Table II. Effect of mouse nerve growth factor on $^{35}S$ incorporation into chick pelvic cartilage

| Experiment | Test sample | $^{35}S$ incorporation ± SEM (cpm × $10^{-3}$/mg cartilage) 0 [a] | 2.5% [a] | 20% [a] |
|---|---|---|---|---|
| 1 | 0.1 ml NGF (100 U/ml) | 8.7 ± 0.8 | — | — |
| | Control | 9.0 ± 1.4 | — | — |
| 3 | 0.1 ml NGF (100 U/ml) | — | 58.8 ± 3.7 | 84.9 ± 3.5 |
| | 0.1 ml NGF (500 U/ml) | — | 47.9 ± 3.7 | 81.6 ± 4.3 |
| | Control | — | 50.8 ± 1.1 | 79.7 ± 3.4 |
| 4 | 0.1 ml NGF (10,000 U/ml) | — | 33.8 ± 2.2 | 57.3 ± 2.2 |
| | Control | — | 32.6 ± 2.9 | 59.0 ± 1.1 |

Six cartilages were used for each observation. Incubation volume = 1 ml.
[a] Serum concentration in medium.

These results indicate that neither CSF obtained from mouse uterus nor NGF prepared from mouse salivary glands stimulate proteoglycan synthesis in chick embryo cartilage, even when used at concentrations well in excess of those which are effective in promoting colony formation or neural growth in vitro. In addition, they indicate that mouse NGF has no appreciable colony stimulating activity. The findings are in agreement with the observation by METCALF et al.[10] that CSF had no effect on the growth of fibroblasts and several other cell-lines in culture. It therefore appears that neither NGF nor CSF have the more general biological effects which have been attributed to SM.

Table III. Effect of mouse nerve growth factor on colony formation by mouse bone-marrow cells

| Test sample | No. of colonies |
|---|---|
| 0.1 ml NGF (625 U/ml) | 0,4 |
| 0.1 ml NGF (2500 U/ml) | 0,0 |
| 0.1 ml NGF (10,000 U/ml) | 0,4 |
| 0.1 ml $H_2O$ | 0,2 |
| 0.05 ml CSF | 221,202 |
| 0.05 ml CSF + 0.05 ml NGF (5000 U/ml) | 201,208 |

Results obtained with mouse colony stimulating factor are also shown.

*Résumé.* Le facteur nerveux de croissance des souris (mouse nerve growth factor: NGF) et le «colony stimulating factor» (CSF) furent testés pour contrôler l'activité de la somatomédine, en mesurant l'incorporation du $S^{35}$ dans du cartilage d'embryon de poulet. Aucune de ces deux préparations ne stimulait cette incorporation. De plus, le NGF n'avait pas d'effet sur la formation des colonies de moelle osseuse. Ces découvertes indiquent que ni le NGF ni le CSF ne participent aux effets biologiques généraux attribués à la somatomédine.

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## Plasma Thyrotrophin Concentration in the Foetal Calf

Previous observations on thyroxine levels in foetal calves with indwelling vascular catheters have shown that total plasma thyroxine falls in the last 3 days of intrauterine life, while the concentration of total free thyroxine does not change significantly[1]. These findings suggest that the pituitary-thyroid axis adjusts the level of secretion of thyroxine to maintain a constant circulating concentration of free thyroxine. The present paper reports the foetal plasma thyrotrophin (TSH) concentrations in the same 7 animals, and the changes in TSH, cortisol and thyroxine which occurred when parturition was induced with Synacthen.

*Materials and methods.* Plasma samples were obtained from chronically catheterized foetuses during the last 15 days of gestation. The calves were born between 261 and 283 days gestation (term 280 days)[2]. Premature parturition was induced in 2 cows by the administration of 0.06–0.125 mg Synacthen $\beta$ 1–24 Corticotrophin (CIBA) 4 times daily i.m. to the foetus. In a 3rd cow the foetus was given cortisol (Efcortelan, Glaxo, i.v., 4 times daily) to initiate delivery. Gestational age was between 250 and 260 days in these induced pregnancies.

