Ontogeny of the glucocorticoid receptor and its relationship to tyrosine aminotransferase induction in cultured foetal hepatocytes

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The glucocorticoid receptor activity that can be detected in the liver from 15-day foetal rats would appear to be associated with the haemopoietic cells. In hepatocytes, purified by culture for 1–2 days from 15-day foetal rats, the glucocorticoid receptor activity is low and dexamethasone does not induce the enzyme tyrosine aminotransferase. If culture is continued both receptor activity and steroid responsiveness are acquired. Cultured hepatocytes from 19-day foetal liver contain receptor from the first day of culture and, furthermore, the subsequent level of response to glucocorticoids is directly correlated with the actual receptor concentration. It would appear that the glucocorticoid receptor is not acquired by hepatocytes until after 18 days of gestation. Nevertheless, the fact that bromodeoxyuridine has no effect on the rate of accumulation of receptor in hepatocytes suggests that the differentiative event leading to the subsequent appearance of the receptor has already occurred before day 15 of gestation. However, the acquisition of the receptor would appear to be dependent on mitosis as cytosine arabinoside can inhibit the process.

The administration of glucocorticoids to post-natal rats causes a marked increase in the activity of hepatic tyrosine aminotransferase (EC 2.6.1.5) but fails to induce the enzyme when administered to foetal rats *in utero* (Sereni *et al.*, 1959; Franz & Knox, 1967; Greengard, 1969; Cake *et al.*, 1973). The unresponsiveness of the foetus *in utero* is not due to impaired hepatic accumulation of the steroid. Singer & Litwack (1971) have shown that, although the liver of foetuses concentrates the hormone less efficiently than those of postnatal animals, enzyme induction would be expected from the amount of hormone accumulated. Both cytoplasmic and nuclear receptors have been demonstrated in foetal liver *in vitro* (Feldman, 1974; Giannopoulos, 1975; Cake *et al.*, 1979) and *in vivo* (Parchman *et al.*, 1978) suggesting that glucocorticoids are unable to induce tyrosine aminotransferase in foetal animals *in utero* despite the presence of a functional glucocorticoid receptor system.

However, these studies have been made using whole liver and it is well-established that foetal rat liver contains, in addition to the parenchymal cells, a large population of haemopoietic cells (Oliver *et al.*, 1963; Greengard *et al.*, 1972). Recently, haemopoietic cells have been shown to be glucocorticoid-sensitive (Golde *et al.*, 1976) and to contain a

glucocorticoid receptor (Cake *et al.*, 1978). Accordingly, the foetal parenchymal cells might fail to respond to steroid because such cells have no receptors, the receptor activity of whole liver being associated exclusively with the haemopoietic cells.

The present paper reports results from experiments with cultured foetal hepatocytes grown under conditions that essentially eliminate contamination by haemopoietic cells. This has enabled the examination of the relationship between the presence of the glucocorticoid receptor and responsiveness of the hepatocytes to glucocorticoids. Since it has been shown that cytosine arabinoside and bromodeoxyuridine block the appearance of tyrosine aminotransferase in cultured foetal hepatocytes (Yeoh & Oliver, 1980), the effect of both of these agents on the accumulation of receptor is included in the present study.

Materials and methods

Animals

Rats of the Wistar albino strain of *Rattus norvegicus* were used. Gestational age was determined from the time of a positive vaginal smear for spermatozoa and is accurate to ± 8 h. These animals have a gestation period of 22 days.

Chemicals

L-[3,5-³H]Tyrosine, [1,2-³H]corticosterone and [1,2-³H]dexamethasone were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. L-Tyrosine and sodium diethyldithiocarbamate were from Merck, Darmstadt, Germany. Pyridoxal 5'-phosphate, 2-oxoglutarate, 2,5-diphenyloxazole, cytosine arabinoside, bromodeoxyuridine, dexamethasone, calf thymus DNA and activated charcoal were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Collagenase (grade II) was purchased from Boehringer Mannheim, Melbourne, Victoria, Australia. 2,4-Dinitrophenylhydrazine was supplied by Ajax Chemical Co., Sydney, N.S.W., Australia, and diaminobenzoic acid by the Eastman Kodak Co., Rochester, NY, U.S.A. Eagle's minimal essential medium was obtained in powder form from Flow Laboratories, Annandale, N.S.W., Australia, and foetal calf serum and guinea-pig complement were from Commonwealth Serum Laboratories, Parkville, Victoria, Australia. Fungizone, penicillin/streptomycin and glutamine were obtained from Grand Island Biological Co., Grand Island, NY, U.S.A.

