Role of adrenaline and cyclic AMP in appearance of tyrosine aminotransferase in perinatal rat liver

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1. Adrenaline increased hepatic tyrosine aminotransferase activity when injected into foetal rats or 2-day-old rats. 2. The inhibition of the postnatal increase in tyrosine aminotransferase activity which occurred in adrenalectomized newborn rats was rapidly overcome by injection of adrenaline or dibutyryl cyclic AMP. 3. The effect of adrenaline or dibutyryl cyclic AMP on the tyrosine aminotransferase activity in foetal, adrenalectomized newborn and 2-day-old rats could be partially or completely blocked by prior treatment with actinomycin D. 4. Dibutyryl cyclic AMP induced tyrosine aminotransferase activity in hepatocytes cultured from 15-day foetal rats in glucocorticoid-free medium. 5. Actinomycin D at 0.2 µg/ml in the culture medium completely prevented the induction of tyrosine aminotransferase activity by dibutyryl cyclic AMP in cultured cells. 6. The results suggest that adrenaline and cyclic AMP stimulate a transcriptional event during induction of tyrosine aminotransferase in perinatal liver.

Tyrosine aminotransferase (EC 2.6.1.5) is absent from the liver of foetal rats, but the activity of the enzyme rises rapidly within the first 5 h of birth (Sereni *et al.*, 1959; Di Marco *et al.*, 1976) or after premature delivery of the animals (Holt & Oliver, 1968a; Di Marco *et al.*, 1976). Although the mechanism for the appearance of tyrosine aminotransferase has not yet been elucidated, the results of experiments involving the use of an inhibitor of transcription suggest that the induction is dependent on a transcriptional event which occurs within a few hours of birth. The administration of actinomycin D to foetal rats 1 h before surgical delivery completely abolishes the accumulation of tyrosine aminotransferase activity in the livers after delivery (Holt & Oliver, 1968a). In view of the changes in plasma concentrations of hormones from the adrenal glands and the pancreas after birth (Girard *et al.*, 1973; Di Marco *et al.*, 1978), these hormones have been implicated in the mechanism of induction of the enzyme activity (Greengard, 1970; Oliver, 1974).

Glucocorticoids have been proposed as being necessary for the normal development of hepatic tyrosine aminotransferase activity, since Sereni *et al.* (1959) and Holt & Oliver (1969) have demon-

strated that adrenalectomy at birth markedly decreases the postnatal increase in tyrosine aminotransferase activity. Administration of glucocorticoids completely restores the activity. However, little change occurs in the concentrations of free or total corticosteroids in the plasma or the liver of newborn full-term rats (Di Marco *et al.*, 1978; cf. Holt & Oliver, 1968b; Corbier & Roffi, 1974; Dupouy *et al.*, 1975). Furthermore, tyrosine aminotransferase cannot be induced by administration *in utero* of either cortisol (Sereni *et al.*, 1959; Franz & Knox, 1967; Greengard & Dewey, 1967) or the fluorinated glucocorticoid dexamethasone (Cake *et al.*, 1973; Yeoh *et al.*, 1979b), whereas the enzyme activity can be induced in foetal rats 2 days before term with glucagon or dibutyryl cyclic AMP (Holt & Oliver, 1968a; Greengard, 1970).

The hormonal signals for the synthesis of tyrosine aminotransferase may include the catecholamines, secreted as a result of stress at birth (Di Marco *et al.*, 1976) together with an increase in the concentration of glucagon and a fall in that of plasma insulin within 1 h of birth (Girard *et al.*, 1973; Oliver, 1974). The present paper reports experiments on the effects of adrenaline and cyclic AMP on tyrosine aminotransferase activity in the liver. The effect of adrenaline on tyrosine aminotransferase activity was tested in foetuses *in utero*

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during the last day of gestation or after adrenal-ectomy at surgical delivery. In addition, the response of the enzyme activity to treatment with dibutyryl cyclic AMP was tested in hepatocytes which were isolated from foetal liver and cultured in the absence of glucocorticoids.

Materials and methods

Animals

Rats of the Wistar albino strain of *Rattus norvegicus* were used. These animals have a gestation period of 22 days. The gestational age of foetal animals was determined as described by Di Marco *et al.* (1976).

