for 180 days. The table shows, in fact, that in controls, at the age of 225 days there is a significant reduction of β-glucuronidase activity in the hypothalamus ($p < 0.02$).
In the cerebral cortex none of these treatments had any effect either on protein concentration or on β-glucuronidase activity, which apparently convalidates the specificity of the effects observed in the hypothalamus.
These results may mean that β-glucuronidase activity in the hypothalamus of male mouse is androgen-dependent. Firstly because the antiandrogen greatly decreases its activity and secondly the androgen-replacement therapy stimulates it. The androgen-dependence of β-glucuronidase activity in the hypothalamus is not restricted to mammalian species, however. We earlier demonstrated this phenomenon in the hypothalamus of the male frog[4]. Furthermore the effects of CA seem to be reversible, although longer CA treatments need longer recovery periods. These data also indicate that the recovery capacity of the animal after withdrawal of CA seems to be proportional to the duration of recovery, and inversely related to the duration of treatment.

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A human calcitonin-like molecule in the ultimobranchial body of the amphibia *(Rana pipiens)*

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Summary. As assessed by radioimmunoassay, and high performance liquid chromatography (HPLC), frog ultimobranchial calcitonin was found to be similar to synthetic human calcitonin but completely different from synthetic salmon calcitonin.

Frogs have a well developed ultimobranchial gland (UBG)[2] which is thought to contain calcitonin (CT). Extracts of frog UBG may induce hypocalcemia in a rat bioassay for CT[3]. Induced hypercalcemia will increase the secretory activity[4], while ultimobranchialectomy leads to short-lived hypercalcemia, hypercalciuria, increased osteoclastic bone activity and eventual osteopenia[5]. There is a marked seasonal variation in the activity of the UBG with maximal activity in the summer months while much less in the winter[6,7].
Amphibian growth hormone and prolactin are immunochemically related to rat growth hormone[8]. And toad LHRH is identical by radioimmunoassay and chromatography criteria to mammalian LHRH yet different from more nearly related species[9]. We have studied the immunochemical characteristics of frog UBG calcitonin to clarify whether it is more closely related to the primate-rodent group or to the teleost group of calcitonins.
Materials and methods. Adult frogs *Rana pipiens*, of both sexes were obtained in November, before the hibernation, from T. Gerrard & Co., Surrey, England. Groups of 25 were anesthetized with ether, and the UBG in the adjacent area to the glottis was removed, immediately frozen and stored at $-20\,^{\circ}C$. Pooled UBG were extracted and prepared for RIA as described before[3], except that the tissue was extracted first by 0.1 M HCl, 1% NaCl.
Human CT RIA was performed using 2 antihuman CT antisera. Antiserum 827/4 has mainly a mid molecule specificity, while antiserum 336/6 has both C-terminal and mid portion specificities[11]. Standards and labelled hormone of synthetic human CT and assay procedure were as described before[11]. Antiserum 827/4 was used in a dilution of 1:60,000, gave a sensitivity of 8 pg/tube after 5 days of incubation and antiserum 336/6 was used in a dilution of 1:24,000[13]. Salmon CT RIA was done as previously described[14]. Specific antibody to synthetic salmon CT was a gift from Dr L.J. Deftos, used in a final dilution 1:25,000. In addition the extract was applied to a reverse phase high performance liquid chromatography (HPLC)[15] and the fractions were assayed for both human CT and salmon CT immunoreactivity.
Results and discussion. Approximately 80% of labelled CT can be recovered by this hydrophilic tissue extraction method. Frog UBG's contained significant quantities of human CT-like immunoreactivity. The mean level was 16 ng/UB region, expressed as human CT. Extracts gave an

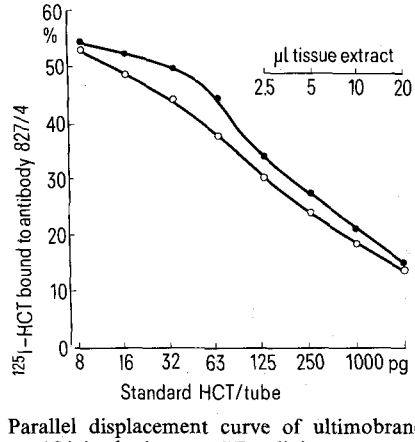

Figure 1. Parallel displacement curve of ultimobranchial gland tussue extract (●) in the human CT radioimmunoassay. Synthetic human calcitonin (○). Antiserum 827/4.