Hepatocyte isolation and culture

Livers from 15-day foetal rats were removed aseptically and disaggregated in balanced salts solution (Hanks & Wallace, 1949) by dispersion in a Pasteur pipette, as previously described (Yeoh *et al.*, 1979). To increase the yield of cells from livers of 19-day foetal rats a collagenase treatment was included (Yeoh *et al.*, 1979). This modification did not alter the response of the cultured cells to glucocorticoids. Cells were harvested and washed twice in balanced salts solution by centrifugation at 50g for 2min, then suspended in culture medium and dispensed into 90mm-diameter plastic culture dishes previously coated with collagen.

The basal culture medium was modified Eagle's minimal essential medium supplemented with 10% charcoal-treated foetal calf serum, glutamine (2.4mM final concentration), fungizone (2.8µg/ml) and penicillin/streptomycin (57 units/ml and 570µg/ml respectively). To remove endogenous steroids, the foetal calf serum was charcoal-treated before medium supplementation. The serum was incubated for 30min at 37°C in the presence of activated charcoal (50mg/ml of plasma), cooled and then centrifuged (20000g for 20min at 4°C). The charcoal-treated serum was stored frozen. Using a tracer dose of [³H]corticosterone it was shown that this process removed more than 99% of the endogenous corticosteroids.

The cultures were maintained at 37°C in a water-saturated atmosphere of CO₂/air (1:19) and were given fresh medium 24h after plating and thereafter every 48h. The hepatocytes formed a

monolayer attached to the collagen substratum but most of the haemopoietic cells remained in suspension and were discarded when the medium was replaced 24h after inoculation. The residual haemopoietic cells were completely removed by subsequent media replacement.

Dexamethasone dissolved in propylene glycol was added to the medium at a final concentration of 5µM. Cytosine arabinoside and bromodeoxyuridine were dissolved in balanced salts solution, sterilized by Millipore filtration and added to cultures at the time of plating and after addition of fresh medium.

Removal of haemopoietic cells by antibody treatment

Livers from 15–19-day foetal rats were removed and disaggregated by using collagenase treatment as previously described (Yeoh *et al.*, 1979). The isolated cells were incubated with guinea-pig complement (10mg of freeze-dried powder/ml of buffered salts solution) and then with an antiserum raised in rabbits against rat erythrocyte ghosts. Both the complement solution and the antiserum were previously treated with charcoal (as above) to remove endogenous corticosteroids. This antibody treatment caused lysis of the haemopoietic cells and resulted in a hepatocyte cell population essentially free of haemopoietic cells. Less than 1% of the cells were positive to benzidine staining. The hepatocyte preparation was washed several times with buffered salts solution before being assayed for glucocorticoid receptor activity.

Preparation of hepatocyte extracts

Cells were harvested using a Teflon scraper, suspended in balanced salts solution and collected by centrifugation at 1600g for 2min. The cellular pellet was resuspended in either 0.7ml of 0.1M-potassium phosphate buffer, pH 7.4, containing 0.3mM-pyridoxal 5'-phosphate, 1mM-dithiothreitol, 0.5mM-2-oxoglutarate and 1mM-EDTA for enzyme assay or 4vol. of 50mM-Tris/HCl, pH 7.6, containing 3mM-MgCl₂ and 10mM-2-mercaptoethanol for glucocorticoid receptor assay. The cell suspensions were sonicated in a Branson model B 12 sonifier (Branson Instruments, CN, U.S.A.) at 2A for 15s using a microprobe. A sample of the sonicated preparation was taken for DNA estimation. The remainder was centrifuged in a Beckman model L5-50 ultracentrifuge (Beckman Instruments, Palo Alto, CA, U.S.A.) at 300000g_{max} for 20min in an SW 50.1 rotor at 0–4°C.