Chemicals

L-[3,5-³H]Tyrosine and [1,2,6,7-³H]cortico-sterone were from The Radiochemical Centre, Amersham, Bucks., U.K. L-Tyrosine, from Merck, Darmstadt, Germany, was recrystallized from boiling water. Activated charcoal, dexamethasone, dithiothreitol, actinomycin D, bovine serum albumin and dibutyryl cyclic AMP (*N*⁶, *O*^{2'}-dibutyryl adenosine 3':5'-cyclic monophosphate) were from Sigma Chemical Co., St. Louis, MO, U.S.A. Adrenaline bitartrate was from Parke-Davis, Sydney, Australia. CM-Sephadex (C-50) was supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. Cell-culture reagents, Fungizone (amphoterecine B), penicillin/streptomycin and glutamine were obtained from Grand Island Biological Co., Grand Island, NY, U.S.A. Eagle's minimal essential medium and foetal calf serum were from Flow Laboratories, Annandale, N.S.W., Australia. Plastic culture dishes were from Sterilin Products, Teddington, Middx., U.K.

Surgical procedures

Premature animals were delivered by Caesarian section, as described by Di Marco *et al.* (1976). The pregnant rat was killed by a blow on the head and cervical fracture. The foetuses were immediately removed from the uterus, excised from the amniotic sacs and the umbilical cords tied. Prematurely delivered animals were maintained in a Humidicrib at 37°C under a stream of O₂/CO₂ (19:1) without feeding for the duration of the experiments.

When the prematurely delivered rats were to be adrenalectomized, the dam was maintained under diethyl ether anaesthesia while the foetuses were delivered two at a time, weighed and immediately anaesthetized by packing in ice. A dorsal skin incision was made over the vertebral column in the abdominal region and each adrenal was removed, by using an electro-cautery knife through separate incisions in the body wall on either side of the spine. These incisions were not closed, but the skin was then tightly sutured with surgical thread. In the

sham-operated animals (the second of each pair), the adrenals were left intact after the incisions were made in the body wall. The hypothermized animals were warmed under a lamp until respiration commenced and then placed in the Humidicrib. Surgery on one litter was completed within 30 min, and the survival rate was greater than 90%. The animals were examined for residual adrenal tissue at the time of killing.

Newborn and 2-day-old rats were injected intra-peritoneally, as described by Di Marco & Oliver (1978). The doses of actinomycin D and adrenaline administered were 2 µg/g body wt. and the dose of dibutyryl cyclic AMP given was 62.5 µg/g body wt., each dissolved in 50 µl of 0.15 M-NaCl. Control animals received two injections of saline, whereas their littermates were injected with either actinomycin D or saline 30–60 min before receiving adrenaline.

Foetal animals were injected *in utero* as described by Yeung *et al.* (1967). The pregnant rat was anaesthetized with ether and the uterus exposed by partial laparotomy. Three foetuses at opposite ends of the uterus were injected with actinomycin D and the remaining foetuses were injected with saline. The maternal body wall and skin were stitched and the animal recovered rapidly from the anaesthesia. Then 1 h later the dam was re-anaesthetized, and all the foetuses in one uterine horn were injected with adrenaline and the foetuses in the other uterine horn were injected with saline.

Hepatocyte isolation

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.*, 1979a). Cells were harvested and washed twice in balanced salts solution by centrifugation at 50 g for 2 min, then suspended in culture medium. The cell suspension was then pipetted in samples of 10 ml, containing the cells from the livers of ten foetal rats, into 90 mm-diameter plastic dishes previously coated with collagen.

Culture medium and conditions

The basal culture medium was modified Eagle's minimal essential medium supplemented with 10% foetal calf serum, glutamine (2.4 mM final concn.), Fungizone (28 µg/ml) and penicillin/streptomycin (57 units/ml and 570 µg/ml respectively). Further additions to the medium are indicated in the text.

