PRECOCIOUS DEVELOPMENT IN VIVO OF UDP-GLUCURONYLTRANSFERASE AND ANILINE HYDROXYLASE BY CORTICOSTEROIDS AND ACTH, USING A SIMPLE NEW 'CONTINUOUS FLOW' TECHNIQUE

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Summary: Precocious induction from zero to above adult levels of UDPglucuronyltransferase activity towards o-aminophenol has been demonstrated in chick embryo liver following administration of corticosterone, hydrocortisone, aldosterone or ACTH but not of progesterone, testosterone, insulin, thyroxine or other agents tried. Precocious development to adult levels of aniline hydroxylase also occurred. This is the first demonstration of known endogenous compounds initiating development of the transferase. Its relationship to natural development is discussed. Demonstration was made possible by allowing the active agents to flow continuously down a paper strip on to the exposed chorioallantoic membrane. This new technique, superior to repeated injection of compounds with short biological half-life, is described and its potentialities suggested.

INTRODUCTION

We have previously shown in this laboratory (1) that microsomal UDPglucuronyltransferase (E.C. 2.4.1.17) and the mixed-function oxidase aniline hydroxylase (E.C. 1.14.14.1) will develop precociously in chick embryo liver following the grafting of pituitary gland from chickens or hatching birds on to the chorioallantoic membrane. We pointed out the importance of studying the natural induction of these enzymes, whose delayed development in man and other vertebrates gives rise to toxicity from many routine metabolites, drugs and environmental pollutants. We suggested that this first demonstration of an endogenous perinatal inducing mechanism would lead to identification of the molecules involved.

Grafting had been undertaken because we could not conclusively achieve induction of the transferase by injecting endocrine extracts or known hormones

(1,2,3). Too large an injection into airspace, yolk-sac or embryo killed the animal, and smaller doses were ineffective possibly from rapid breakdown. Repeated injections every 3 hr over a 2-4 day period occasionally produced induction by a few endogenous compounds, but this procedure was time-demanding and uncertain. It appeared most unsuitable for the screening of possible inducing compounds produced by, or in response to, the graft.

We describe here a new simple and rapid method of ensuring continuous delivery of the compound under test, in a manner approaching more closely to the physiological release of hormones occurring in the grafted host. We report briefly the use of this method to demonstrate the first induction of UDPglucuronyltransferase, from virtually zero to above adult levels, by known endogenous compounds; aniline hydroxylase was also raised up to or above adult levels using this method, which should find wide application in similar work with compounds of short biological half-life.

METHODS

A thin strip of chromatography paper cut to a point at the lower end was placed (Fig. 1) in the chamber of a plastic fermentation lock (Boots Ltd., Nottingham, England). The shell of a 13-day embryonated White Leghorn egg was opened above the air-space and the inner shell membrane cut back to expose 1 sq cm of chorioallantoic membrane. The lock, complete with strip, was then taped to the egg as shown in Fig. 1, so that 1 mm of the strip tip lay centrally on the exposed chorioallantoic membrane. 2 to 3 ml of sample solution was placed in the lock chamber and final adjustments ensured that solution from the strip would flow directly on to the membrane. The egg was then returned to incubation. Control eggs were identically treated, but the solution did not contain the test compound. Asepsis is described under Fig. 1.

This technique preserves high humidity in lock and air-space, but avoids the 'drowning' which occurs if the egg itself is incubated above 55-60% humidity. Type of paper and strip width allow adjustment of flow

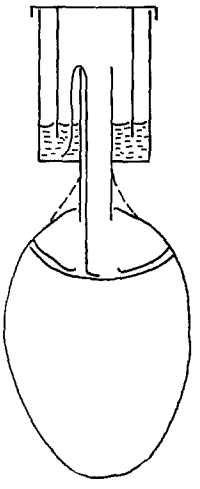

Figure 1. Diagram of 'continuous-flow' technique for application of hormones etc. *in ovo*. The plastic fermentation lock, sterilised by immersion in 70% aqueous ethanol for 4 hr, is washed thoroughly with sterile distilled water and dried in warm air under sterile conditions. The autoclaved paper strip is placed in the lock and the whole assembly positioned as shown, so that the strip rests on the exposed chorio-allantoic membrane of an ethanol-washed egg. Sample solution sterilised by ultra-filtration is added last. See text for more general procedure.