Tyrosine aminotransferase assay

The enzyme activity was estimated by a modification of the methods of Miller & Thompson (1972) and Lees & Weiner (1975). The initial reaction mixture contained 5mM-2-oxoglutarate, 0.13mM-

pyridoxal 5'-phosphate, 53 mM-diethyl dithiocarbamate and 3 mM-L-tyrosine, in 0.1 M-potassium phosphate buffer, pH 7.4. L-[3,4-³H]Tyrosine (approx. 1 μ Ci) was added to each ml of this mixture. The radioactivity was determined in a 100 μ l sample that was counted in 10 ml of scintillant solution consisting of 180 g of naphthalene, 4 g of 2,5-diphenyloxazole per litre of dioxan. The reaction mixture (100 μ l) was pre-incubated at 37°C in a shaking water bath and the reaction started by addition of enzyme. The reaction was stopped by the addition of 50 μ l of 15 mM-2,4-dinitrophenylhydrazine in 1 M-H₂SO₄. Blanks were prepared by addition of this solution before the addition of enzyme. The product was extracted into 1 ml of toluene/ethyl acetate (10:1, v/v) and the solution was centrifuged at 1600 g for 15 min. The organic phase (0.7 ml) was added to 0.3 M-HCl (0.5 ml), mixed and centrifuged. The radioactivity in the organic phase was determined in an Isocap 300 liquid-scintillation counter (Nuclear-Chicago) using a scintillant containing 6 g of 2,5-diphenyloxazole and 5.0 ml of ethanol per litre of toluene. The efficiency of counting was 40%. Tyrosine aminotransferase is expressed as nmol of *p*-hydroxyphenylpyruvate/h per μ g of DNA.

Glucocorticoid receptor assay

The hepatocyte cytosol was incubated with 50 nM-[³H]dexamethasone in the presence and absence of a 667-fold excess of non-radioactive steroid. After a 3 h incubation at 0°C, when steroid binding to receptor was essentially complete, the specific macromolecular-bound fraction was determined using the dextran-coated charcoal technique (Beato & Feigelson, 1972). Results are expressed as pmol bound/mg of DNA.

An exchange assay capable of measuring both cytoplasmic and nuclear receptor levels would have been preferred but such an assay is not presently available for detecting the hepatic glucocorticoid receptor. To circumvent this problem the cells were grown in a medium devoid of glucocorticoids (the foetal calf serum was charcoal treated to remove steroid) a situation that would ensure that the receptor was located totally in the cytoplasm (Rousseau *et al.*, 1973) and not 'masked' by endogenous hormone.

DNA estimation

DNA was measured by the method of Hinegardner (1971). Portions of the cell extracts were dried overnight at 50°C, then redissolved in 100 μ l of diaminobenzoic acid hydrochloride solution (0.4 g/ml of water). This was heated at 50°C for 40 min then 1.5 ml of 1 M-HCl was added and mixed. The fluorescence was measured using a fluorimeter (G. K. Turner and Assoc., Palo Alto, CA, U.S.A.)

with type 405 primary filter and combined 2A-12 and 65A secondary filters. Higher polymerized calf thymus DNA was used as a standard.

Results

The presence of endogenous glucocorticoid in the foetal calf serum used as a medium supplement has been shown to cause induction of tyrosine aminotransferase even in control cultures (Yeoh *et al.*, 1979). In preliminary experiments (results not shown) we have found that endogenous steroid also causes an underestimation of glucocorticoid receptor activity in cultured hepatocytes most likely as a result of competition. However, if the foetal calf serum is charcoal-treated (a procedure that removes more than 99% of the steroid) before medium supplementation then optimal receptor activity is determined and the enzyme tyrosine aminotransferase is not induced in control cultures. For these reasons all the studies presented in the present paper have been made using charcoal-treated foetal calf serum.

Previous studies have shown that glucocorticoid receptor activity can be detected in whole foetal rat liver from animals at an early stage of development (Giannopoulos, 1975; Cake *et al.*, 1979). In contrast, when liver cells derived from rats of 15 days gestation are cultured under conditions that yield hepatocytes essentially free of haemopoietic cells, the receptor activity measured within 48 h of culture is very low (Fig. 1a). If the culture is maintained for 72 h these cells acquire glucocorticoid receptor activity. Hepatocytes derived from rats of 19 days gestation have glucocorticoid receptor at all stages of culture and the concentration increases with time in culture. The K_d of the glucocorticoid receptor of cultured foetal hepatocytes was estimated to be 8.2 nM.