Plasma TSH was measured in a double antibody radioimmunoassay. The coefficient of variation of the assay was 7.4% and the recovery of added TSH from calf plasma was 96.1% over the range 1.25–25 μU/ml. Cross

[1] P. W. NATHANIELSZ, R. S. COMLINE, M. SILVER and A. L. THOMAS, J. Endocr. *58*, 21 (1974).
[2] R. S. COMLINE, L. HALL, R. B. LAVELLE, P. W. NATHANIELSZ and M. SILVER, J. Endocr., in press.

reactivity with luteinizing hormone was less than 1%. Plasma thyroxine and cortisol were estimated as described previously[3].

*Results and discussion.* Figure 1. shows the mean changes in foetal plasma TSH during the last 15 days of gestation together with the mean percentage changes in total plasma thyroxine in the same animals. TSH levels varied widely until a week before delivery; the values then fell gradually until the day before birth. Samples taken on the day of delivery within the last 4 h of intra-uterine life have been excluded from the present series since there was evidence that the thyroid axis was activated by the actual processes of delivery. All samples taken at other times on the last day of foetal life had TSH concentrations below the limits of sensitivity of the assay (0.25 μU/ml). The results indicate that the fall in total plasma thyroxine is preceded by a drop in plasma TSH before birth in this species.

A gradual rise in foetal plasma cortisol begins about 6 days before parturition in the calf[2], and in Figure 1 the increase in mean plasma cortisol in the present series of calves is shown in relation to the changes in mean plasma TSH concentration.

In both the animals which received foetal injections of Synacthen there was a rise in plasma cortisol and fall in plasma thyroxine and TSH comparable with that observed in untreated catheterized animals before delivery (Figure 2). In 1 animal (Figure 2b) TSH concentration on the morning of commencement of Synacthen administration was $< 0.25$ μU/ml. However, this finding was occasionally observed in untreated animals and this was the only low value obtained prior to the Synacthen treatment. After Synacthen administration, TSH was undetectable during the last 3 days of intra-uterine life. The rise in cortisol preceded the changes in thyroxine by at least 24 h.

[3] P. W. Nathanielsz, R. S. Comline, M. Silver and A. L. Thomas, J. Endocr. *58*, 535 (1973).

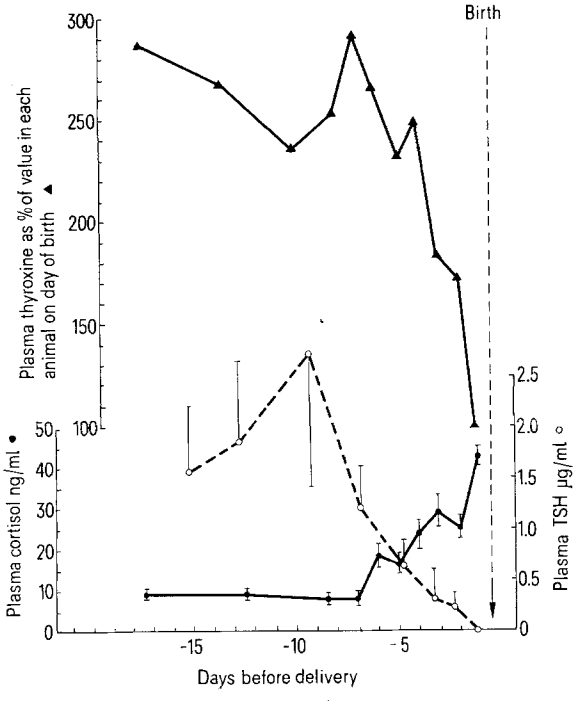


Fig. 1. Plasma TSH, cortisol and thyroxine in 7 foetal calves during the last 18 days of intrauterine life. All values for TSH are the mean and SEM of at least 4 animals. Plasma thyroxine for each animal was expressed as a percentage of the value observed in the last day of intrauterine life. Thyroxine values are the mean of 7 animals.