In order to remove endogenous steroids, the foetal calf serum was charcoal-treated before supplementation of the medium. The serum was incubated for 30 min at 37°C in the presence of activated charcoal (50 mg/ml of plasma), cooled and then centrifuged (116 000 g_{max} for 20 min at 0–4°C) to

remove the charcoal. The charcoal-treated serum was stored frozen. By using a tracer dose of [^3H]corticosterone it was shown that this procedure removed more than 99% of the endogenous corticosteroids; any endogenous corticosterone that remained in the charcoal-treated foetal calf serum was present in a concentration that could not be detected by using a protein-binding assay (Martin *et al.*, 1977).

The cultures were maintained at 37°C in a water-saturated atmosphere of air/CO₂ (19:1) and were given fresh medium 24 h and 48 h after plating. The hepatocytes formed a monolayer attached to the collagen substratum, but most of the haematopoietic cells remained in suspension and were discarded when the medium was replaced after 1 day of culture. The residual haematopoietic cells were removed when the monolayer of hepatocytes was vigorously washed and given fresh medium.

Assay of tyrosine aminotransferase

(a) *In whole liver.* Livers were homogenized in ice-cold 0.25 M-sucrose and the homogenate was centrifuged at 25 000 g_{max} for 25 min at 4°C in the International PR-6 centrifuge. Tyrosine aminotransferase activity was determined in individual livers from prematurely delivered or adrenalectomized animals, or in the pooled livers from three littermates of foetal rats, by the method of Diamondstone (1966), as modified by Cake *et al.* (1973). The enzyme activity is expressed as units per mg of liver, where a unit of tyrosine aminotransferase activity is defined as the amount of enzyme that produces 1 nmol of *p*-hydroxyphenylpyruvate/h at 37°C.

(b) *In cultured hepatocytes.* Cells were removed from the dishes with a Teflon scraper, suspended in balanced salts solution and collected by centrifugation at 500 g for 2 min. The cellular pellet was resuspended in 0.05 M-potassium phosphate buffer, pH 6.5, containing 1 mM-dithiothreitol. This cell suspension was sonicated in a Branson model L5-75 sonifier (Branson Instruments, Danbury, CT,

U.S.A.) at 2A for 15 s by using a micro-probe, and the sonicated preparation was incubated at 4°C for 45 min before centrifugation in a Beckman Airfuge at approx. 210 kPa (165 000 g_{max}) for 12 min in the A-100 rotor.

Tyrosine aminotransferase was separated from aspartate aminotransferase (EC 2.6.1.1) in the hepatocyte extracts by chromatography on CM-Sephadex C-50 at 4°C, as described by Yeoh *et al.* (1979a). Samples (100 μl) of hepatocyte extract were run into columns (0.7 cm $\times$ 3 cm), washed with 1 ml of 0.05 M-potassium phosphate buffer, pH 6.5, then left at 4°C for 15 min. Aspartate aminotransferase was removed by elution with 5 ml of 0.05 M-KCl/0.05 M-potassium phosphate buffer, pH 6.5. Tyrosine aminotransferase was then eluted with two 1 ml portions of 0.33 M-KCl/0.05 M-potassium phosphate, pH 6.5. The tyrosine aminotransferase activity and the protein content were determined in this second eluate, which contained most of the enzyme activity (Yeoh *et al.*, 1979a). The enzyme activity was measured by a modification of the methods of Miller & Thompson (1972) and Lees & Weiner (1975), as described by Yeoh *et al.* (1979a). The protein content of the column eluate was determined by the dye-binding method of Bradford (1976), incorporating the modifications of Bearden (1978), and with bovine serum albumin as a standard.

The enzyme activity is expressed as units (as above) per mg of protein in the eluate.

Results

The intraperitoneal injection of adrenaline into foetal rats 1 day before term caused a marked increase in the hepatic tyrosine aminotransferase activity within 5 h (Table 1). Normally there is very little if any tyrosine aminotransferase activity present in the liver at this stage of development (Sereni *et al.*, 1959; Di Marco *et al.*, 1976). The enzyme activity measured in the control animals

Table 1. *Effect of actinomycin D on induction of tyrosine aminotransferase in utero or in 2-day-old rats by adrenaline*
Foetal animals at day 21 of gestation or 2-day-old rats were injected with saline (controls), saline and adrenaline, or actinomycin D and adrenaline, as described in the Materials and methods section. The hepatic tyrosine aminotransferase activity was determined 5 h after adrenaline administration to foetal rats and 3 h after adrenaline injection into 2-day-old rats. The results for each treatment represent the mean $\pm$ S.E.M. for three litters of foetal rats or six animals which were 2 days old.

