choline was omitted no 1-^{14}C-ACh was formed. Further identification that the enzyme was ChAc was obtained by inhibitor studies. Cl Ach and BrACh were prepared just prior to transfer into incubation tubes because of their instability in aqueous solution[17]. At a concentration of 5×10^{-6} *M* both halogenated ACh derivatives inhibited the enzymatic 1-^{14}C-acetyl transfer by about 90% and 100% in two placenta homogenates (Table I). NVP solutions were handled in a darkroom only[18]. This drug inhibited the enzyme of immature placentae by 75% at 2.5×10^{-4} *M* or 15 and 25% at 2.5×10^{-5} *M* (Table I). The results obtained with these three specific ChAc inhibitors strongly supported the conclusion that the early placentae examined contained this enzyme at 6–9 weeks of gestation. ChAc activity at this stage of pregnancy was already higher than in term placenta (Table II) and gradually increased. All values shown after 12 weeks are single determinations from elective abortions (13 and 14 weeks) or premature deliveries (27–37 weeks). Elective terminations of pregnancy under the 1973 United States Supreme Court ruling are preferably performed between 7–10 weeks which made such specimens more readily available.

Table II. ChAc activity of human placenta at various lengths of pregnancy

Length of pregnancy (weeks)	ACh synthesized	
	Moles $\times 10^{-6}$/g fresh tissue $\times$ h^{-1}	Moles $\times 10^{-9}$/mg protein $\times$ h^{-1}
6–7	17.34 ± 2.31 (5)	245 ± 54 (5)
8	16.64 ± 3.16 (9)	291 ± 37 (9)
9	20.47 ± 3.67 (5)	388 ± 40 (5)
10	44.70 ± 14.93 (3)	643 ± 67 (3)
11	44.53 ± 6.89 (3)	672 ± 52 (3)
12	37.88 ± 2.88 (2)	751 ± 17 (2)
13	34.40	882
14	55.59	794
27	24.10	199
32	32.50	262
36	20.33	289
37	12.64	177
41–42 (term)	7.85 ± 1.04 (6)	60.0 ± 8.02 (4)

Values represent mean ± S.E. of the mean. The number of placentae is indicated in brackets. All determinations from pregnancies longer than 12 weeks are single observations with exception of the term placentae. ChAc activity is expressed in moles of ACh synthesized per gram fresh tissue or mg protein as specified.

The results allow the speculation that ChAc is present in human placenta as soon as placentation begins. The development of ChAc activity during the second and third trimester resembled previous observations[4] and enzyme activity seemed quite comparable when calculated back to a fresh weight basis in both immature and term placenta[2–4]. There was a rapid decline of ChAc towards the normal end of pregnancy, the activity being lower in the term placenta than at any previous point of measurement (Table II).

The ChAc of early placental villous tissue was easily released upon homogenization. The addition of butanol[19] or ether, which greatly increased the enzyme activity in brain tissue[20, 21] did not result in formation of more 1-^{14}C-ACh, an observation which is in agreement with observations on term placenta[2].

The presence of ChAc with high specific activity in immature placenta suggests a function of ACh throughout pregnancy rather than a role during the delivery process only[5]. The highest enzyme activity was observed long before term (Table II and refs.[3, 4]). These findings support previous speculations[4, 6] that ACh (which was detected in significant concentrations by pyrolysis gas chromatography in 8-9-week-old placentae, unpublished observations) might have some function in permeability and transport regulation. The present results would suggest that such as yet undefined cholinergic modulation of placenta functions could become active at a very early stage of fetal development.

Zusammenfassung. Cholinacetyltransferase (ChAc) wurde mit radiometrischer Methode im Homogenat unreifer menschlicher Plazenta bestimmt. Das Enzym war bereits nach 6–7 Wochen Gravidität eindeutig messbar und hatte höhere spezifische Aktivität als dasjenige der reifen Plazenta. Die Ergebnisse lassen vermuten, dass ChAc schon mit Beginn der Plazentation auftritt und unterstützen früher geäusserte Vermutungen, dass ACh in der menschlichen Plazenta bisher noch nicht genauer definierte Aufgaben in der Regulation von Membranpermeabilität und aktivem Transport haben könnte.

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[22] Supported by USPHS grant No. HD 07091-02. The technical assistance of Mrs. Cynthia Knight is gratefully acknowledged.

The Role of Thyroxine in the Maintenance of a Normal Glycogenolytic Response to Splanchnic Nerve Stimulation in Adrenalectomized Calves

Stimulation of splanchnic sympathetic efferent fibres at physiological frequencies causes rapid mobilization of liver glycogen in the calf. Three separate pathways mediate this response: direct activation of the hepatic sympathetic innervation[1], release of glucagon from the pancreatic islets[2] and the release of catecholamines from the adrenal medullae[3]. The experiments described here were devised to elucidate the extent to which thyroxine might influence the first two of these mechanisms.