There is a very close correlation between glucocorticoid receptor concentration and the ability of exogenously administered dexamethasone to induce the enzyme tyrosine aminotransferase in these cells (Fig. 1). After 2 days of culture, when cells derived from 15-day foetuses lack receptor, dexamethasone does not induce the enzyme. By day 3 of culture, when receptor has been acquired, dexamethasone effectively induces enzyme activity. The receptor detected after 1 day of culture is probably due to residual contamination by haemopoietic cells and the cells are not inducible. Hepatocytes derived from 19-day foetuses are responsive to dexamethasone at all stages studied and, although the absolute amount of receptor varies between different cell preparations from animals of the same developmental stage, the degree of response is proportional to the concentration of receptor. In fact, when all the data of Fig. 1 were subjected to linear regression analysis it

Fig. 1. Glucocorticoid receptor concentration and dexamethasone induction of tyrosine aminotransferase in cultured hepatocytes

Hepatocytes from 15-day (●) and 19-day (○) foetal rats were cultured as described in the Materials and methods section. At daily intervals receptor concentration was determined in control cultures (a) and tyrosine aminotransferase activity was determined in both control cultures (control) and cultures supplemented with 5 μ M-dexamethasone (test) (b). Receptor concentration is expressed as pmol of dexamethasone bound/mg of DNA and dexamethasone induction of tyrosine aminotransferase is expressed as the ratio of test/control activity. Data are means $\pm$ S.E.M. of determinations on at least three independent cell preparations at each time.

was established that there is a significant correlation between inducibility (test/control) and receptor concentration ($r = 0.988$; $P < 0.001$).

Hepatocytes from foetuses of various stages of gestation were also isolated by a procedure not involving cell culture. Livers were dispersed by collagenase treatment and then incubated with steroid-free complement and antiserum raised against rat erythrocyte ghosts. This treatment caused lysis of the haemopoietic cells and resulted in a hepatocyte population essentially free of haemopoietic cells; less than 1% of the cells stained

Table 1. Glucocorticoid receptor activity of hepatocytes from foetuses of various gestational ages

Livers from 15–19-day foetal rats were removed and disaggregated before being treated with an antiserum raised against rat erythrocyte ghosts. This treatment caused lysis of the haemopoietic cells and resulted in a hepatocyte cell population essentially free of haemopoietic cells. Cytosol was prepared from these hepatocytes and assayed for glucocorticoid receptor activity.

Gestational age (days)	Receptor activity (pmol bound/mg of DNA)
15	0.04, 0.07
16	0.05, 0.16
17	0.02, 0.11
18	0.04, 0.06
19	0.54, 0.28, 0.30

positively with benzidine. In Table 1 it can be seen that hepatocytes prepared in this way have low glucocorticoid receptor activity if isolated from foetuses before day 18 of gestation. On day 19 of gestation there is a significant increase in the level of glucocorticoid receptor of hepatocytes. The data suggest that the glucocorticoid receptor activity previously detected in whole foetal liver in the earlier stages of gestation is associated almost exclusively with the haemopoietic cells.

Indeed, haemopoietic cells have been shown to contain a glucocorticoid receptor (Cake *et al.*, 1978) and it has been shown that haemopoietic tissue from 14-day foetal liver is responsive to glucocorticoids with respect to cellular maturation (Billat *et al.*, 1980).

Table 2 compares the receptor activity in hepatocytes derived from 15-day foetuses and cultured in the presence of cytosine arabinoside (7.5 μ g/ml) or bromodeoxyuridine (150 μ g/ml) with that of hepatocytes grown in control medium. The mitotic inhibitor, cytosine arabinoside, largely prevented the appearance of the glucocorticoid receptor (71% inhibition). On the other hand, bromodeoxyuridine, which effectively blocks terminal differentiation in a number of cell types (Bischoff & Holtzer, 1970; Miura & Wilt, 1971; Mayne *et al.*, 1973), had no effect on the glucocorticoid receptor activity of these hepatocytes. Neither cytosine arabinoside nor bromodeoxyuridine had any significant effect on the receptor level in hepatocytes derived from 19-day foetuses.

To determine whether or not the effect of cytosine arabinoside was simply a cytotoxic effect, hepatocytes from 15-day foetuses were cultured for 3 days in the presence of the mitotic inhibitor and then returned to control medium for another 3 days. The results of Table 3 show that whereas cytosine arabinoside-treated cells have markedly decreased

Table 2. *Effect of cytosine arabinoside and bromodeoxyuridine on glucocorticoid receptor activity of cultured foetal hepatocytes*

Hepatocytes from 15–19-day-foetal liver were cultured as described in the Materials and methods section. At the time of plating cytosine arabinoside (7.5 µg/ml) and bromodeoxyuridine (150 µg/ml) were added to some of the cultures as indicated. The cells were given fresh medium late the following day with appropriate supplements and then harvested on day 3 for receptor assay. Data are means ± S.E.M. with numbers of independent cell preparations indicated in parentheses. NS, not significantly different from control.