Age	Treatment	Tyrosine aminotransferase activity (units/mg of liver)
Day 21 of gestation	Controls	23.6 $\pm$ 4.8
	Adrenaline	77.2 $\pm$ 14.1
	Actinomycin D + adrenaline	9.8 $\pm$ 2.1
2 days old	Controls	36.0 $\pm$ 6.5
	Adrenaline	297.2 $\pm$ 32.9
	Actinomycin D + adrenaline	108.7 $\pm$ 9.6

Table 2. *Effect of adrenalectomy and adrenaline or dibutyryl cyclic AMP on induction of tyrosine aminotransferase after premature delivery*

Animals were prematurely delivered at day 21 of gestation and were either adrenalectomized or sham-operated, as described in the Materials and methods section. Some adrenalectomized newborns were injected with actinomycin D 2½ h after delivery. At 3 h after delivery the adrenalectomized animals were injected with adrenaline, dibutyryl cyclic AMP or saline. The hepatic tyrosine aminotransferase activity was determined 6 h after delivery (3 h after injection of adrenaline or dibutyryl cyclic AMP) or 8 h after delivery (5 h after adrenaline administration). The results for each treatment represent the mean $\pm$ S.E.M. for the number of animals indicated. Two litters were used for the experiment with dibutyryl cyclic AMP, rats from four litters were injected with adrenaline and killed 6 h after delivery and five litters were used where the newborns were killed 8 h after delivery.

Treatment	Tyrosine aminotransferase activity (units/mg of liver)	
	6 h after delivery	8 h after delivery
Sham-operated	125.2 $\pm$ 10.5 (15)	192.0 $\pm$ 14.4 (14)
Adrenalectomized	22.0 $\pm$ 1.8 (19)	12.2 $\pm$ 2.0 (13)
Adrenalectomized + adrenaline	86.1 $\pm$ 12.3 (9)	169.8 $\pm$ 38.2 (15)
Adrenalectomized + actinomycin D + adrenaline	19.8 $\pm$ 3.5 (10)	50.5 $\pm$ 9.8 (4)
Adrenalectomized + dibutyryl cyclic AMP	122.5 $\pm$ 30.1 (4)	—
Adrenalectomized + actinomycin D + dibutyryl cyclic AMP	55.3 $\pm$ 9.1 (5)	—

(Table 1) is likely to have been induced as a result of ether anaesthesia of the pregnant dam during the injection procedure (Di Marco *et al.*, 1976) or transfer of some adrenaline from the hormone-injected animals to the control animals in the other uterine horn.

Similarly, adrenaline injection increased the hepatic tyrosine aminotransferase activity in 2-day-old rats (Table 1), but a time-course showed that the effect was more rapid than in the foetal animals; the maximal enzyme activity was detected 5 h after adrenaline injection *in utero*, but only 3 h after hormone administration in 2-day-olds (time-course not shown). Actinomycin D, when administered before adrenaline, completely blocked the effect of the hormone in the foetal animals and diminished the increase in the enzyme activity in the 2-day-old animals by 72% (Table 1).

The effect of adrenaline on the hepatic tyrosine aminotransferase activity in animals that had been adrenalectomized at surgical delivery is shown in Table 2. When adrenaline was administered 3 h after adrenalectomy, the tyrosine aminotransferase activity increased during the subsequent 3 h to 69% of the enzyme activity that accumulated in the sham-operated animals during the 6 h period. Exposure to adrenaline for 5 h increased the tyrosine aminotransferase activity to 88% of the activity that developed over an 8 h period in the control animals. Prior treatment with actinomycin D completely abolished the effect of adrenaline on the tyrosine aminotransferase activity during 3 h after hormone

Table 3. *Induction of tyrosine aminotransferase in cultured hepatocytes derived from 15-day foetal liver by dibutyryl cyclic AMP or dexamethasone*

Hepatocytes from 15-day foetal liver were cultured for 60 h as described in the Materials and methods section. At this time some of the cultures were treated with actinomycin D (0.2 μ g/ml) and 0.5 mM-dibutyryl cyclic AMP or 50 nM-dexamethasone were added, as shown in the Table. After a further 14 h incubation, all cells were harvested and the tyrosine aminotransferase activity was determined. The data represent the means $\pm$ S.E.M. for three plates from each of three independent cell preparations. The lower limit of sensitivity for this assay is 20 units/mg of protein.