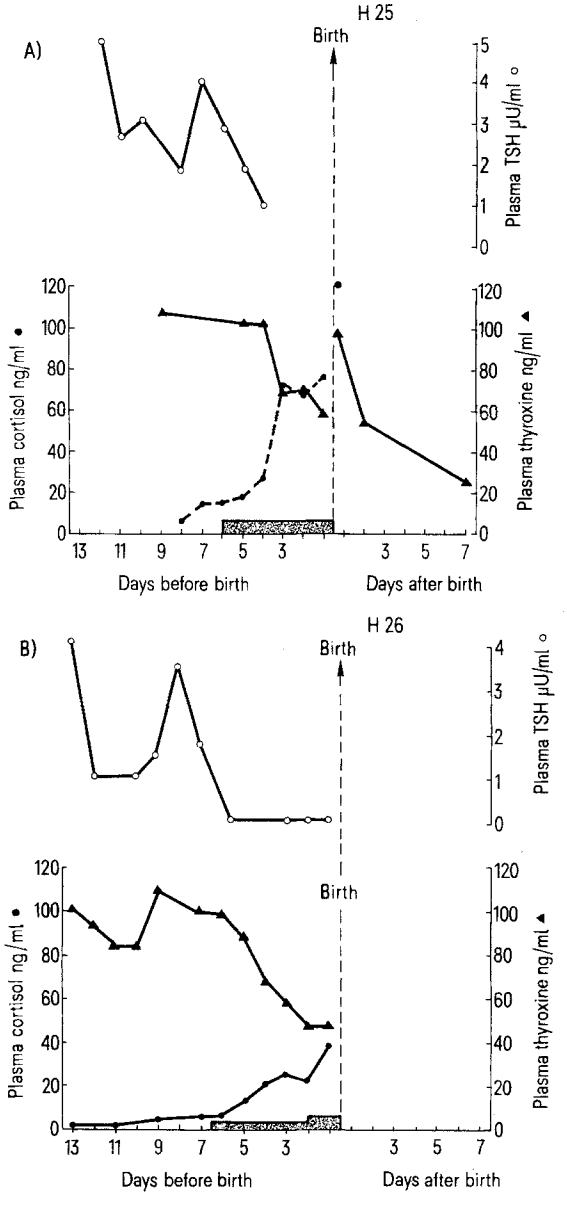


Fig. 2. Changes in plasma TSH, thyroxine and cortisol in 2 calves in which parturition was induced by the foetal administration of Synacthen. A) Parturition occurred at 259 days gestation after 6 days administration of Synacthen at 0.5 mg/day. B) Parturition occurred at 261 days gestation. 0.25 mg/day was administered for 4 days followed by 0.5 mg/day for 2 days before parturition occurred.

In the cow in which the foetus was given cortisol, like those receiving Synacthen, foetal plasma TSH was greatly depressed during the period of treatment (Figure 3). However the prenatal depression of TSH was not sufficient to inhibit the postnatal rise in TSH which occurred within 30 min after birth (Figure 3), although endogenous cortisol secretion remained very low, not demonstrating the usual post-natal rise in the newborn calf[2].

