compound	Amount administered (nmol)	<i>o</i> -Aminophenyl glucuronide formed (nmol/h per mg of protein)
Tri-iodo-L-thyronine	0.8–4.0	1.0 ± 0.5 (7)
	>5.0	Dead (10)
L-Thyroxine	6.0–9.5	11.8, 1.8, 4.4, 3.2*
	11–15	28.8, 1.4, 3.2, 10.9, 1.1*
	>15	Dead (8)

Table 4. Synergistic effect of thyroid hormones applied with corticosterone

Application, assay and expression of results as in Table 2, except that infusion was for 48h over days 14–16; assays were performed on day 16.

Compounds administered		<i>o</i> -Aminophenyl glucuronide formed (nmol/h per mg of protein)
L-Thyroxine (nmol)	Corticosterone acetate (μmol)	
16–80	0	1.8 ± 0.9 (5)
0	0.5–1.1	4.5 ± 1.8 (14)
20–40	0.5–1.0	43.2 ± 18.6 (9)

Table 5. Age of response of embryo liver UDP-glucuronyltransferase to corticosterone

Corticosterone was infused for 4-day periods into eggs at various stages of incubation, and transferase activity assayed in embryo liver at the end of the 4th day. Methods and expression of results are as for Table 2.

Days in 4-day period	Corticosterone added (μmol)	<i>o</i> -Aminophenyl glucuronide formed (nmol/h per mg of protein)
9–13	0.2–0.9	2.0 ± 1.3 (5)
9–13	>1.0	Dead
10–14	0.08–0.1	2.2 ± 1.3 (12)
10–14	0.2	6.2, 6.3
11–15	0.07–0.09	5.3 ± 3.4 (5)
11–15	0.1	17.3
11–15	1.0	13.7, 65.9
12–16	0.07–0.10	25.3 ± 8.9 (4)
13–17	0.08–0.10	51.3 ± 23.0 (4)

Other hormones

Administration of other hormones is summarized in Table 6. Insulin and oxytocin appear completely inactive. The single result above the control range with

Table 6. *Effect of non-steroid, non-tropic hormones on embryo liver UDP-glucuronyltransferase*

Compounds were applied as for Table 1, except those marked (*), which were added in sterile 0.9% NaCl containing 1% (w/v) ascorbic acid. Other conditions and expression of results were as for Table 2.

Compound	Amount administered	<i>o</i> -Aminophenyl glucuronide formed (nmol/h per mg of protein)
Insulin	0.2–35 i.u.	3.2 ± 1.6 (10)
	>35 i.u.	Dead (8)
Glucagon	0.4–3.6 mg	1.6 ± 0.8 (10)
	1.6 mg	11.2
	3.8 mg	13.2
	4.0 mg	9.1
Dibutyl cAMP	0.2–1.5 μ mol	0.6 ± 0.4 (4)
	2 μ mol	16.6, 0.3
Oxytocin	1.7–180 i.u.	0.9 ± 0.6 (7)
	200 i.u.	Dead (2)
Adrenaline*	2–20 nmol	1.7 ± 0.9 (8)
	100–250 nmol	3.5 ± 2.6 (8)
	>250 nmol	Dead (8)
Noradrenaline*	50–300 nmol	1.6 ± 0.9 (7)
	500 nmol	16.6, 3 Dead

Fig. 2. *Activation of UDP-glucuronyltransferase*

Liver homogenates were prepared from 17-day chick embryo (●), from 17-day chick embryo infused with 0.5 μ mol of corticosterone from day 13 (Δ), and from 5-day-old hatched chick (○). Samples of homogenates were then subjected to increasing periods of sonication, in 15 s bursts or less, at 0°C. Transferase activity was then assayed in all samples. Methods are as in the text. Results are from single assays.

jection of these latter compounds proved even less conclusive. So far no compound reproduces the response reported with the 11 β -hydroxy corticosteroids.

Activation of enzyme

Liver UDP-glucuronyltransferase is markedly latent in adult chicken and adult, infant or late-foetal mammals (see Dutton *et al.*, 1976). To check that administration of corticosterone did not result in microsomal membrane change leading to readier activation of the enzyme during its (gentle) preparation and assay, the following experiments were performed. Liver homogenates from control and corticosterone-treated embryos, from pituitary-grafted embryos and from adult birds, were subjected to membrane-perturbation procedures for increasing periods of time, and transferase activities compared. Liver transferase activity from untreated embryos always remained unactivatable by a range of perturbation procedures, whereas the activity evoked by corticosterone (*in ovo* or in culture) or by grafting, like that of the adult bird, increased markedly to a peak with increased perturbation. Fig. 2 shows a typical result. We conclude that activation of a latent embryo enzyme does not explain the stimulation of transferase activity by corticosterone, pituitary-grafting or hatching; and that induction of new and activatable enzyme [as demonstrated for phenobarbital-stimulated transferase (Burchell *et al.*, 1972)] is more likely.

noradrenaline probably derives from stress. Similar raised values with glucagon and possibly dibutyl cAMP may warrant further investigation. In-

Discussion

The above results indicate that adrenocorticotrophic hormone, and certain corticosteroids possessing a 11 β -hydroxyl or 11-oxo group, can precociously evoke development of UDP-glucuronyltransferase activity. These results appear to be the first to link known endogenous compounds with this process. They further suggest that the major responsible 'factor' released by the pituitary grafts of Wishart & Dutton (1975b) is adrenocorticotrophic hormone, with thyrotrophic hormone speeding its effect. The possibility of this mechanism operating *in vivo* over hatching may now be discussed.