Addition to medium	Tyrosine aminotransferase activity (units/mg of protein)
Nil	<20
Actinomycin D	<20
Dexamethasone	80 $\pm$ 11
Dibutyryl cyclic AMP	925 $\pm$ 53
Dibutyryl cyclic AMP + actinomycin D	<20
Dibutyryl cyclic AMP + dexamethasone	936 $\pm$ 80

injection, and blocked by 75% the effect of exposure to adrenaline for 5 h (Table 2).

Similarly, the inhibitory effect of adrenalectomy at delivery on the development of tyrosine aminotransferase activity could be completely reversed by treatment with dibutyryl cyclic AMP for 3 h (Table 2). As with the effect of adrenaline, this increase in

enzyme activity induced by dibutyryl cyclic AMP was markedly decreased (67%) by actinomycin D.

The activity of tyrosine aminotransferase in 3-day cultures of hepatocytes obtained from 15-day foetuses is shown in Table 3. Addition of dibutyryl cyclic AMP to the culture medium dramatically increased the tyrosine aminotransferase activity; this induction was completely prevented when actinomycin D (0.2 µg/ml) was present in the culture medium. As previously reported (Yeoh *et al.*, 1979a), dexamethasone induced only a low tyrosine aminotransferase activity in cultured hepatocytes obtained at this stage of development. The simultaneous addition of both dibutyryl cyclic AMP and dexamethasone to the culture medium did not significantly enhance the induction of tyrosine aminotransferase activity that occurred in the presence of the cyclic nucleotide alone.

Discussion

These results give further support to the proposal that the secretion of adrenaline at birth (Girard *et al.*, 1973; Di Marco *et al.*, 1976) may act as the major signal for the appearance of tyrosine aminotransferase in the liver. Furthermore, it is also proposed that the increase in cyclic AMP which occurs within 1 h of delivery (Di Marco & Oliver, 1978) stimulates the transcription of mRNA molecules which are required for the synthesis of tyrosine aminotransferase.

In previous studies the possibility that a hormone is involved in the induction of tyrosine aminotransferase has been tested by using the two experimental approaches of ablation of the endocrine glands or administration of the putative inducer to foetal rats *in utero* (Sereni *et al.*, 1959; Greengard, 1969; Oliver, 1974). The initial finding (Sereni *et al.*, 1959) that cortisol administration after adrenalectomy at birth restored normal development of tyrosine aminotransferase activity provided the basis for the hypothesis that glucocorticoids are required for normal development of enzyme activity (Sereni *et al.*, 1959; Holt & Oliver, 1969; Greengard, 1970). In the present study we report that the effect of adrenalectomy on the increase in enzyme activity after surgical delivery could also be rapidly reversed by adrenaline administration. Furthermore, administration of dibutyryl cyclic AMP completely restored the tyrosine aminotransferase activity in the adrenalectomized animals, consistent with the presumed role of cyclic AMP as the intracellular mediator of adrenaline and glucagon action in the liver of the foetal rat (Greengard, 1970; Oliver, 1974; Di Marco & Oliver, 1978; Bulanyi *et al.*, 1979). In addition, adrenaline induced tyrosine aminotransferase activity *in utero* [in agreement with Greengard (1970), but in contrast with Holt &

Oliver (1968a)], and adrenaline injection dramatically increased the enzyme activity in 2-day-old animals (Holt & Oliver, 1969; Table 1).