Foetal age (days)	Culture time (days)	Receptor activity (pmol bound/mg of DNA)		
		Control	Cytosine arabinoside	Bromodeoxyuridine
15	3	0.62 ± 0.11 (7)	0.18 ± 0.08 (7)**	0.77 ± 0.28 (3) (NS)
19	3	0.69 ± 0.09 (5)	0.81 ± 0.08 (5) (NS)	0.59 ± 0.08 (3) (NS)

** Significantly different from control = $P < 0.01$.

Table 3. *Reversal of the effect of cytosine arabinoside on glucocorticoid receptor activity of hepatocytes cultured from 15-day-foetal liver*

Hepatocyte cultures from 15-day-foetal rat liver were prepared and maintained in the presence or absence of cytosine arabinoside (7.5 µg/ml). Cultures were given fresh medium the following day with appropriate supplementation and half the cultures were harvested on day 3, for receptor assay. The remainder were subsequently maintained in control medium, supplemented with fresh medium on day 5, then harvested on day 6 for receptor assay.

	Receptor activity (pmol bound/mg of DNA)	
	Control	Cytosine arabinoside
Expt. 1		
Day 3	0.37	0
Day 6	1.73	1.21
Expt. 2		
Day 3	0.51	0
Day 6	1.74	1.71

receptor activity on day 3, these cells acquire near normal receptor activity after a further 3 days growth in control medium.

Discussion

Previous studies have established that hepatocytes isolated from 15-day foetal rats and grown in culture are initially unresponsive to glucocorticoids both in respect of enhanced glycogen storage (Plas *et al.*, 1973) and induction of the enzyme tyrosine aminotransferase (Yeoh *et al.*, 1979). However, if the cells are cultured for several days both of these systems become responsive to exogenous glucocorticoid. It is difficult to relate these data with the finding that the glucocorticoid receptor can be detected in foetal rat liver from animals at an early stage of development (Giannopoulos, 1975; Cake *et al.*, 1979). However, one of the problems in interpreting these data on the

receptor is that the studies were made using whole liver and it is well-established that foetal rat liver contains, in addition to the hepatocytes, a large population of haemopoietic cells (Oliver *et al.*, 1963; Greengard *et al.*, 1972). More recently haemopoietic cells have been shown to be glucocorticoid-sensitive (Golde *et al.*, 1976; Billat *et al.*, 1980) and to contain a glucocorticoid receptor (Cake *et al.*, 1978).

In the present report we have shown that, by contrast, hepatocytes prepared from 15-day foetal rats essentially free of haemopoietic cells lack the glucocorticoid receptor. If they are maintained in culture for several days the receptor is acquired and simultaneously the tyrosine aminotransferase system becomes responsive to glucocorticoids. Similarly, there is a close correlation between the acquisition of the receptor and responsiveness of the glycogen storage system to exogenous steroids. Plas *et al.* (1973) have demonstrated that cortisol does not significantly enhance the incorporation of [¹⁴C]-glucose into glycogen in hepatocytes from 15-day foetal liver until the cells have been cultured for 3 days. Hepatocytes from 19-day foetal rats are responsive at all stages of culture and contain the glucocorticoid receptor. Moreover, the degree of response of these cells is directly correlated with the actual concentration of the receptor.

From the data presented in this report it would appear the hepatocytes first acquire the glucocorticoid receptor after 18 days of gestation. It is possible that a small proportion of the cells acquire the receptor before this time but that the total activity is below the sensitivity of the steroid binding assay used in the present study. To determine the exact stage of development will require an immunochemical assay capable of detecting the receptor in individual hepatocytes.

Whether the ontogeny of the receptor in cultured foetal hepatocytes reflects the normal development of receptor activity that occurs in the liver *in vivo*

remains to be established. Certainly there are important differences in studies with cultured hepatocytes when compared with those made in the intact animal. Jost (1966) and Greengard & Dewey (1970) have demonstrated that cortisol induces a significant increase in the liver glycogen content of foetal rats if the animals are injected with cortisol after 16.5 days of gestation and assayed for liver glycogen content 24 h later. Discrepancies that exist between the age at which glycogen synthesis is induced by glucocorticoids might be because cultured hepatocytes are initially devoid of the receptor as a result of the cell dispersion procedure. However, given that the whole-animal studies were made using whole liver, it might also be that the small amount of glycogen that is synthesized in intact fetuses in response to glucocorticoids is associated not with hepatocytes but with the non-parenchymal cells. This particular aspect requires further investigation.