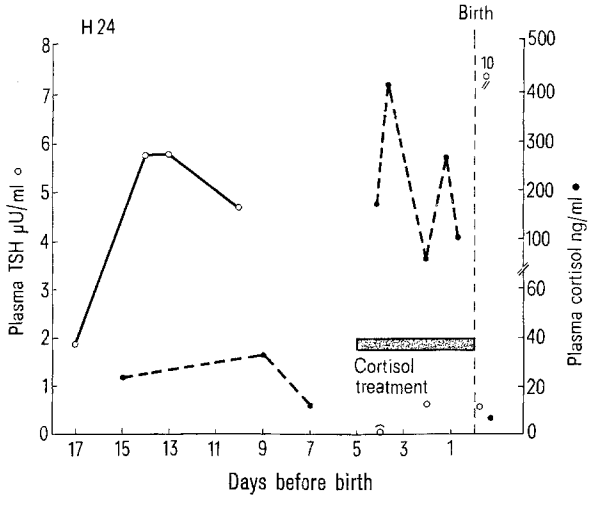


Fig. 3. Changes in plasma TSH and cortisol during initiation of parturition with cortisol (100 mg/day) for 5 days to a foetal calf. Delivery occurred at 258 days gestation.

The present findings in the calf foetus under chronic conditions suggest that the pronounced fall in foetal plasma thyroxine which occurs before birth in this species is preceded by a drop in foetal plasma TSH. These changes contrast with the situation in the foetal lamb in which the picture is far less clear cut: HOPKINS and THORBURN[4] reported a prenatal fall in plasma thyroxine but no change in plasma TSH, whereas stable plasma thyroxine levels were observed in foetal lambs in this laboratory up to the time of parturition[3].

*Résumé.* On a mesuré le taux de thyrotrophine (TSH), thyroxine et cortisol dans le plasma du foetus du veau pendant les 18 jours précédant la naissance. La diminution du TSH a précédé de 6 jours et s'est poursuivie en même temps que celle du taux de thyroxine.

A. L. THOMAS, R. S. COMLINE, MARIAN SILVER and P. W. NATHANIELSZ[5,6]

*Physiological Laboratory, Cambridge CB2 3EG (England), 29 March 1974.*

[4] G. D. THORBURN and P. S. HOPKINS, (1973) *Foetal and Neonatal Physiology*. Proceedings of the Sir Joseph Barcroft Centenary Symposium (Ed. R. S. COMLINE; Cambridge University Press), p. 488.

[5] We are grateful to the Medical Research Council and the Milk Marketing Board for financial support.

[6] The TSH preparation used for iodination in these studies was kindly provided by Dr. J. PIERCE, and the antiserum to Pierce bovine TSH was provided by Dr. G. D. THORBURN. Synacthen was kindly provided by Dr. D. M. BURLEY, CIBA Laboratories, Horsham, Sussex and Efcortelan by Dr. E. S. SNELL,Glaxo Laboratories, Middx.

## THEORIA

## Ligand-Leakage in Affinity Chromatography, a Mathematical Approach

Although cellulose-bound, highly specific substrates have already been used by CAMPBELL and LERMAN[1,2], about 20 years ago, for the isolation and purification of biological macromolecules, the large increase in the application of this technique, named affinity chromatography[3], only started in 1967. The introduction of the cyanogen bromide activation of insoluble polysaccharides for the coupling with ligand molecules by AXÉN et al.[4-6], and the use of beaded agarose[7] as the solid support[8] have certainly been important stimuli for this overwhelming development[8-12]. A further improvement of this technique was obtained by the insertion of spacer-molecules between the ligand and the matrix[3,9].

Several alternatives for agarose as the solid support have been proposed, e.g. glass beads, nylon fibres, polyacrylamide, cellulosederivatives, ethylene-maleic

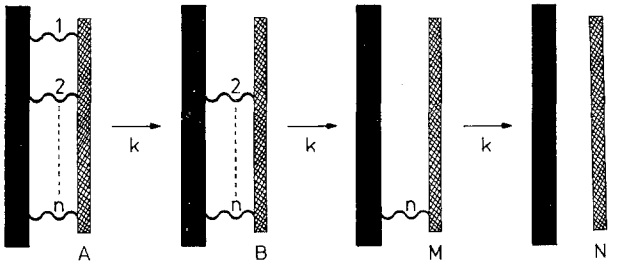


Fig. 1. Schematic representation of the detachment of a multiply bound ligand-molecule from a matrix. ■, matrix; ▒, ligand; *k*, detachment rate constant.

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