Cyclic AMP has been implicated in the control of the termination step during the translation of the mRNA for the synthesis of tyrosine aminotransferase (Chuah & Oliver, 1971, 1972). The ability of actinomycin D to block the accumulation of tyrosine aminotransferase activity after administration of adrenaline or dibutyryl cyclic AMP to adrenalectomized newborn animals suggests that cyclic AMP is also involved in the control of transcription of the gene for tyrosine aminotransferase. However, one uncertainty in interpreting this result is that there may have been some release of glucocorticoids during the operative handling of the adrenal glands (Sereni *et al.*, 1959). To establish whether cyclic AMP could stimulate transcription of the gene for tyrosine aminotransferase in the absence of glucocorticoids, the induction studies were repeated in foetal hepatocytes in culture. Previously it has been shown that hepatocytes isolated from liver at day 15 of gestation have a very low concentration of cytoplasmic receptors for dexamethasone, and the glucocorticoid does not induce tyrosine aminotransferase activity during the first day of culture (Cake *et al.*, 1978; Yeoh *et al.*, 1979a). The glucocorticoid receptor is acquired during 3 days of culture (Cake *et al.*, 1980), and the cells synthesize tyrosine aminotransferase in response to endogenous corticosteroids in the culture medium and added dexamethasone (Yeoh *et al.*, 1979a,b). In the present study the endogenous steroid was removed from the foetal calf serum by treatment with charcoal before supplementation of the medium. Dibutyryl cyclic AMP very effectively induced tyrosine aminotransferase under these culture conditions, indicating that glucocorticoids were not essential for induction of the enzyme by the cyclic nucleotide. This enzyme induction was dependent on RNA synthesis, as it was blocked by the presence of actinomycin D at a concentration that has been shown to block RNA synthesis by 88% but to decrease protein synthesis by only 13% (Yeoh *et al.*, 1979b). Dibutyryl cyclic AMP has been shown previously to increase the amount of functional mRNA for tyrosine aminotransferase in normal (Ernest & Fiegelson, 1978; Ernest *et al.*, 1978) or adrenalectomized (Noguchi *et al.*, 1978) adult rats, and it is proposed that the cyclic nucleotide has the same effect in foetal hepatocytes.

The results in the present paper cast doubt on the original interpretation that adrenal steroids are essential for the normal development of tyrosine aminotransferase activity in the liver (Sereni *et al.*, 1959; Holt & Oliver, 1969; Greengard, 1970). Direct measurement of the amount of mRNA for tyrosine aminotransferase will be necessary to

establish the effect of glucocorticoids during the induction of the enzyme. The concentrations of corticosteroids circulating in the foetus are high several days before term (Holt & Oliver, 1968*b*; Di Marco *et al.*, 1978), and at this late stage of gestation the liver contains hepatocytes which are sensitive to glucocorticoids (Yeoh *et al.*, 1979*a,b*). Neither the endogenous corticosteroid nor exogenously administered glucocorticoids cause tyrosine aminotransferase activity to appear *in utero* (Sereni *et al.*, 1959; Greengard & Dewey, 1967; Cake *et al.*, 1973; Yeoh *et al.*, 1979*b*). However, it has been proposed that, although glucocorticoids may induce the transcription of the gene for the enzyme, the mRNA is translationally blocked *in utero* (Yeoh *et al.*, 1979*b*; Cake *et al.*, 1978). Alternatively, the postnatal development of tyrosine aminotransferase activity may result largely or totally from enzyme-specific mRNA which is transcribed as a result of the hormonal signals at birth, as appears to occur for phosphoenolpyruvate carboxykinase (Garcia Ruiz *et al.*, 1978).

Glucocorticoids, as well as cyclic AMP, are involved in the modulation of tyrosine aminotransferase present in postnatal liver (Kenney, 1962; Rosenfeld & Barrieux, 1979).

However, we conclude from the present studies that, immediately after birth, the increase in the concentration of cyclic AMP in the liver consequent on release of catecholamines completes the signal for appearance of the enzyme activity. Glucocorticoids may be involved in the induction of the enzyme at birth, but appear to be insufficient alone as a signal for the initial appearance of the enzyme.