rate. In the experiments reported, 3 x 15 mm Whatman No. 1 chromatography paper delivered 0.5 - 0.7 ml solution per 24 hr, all of which appeared absorbed by the embryo at this rate. Control embryos survived at least 5 days of this treatment and developed normally. Hormones and reagents were from Sigma. Enzyme assays were as described previously (1).

RESULTS AND DISCUSSION

Table 1 gives typical results, illustrating the superior convenience and response of the constant flow method. When given by this technique or by injection the following compounds evoked no rise in transferase: progesterone, testosterone acetate, estriol, phenobarbital [all at up to 1 μmole; phenobarbital induces at higher dosage (5)], insulin (up to lethal dose), $N^6O^{2'}$-dibutyryl cyclic AMP (0.2 μmole). Thyroxine, near its lethal levels,

Table 1. Development of UDPglucuronyltransferase activity in livers of chick embryos following administration of various compounds to the egg

Total amount of compound administered	Method of administration	nmol o-aminophenyl glucuronide formed mg protein^{-1} hr^{-1}
None	-	0 - 2.7
5 μmoles Corticosterone acetate	(a)	0 - 2.0
0.4 μmoles Corticosterone acetate	(b)	6.2, 11.6
0.2 μmoles Corticosterone acetate	(c)	15.5
1.0 - 1.4 μmoles Corticosterone acetate	(c)	26.9, 61.2, 137.6, 23.1, 63.9, 21.9
1.0 μmoles Hydrocortisone acetate	(c)	35.1, 48.1
0.8 μmoles Aldosterone	(c)	23.3, 22.2
100 i.u. ACTH	(a)	2.5, 1.2
50 i.u. ACTH	(c)	18.9
100 i.u. ACTH	(c)	19.9
200 i.u. ACTH	(c)	38.5, 28.0, 62.0, 61.4

The compounds were administered to 13-day embryos by (a) a single injection into airspace, (b) repeated air-space injections every 3 hr for 3½ days, (c) constant flow method. For the latter, Eagle's Minimum Essential Medium (4) containing 0.25% (wt./vol.) bovine serum albumin was placed in chamber of fermentation lock and flowed on to chorioallantoic membrane at 0.5 - 0.7 ml per 24 hr. Controls received this solution only; test eggs this solution plus hormones (in water or ethanol); final ethanol concentration was 1% (wt./vol.). Enzyme was assayed in homogenates of embryo liver after 4 days; adult activity was 30-60 of above units. Ethanol as given did not affect enzyme activity.

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stimulated the transferase, but not above 30% adult value; FSH and TSH appear so far less effective than ACTH. Aniline hydroxylase activity was raised 3 to 6-fold to post-hatching levels by ACTH and corticosterone, but not by the non-corticoids tested.

These results indicate that aldosterone, corticosterone, hydrocortisone

and ACTH initiate development of UDPglucuronyltransferase in 13-day chick embryo and stimulate that of aniline hydroxylase. They suggest that the factor responsible for precocious development of these enzymes in embryos exposed to grafts of the cephalic portion of pituitary pars distalis (1) is ACTH, operating through the host adrenal gland; as release of this factor coincides with onset of hatching (1; G.J. Wishart and G.J. Dutton, unpublished work), the mechanism reported may initiate the natural surge of these enzymes during the first few days of free life.

The technique described has many applications. For example, yolk-sac retraction has been attributed both to thyroxine and to corticosteroids (6,7,8). On constant-flow, thyroxine but never corticosterone, stimulated grossly premature attempts at yolk-sac retraction.

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