Wishart & Dutton (1975b) found pituitaries from hatching birds to be more effective producers of 'factor' than those from younger or older birds, and allowing time for vascularization of graft and synthesis of enzyme they considered that release of factor from grafted pituitary reproduced its natural release from the embryo's own pituitary during hatching at 20–21 days. If the factor is adrenocorticotrophic hormone one might expect to find the concentration of this hormone raised in plasma during hatching. However, it has not yet been measured in embryo plasma. Embryo pituitary secretes increasing amounts of adrenocorticotrophic hormone up to 16 days (Pedernera, 1972), but the study was not continued during hatching.

As our results indicate that corticosterone concentrations need only be some 5 times higher than normal to initiate precocious transferase development at 17 days, one might also expect raised plasma corticosteroid concentration over hatching. Again, endocrinological evidence is incomplete. Kalliecharan & Hall (1974) found a peak of total plasma corticosteroid concentration over 15–17 days, but they too ignored the hatching period and they assayed free and bound corticosteroid together; if only free corticosteroid is biologically active (see Brooks, 1975), their results may not be relevant here. Wise & Frye (1973) largely measured free corticosterone and did find a peak at 20 days; but their assay could have included other steroids such as progesterone, known (Kalliecharan & Hall, 1974) to peak at that time. Lacking further evidence, therefore, we may postulate that UDP-glucuronyltransferase induction after hatching is initiated principally by the release of adrenocorticotrophic hormone during hatching and the availability of 11 β -hydroxy corticosteroids to the liver cell at that time. It should be noted that the 'competence' experiments reported above suggest competence at that age is not in supply of corticosteroid via the pituitary–adrenal axis, but in acceptability of the corticosteroid, possibly for transport at or in the liver cell. That corticosteroid operates directly at the liver cell is suggested by the culture experiments. Thyroxine may contribute *in vivo* by

assisting corticosteroid transport in plasma (Fortier *et al.*, 1970) or in the liver itself.

Though rise of the transferase activity from virtually zero to adult values is the most dramatically precocious surge in development that we have noted after graft of pituitary gland or after infusion of adrenocorticotrophic hormone or corticosteroid, it is not the only one. We have found (G. J. Wishart, J. E. A. Leakey & G. J. Dutton, unpublished work) a similarly controlled surge from embryonic to immediately post-hatching values with the microsomal mixed-function mono-oxygenase enzymes aniline hydroxylase (EC 1.14.14.1) and aminopyrine demethylase, and the lag with these enzymes is some 24 h less than observed with the transferase, consistent with the longer half-life of this enzyme (Burchell *et al.*, 1972).

We thank Mrs. Agnes Donald for skilled assistance, Eastwood Hatcheries for invaluable help in obtaining eggs, N.I.H. Hormone Distribution Program for a gift of tropic hormones, Dr. J. K. Grant for a gift of Synacthen, and the M.R.C. for a studentship to J. E. A. L. and a grant to G. J. D.

References

- Bennett, R. D. & Heftmann, E. (1962) *J. Chromatogr.* **9**, 348–353
- Betz, T. W. (1965) Ph.D. Thesis, University of Illinois
- Brooks, R. V. (1975) in *Biochemistry of Steroid Hormones* (Makin, H. L. J., ed.), pp. 289–311, Blackwell, Oxford
- Bunim, J. J., Black, R. L., Lutwak, L., Peterson, R. E. & Whedon, G. D. (1958) *Arthritis Rheum.* **1**, 315–319
- Burchell, B., Dutton, G. J. & Nemeth, A. M. (1972) *J. Cell Biol.* **55**, 448–456
- Dutton, G. J. & Burchell, B. (1976) in *Progress in Drug Metabolism* (Bridges, J. W. & Chasseaud, L. F., eds.), vol. 2, John Wiley, London, in the press
- Dutton, G. J. & Ko, V. (1966) *Biochem. J.* **99**, 550–556
- Dutton, G. J. & Storey, I. D. E. (1962) *Methods Enzymol.* **5**, 159–164
- Dutton, G. J., Wishart, G. J., Leakey, J. E. A. & Goheer, M. A. (1976) in *Proc. Symp. Drug Metab., Guildford, 1976* (Parke, D. V. & Smith, R. L., eds.), Taylor and Francis, Basingstoke, in the press
- Fortier, C., Labrie, F., Pelletier, G., Raynaud, J.-P., Delgado, A., Labrie, C. & Ho-Kim, M.-A. (1970) in *Control Processes in Multi-Cellular Organisms* (Wolstenholme, G. E. W. & Knight, J., eds.), pp. 178–200, Churchill, London
- Fyffe, J. & Dutton, G. J. (1975) *Biochim. Biophys. Acta* **411**, 41–49
- Kalliecharan, R. & Hall, B. K. (1974) *Gen. Comp. Endocrinol.* **24**, 364–372
- Ko, V., Dutton, G. J. & Nemeth, A. M. (1967) *Biochem. J.* **104**, 991–998
- Leakey, J. E. A. & Dutton, G. J. (1975) *Biochem. Biophys. Res. Commun.* **66**, 250–254
- Leakey, J. E. A. & Dutton, G. J. (1976) *Biochem. Soc. Trans.* **4**, 261–262

- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Pedernera, E. A. (1972) *Gen. Comp. Endocrinol.* **19**, 589-591
- Skea, B. R. & Nemeth, A. M. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 795-802
- Ungar, F. & Van Pilsum, J. F. (1966) *Endocrinology* **79**, 1143-1148
- Winsnes, A. (1969) *Biochim. Biophys. Acta* **191**, 279-291
- Wise, P. M. & Frye, B. E. (1973) *J. Exp. Zool.* **185**, 277-292
- Wishart, G. J. & Dutton, G. J. (1975a) *Biochem. Pharmacol.* **24**, 451-454
- Wishart, G. J. & Dutton, G. J. (1975b) *Biochem. J.* **152**, 325-331