Five pedigree Jersey calves were thyroidectomized between 7 and 15 days of age and maintained on a milk diet. 3 weeks later the animals were anaesthetized with

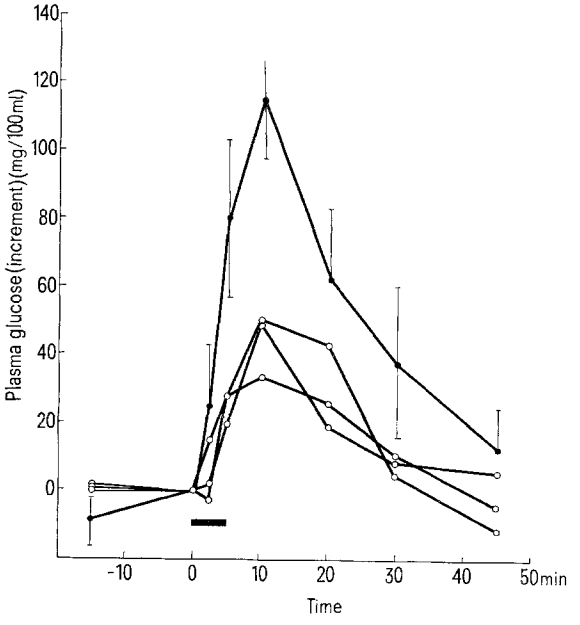

Fig. 1. Comparison of the changes in plasma glucose concentration in response to stimulation of the peripheral ends of the splanchnic nerves (4.0 c/s; 20 V, 1 msec square-wave stimulus) in adrenalectomized calves. ○, thyroidectomized; ●, thyroid intact[4] (n = 4). Vertical bars = S.E. of mean increments. Horizontal bar: duration of stimulus.

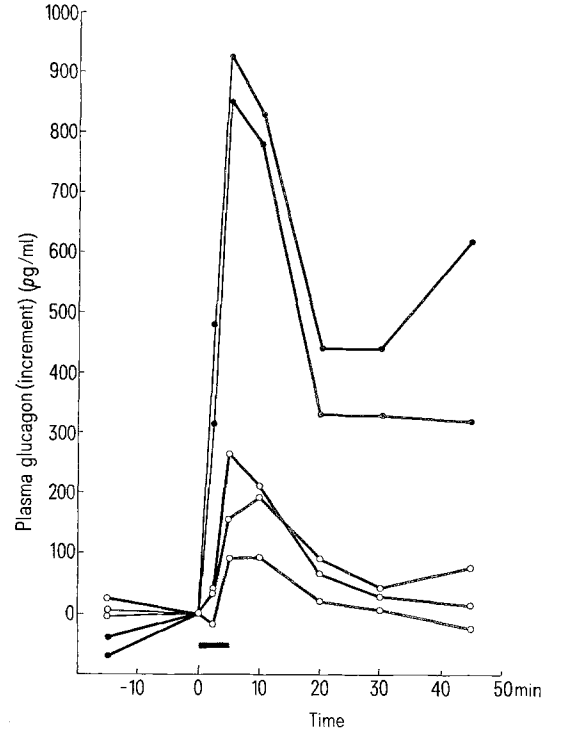

Fig. 2. Comparison of the changes in plasma glucagon concentration in response to stimulation of the peripheral ends of the splanchnic nerves in adrenalectomized calves (4.0 c/s; 20 V, 1 msec square wave stimulus). ○, thyroidectomized; ●, thyroidectomized, thyroxine-replenished. Horizontal bar: duration of stimulus.

sodium pentobarbitone, both adrenal glands were removed and the peripheral ends of both splanchnic nerves attached to fluid electrodes as described previously[1]. In control experiments 2 of these thyroidectomized calves were given i.m. injections of exogenous thyroxine (50 $\mu g\ kg^{-1}day^{-1}$) for 7 days prior to testing. Samples of jugular blood were collected each day for thyroxine estimations and the operation site was scrutinized at post mortem to ensure that the thyroid had been completely removed in each animal. Plasma glucose was estimated with glucose oxidase and plasma glucagon by means of an antibody, specific for pancreatic glucagon, and which did not react with enteroglucagon.

The results show that removal of the thyroid gland substantially reduces the rise in plasma glucose concentration which occurs in response to stimulation of the splanchnic nerves at 4.0 c/s (Figure 1). Each of these animals was found to have a liver glycogen concentration in excess of 10 mg/g at the end of the experiment. The changes in plasma glucose concentration in thyroidectomized, thyroxine-replenished animals are not shown as the liver glycogen concentrations were found to be uniformly low ($<$ 4.0 mg/g).

The rises in plasma glucagon concentration during these experiments were also much less than those obtained previously in control animals of the same age[2]. In the previous study stimulation was continued for 10 min; direct comparison must therefore be restricted to the rise which had occurred 5 min after onset of stimulation. In calves with intact thyroid glands mean plasma glucagon concentration had risen by 675 $\pm$ 40 pg/ml (n = 4) whereas the corresponding values in 3 thyroidectomized calves ranged between 90 and 270 pg/ml (Figure 2). In contrast, the plasma glucagon concentration of 2 thyroidectomized, thyroxine-replenished calves had risen by 850 and 920 pg/ml respectively, 5 min after onset of stimulation (Figure 2). The changes in mean aortic blood pressure and the percentage change in haematocrit, which occurred during these experiments in response to splanchnic nerve stimulation, were apparently unaffected by prior removal of the thyroid gland and closely resembled those in control animals[4].

It is concluded that both the release of pancreatic glucagon and the mobilization of liver glycogen which occurs in response to splanchnic nerve stimulation depend to some extent upon the presence of thyroxine.

Résumé. La sécrétion de glucagon pancréatique aussi bien que la mobilisation du glucogène du foie, provoqués par la stimulation des nerfs splanchniques des veaux surrénalectomisées sont sensiblement diminués par la résection préable de la thyroïde.

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[5] This work was supported by grants from the Agricultural Research Council, the Smith, Kline and French Foundation, and the British Diabetic Association. It is a particular pleasure to acknowledge the skilled technical assistance provided by Mr. P. M. M. BIRCHAM.