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References

- Bearden, J. C. (1978) *Biochim. Biophys. Acta* **533**, 525–529
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Bulanyi, G. S., Steele, J. G., McGrath, M. C., Yeoh, G. C. T. & Oliver, I. T. (1979) *Eur. J. Biochem.* **102**, 93–100
- Cake, M. H., Ghisalberti, A. V. & Oliver, I. T. (1973) *Biochem. Biophys. Res. Commun.* **54**, 983–990
- Cake, M. H., Yeoh, G. & Oliver, I. T. (1978) *Proc. Aust. Biochem. Soc.* **11**, 46
- Cake, M. H., Yeoh, G. C. T., Oliver, I. T. & Litwack, G. (1980) in *Development of Responsiveness to Steroid Hormones* (Kaye, A. M. and Kaye, M., eds.), pp. 263–272, Pergamon Press, Oxford
- Chuah, C. C. & Oliver, I. T. (1971) *Biochemistry* **10**, 2990–3001
- Chuah, C. C. & Oliver, I. T. (1972) *Biochemistry* **11**, 2547–2553
- Corbier, P. & Roffi, J. (1974) *C.R. Hebd. Seances Acad. Sci., Ser. D.* **278**, 99–102
- Diamondstone, T. I. (1966) *Anal. Biochem.* **16**, 395–401
- Di Marco, P. N. & Oliver, I. T. (1978) *Eur. J. Biochem.* **87**, 235–241
- Di Marco, P. N., Ghisalberti, A. V., Pearce, P. H. & Oliver, I. T. (1976) *Biol. Neonate* **30**, 205–215
- Di Marco, P. N., Ghisalberti, A. V., Martin, C. E. & Oliver, I. T. (1978) *Eur. J. Biochem.* **87**, 243–247
- Dupouy, J. P., Coffigny, H. & Magre, S. (1975) *J. Endocrinol.* **65**, 347–352
- Ernest, M. J. & Fiegelson, P. (1978) *J. Biol. Chem.* **253**, 319–322
- Ernest, M. J., Delap, L. & Fiegelson, P. (1978) *J. Biol. Chem.* **253**, 2895–2897
- Franz, J. M. & Knox, W. E. (1967) *Biochemistry* **6**, 3464–3471
- Garcia Ruiz, J. P., Ingram, R. & Hanson, R. W. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4189–4193
- Girard, J. R., Caquet, D., Bal, D. & Guillet, I. (1973) *Enzyme* **15**, 272–285
- Greengard, O. (1969) *Science* **163**, 891–895
- Greengard, O. (1970) in *Biochemical Action of Hormones* (Litwack, G., ed.), vol. 1, pp. 53–87, Academic Press, New York and London
- Greengard, O. & Dewey, H. K. (1967) *J. Biol. Chem.* **242**, 2986–2991
- Hanks, J. H. & Wallace, R. E. (1949) *Proc. Soc. Exp. Biol. Med.* **71**, 196–200
- Holt, P. G. & Oliver, I. T. (1968*a*) *Biochem. J.* **108**, 333–338
- Holt, P. G. & Oliver, I. T. (1968*b*) *Biochem. J.* **108**, 339–341
- Holt, P. G. & Oliver, I. T. (1969) *Biochemistry* **8**, 1429–1437
- Kenney, F. T. (1962) *J. Biol. Chem.* **237**, 3495–3498
- Lees, G. J. & Weiner, N. (1975) *J. Neurochem.* **25**, 315–322
- Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977) *Acta Endocrinol. (Copenhagen)* **84**, 167–176
- Miller, J. V. & Thompson, E. B. (1972) *Anal. Biochem.* **47**, 487–494
- Noguchi, T., Diesterhaft, M. & Granner, D. (1978) *J. Biol. Chem.* **253**, 1332–1335
- Oliver, I. T. (1974) in *Concepts of Development* (Lash, J. & Whittaker, J. R., eds.), pp. 295–320, Sinauer Associates, Stamford, U.S.A.
- Rosenfeld, M. G. & Barrieux, A. (1979) *Adv. Cyclic Nucleotide Res.* **11**, 205–264
- Sereni, F., Kenney, F. T. & Kretchmer, N. (1959) *J. Biol. Chem.* **234**, 609–612
- Yeoh, G. C. T., Bennett, F. A. & Oliver, I. T. (1979*a*) *Biochem. J.* **180**, 153–160
- Yeoh, G. C. T., Arbuckle, T. & Oliver, I. T. (1979*b*) *Biochem. J.* **180**, 545–549
- Yeung, D., Stanley, R. S. & Oliver, I. T. (1967) *Biochem. J.* **105**, 1219–1227