Yeoh & Oliver (1980) have shown that the acquisition of glucocorticoid inducibility of tyrosine aminotransferase by hepatocytes cultured from 15-day foetal rat liver is blocked by the presence of cytosine arabinoside in the culture medium. Similarly we have shown that the appearance of glucocorticoid receptor in these cells is largely prevented by the presence of the mitotic inhibitor. The fact that cytosine arabinoside has no effect on the receptor activity of hepatocytes cultured from 19-day foetal rat liver eliminates the possibility of a direct interaction between the nucleoside analogue and the receptor. A general cytotoxic effect is also unlikely as it has been shown that both the activities of aspartate aminotransferase and phosphoenolpyruvate carboxykinase in hepatocytes are unaffected by this treatment (Yeoh & Oliver, 1980). Nor is the effect of the inhibitor the result of a selective killing of those hepatocytes destined to commence synthesis of the receptor with continued culture, as it has been shown (Table 3) that the effect of cytosine arabinoside is completely reversible. Hepatocytes grown in the presence of the inhibitor rapidly acquire the receptor once the inhibitor is removed from the culture medium.

Bromodeoxyuridine, which effectively blocks terminal differentiation in a number of cell types (Bischoff & Holtzer, 1970; Miura & Wilt, 1971; Mayne *et al.*, 1973), had no effect on the accumulation of receptor in hepatocytes derived from 15-day fetuses (Table 2). In contrast, Yeoh & Oliver (1980) have shown that the acquisition of enzyme inducibility in these cells is markedly inhibited by the presence of bromodeoxyuridine in the culture medium. This suggests the possibility that the mechanisms for control of receptor synthesis and tyrosine aminotransferase synthesis are separately acquired during hepatocyte differentiation. A somewhat similar situation has been encountered in a

study of cartilage differentiation. Whereas bromodeoxyuridine-treated cells have a markedly decreased capacity for the synthesis of chondroitin sulphate glycosaminoglycan, collagen synthesis by these cells is the same as, if not greater than, that measured in untreated cells (Levitt & Dorfman, 1972). An alternative explanation for the different response to bromodeoxyuridine of the receptor and enzyme inducibility is that the incorporation of bromodeoxyuridine into DNA might prevent the interaction of the glucocorticoid-receptor complexes with the appropriate nuclear acceptor sites necessary for activation of the tyrosine aminotransferase gene. Certainly the binding characteristics of the glucocorticoid-receptor complexes to the nuclei are altered, as we have shown in preliminary experiments (results not shown) that the degree of nuclear uptake of the complexes is enhanced in hepatocytes grown in the presence of bromodeoxyuridine. Somewhat analogous to this is the finding that the oestrogen receptor has an increased affinity for bromodeoxyuridine-substituted DNA (Kallós *et al.*, 1978).

Whatever the reason, the fact that bromodeoxyuridine does not alter the rate of accumulation of receptor suggests that the differentiative event leading to the appearance of the glucocorticoid receptor has already occurred before day 15 of gestation. Nevertheless, the subsequent appearance of the receptor in cultured hepatocytes would appear to be dependent on mitosis as it is prevented by culturing the cells in the presence of cytosine arabinoside.

The data in the present report suggest that the unresponsiveness of the foetal liver to glucocorticoids in rats before day 18 of gestation may well be due to a lack of the specific glucocorticoid receptor from hepatocytes. However, the fact that the tyrosine aminotransferase system does not respond to exogenous steroid until after delivery indicates that other factors must be contributing to the unresponsiveness of foetal rats close to term. Indeed, it has been shown that glucocorticoids can induce tyrosine aminotransferase in foetal rat liver if the liver is first removed from the environment *in utero* and cultured either as an explant (Wicks, 1968) or as a monolayer of cells (Croizat *et al.*, 1974; Yeoh *et al.*, 1979), but only if they contain the glucocorticoid receptor (Fig. 1). If hepatocytes, which are normally glucocorticoid responsive, are cultured in the presence of physiological concentrations of insulin the induction of the enzyme by glucocorticoid is largely prevented (Cake *et al.*, 1980). Since circulating insulin concentrations are known to be high in the foetus and fall soon after birth (Girard *et al.*, 1977) this may well account for the inability of glucocorticoids to induce tyrosine aminotransferase in foetal rats near term.

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