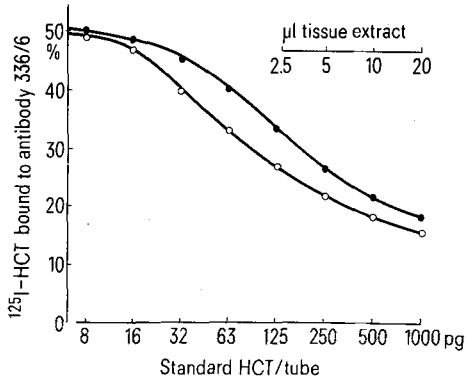

Figure 2. Parallel displacement curve of ultimobranchial gland tissue extract (●) in the human CT radioimmunoassay. Synthetic human calcitonin (○). Antiserum 336/6.

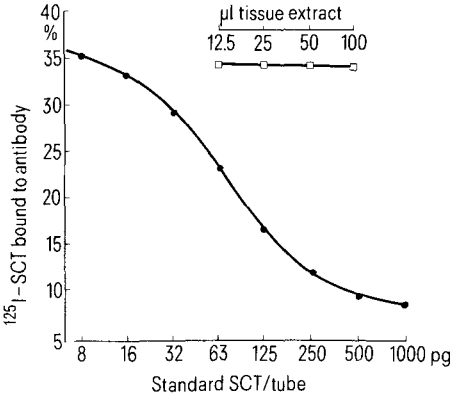

Figure 3. Lack of cross-reactivity of the ultimobranchial gland tissue extract (□) in the salmon CT radioimmunoassay. Synthetic salmon calcitonin (●).

identical dose response curve as synthetic human CT in our assay with both antisera (figs 1-2).

In the salmon CT assay, there was no displacement of tracer even at the highest concentration (fig. 3).

On HPLC, the frog UB region extract appeared as 3 HCT-like immunoreactive peaks, with the major peak eluting very near the elution position of HCT. Furthermore, no SCT immunoreactivity was detected in the same fractions (fig. 4).

The human CT assay was done using 2 well characterized antisera that are specific to the mid portion and C-terminal region of the CT molecule (16-19, 29-32 and to a lesser extent 8-12)[11]. No cross-reaction was seen with the almost identically performed salmon CT assay making it unlikely that tissue extracts contained significant amounts of peptidases which may have damaged the labelled CT leading to an artefact. It is likely that amphibian CT is very similar but not identical to HCT in view of the elution position on HPLC, but it is certainly dissimilar to salmon CT.

In a reported porcine RIA system human CT has been found to give slight but definitive cross-reactivity[16]. The fluorescent localization of CT in frog UBG using antiporcine CT antiserum has been reported[17]. However, this type of study is not quantitative and a very slight degree of cross-reaction, insignificant elsewhere may be seen. Furthermore the salmon CT antiserum used has been shown to cross-react with porcine CT to a limited extent[14]. But the completely negative results obtained with frog UBG extracts in the salmon CT assay suggest that frog CT is not related to salmon or the artiodactyl group of CT.

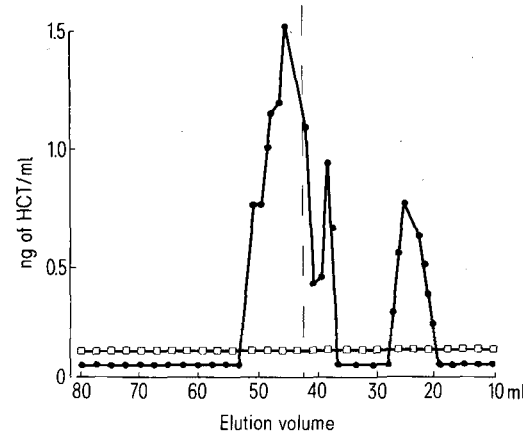

Figure 4. High performance liquid chromatography (HPLC) profile of amphibian ultimobranchial region extract. The fractions were assayed for both SCT (□) and HCT (●) immunoreactivity. A stainless steel column was used (10×0.4 cm), packed with 10 µm ODS silica (Whatman Ltd). The elution fluid was a linear gradient from methanol:water:trifluoroacetic acid (TFA) (40:59:1, by vol) to methanol:water:TFA (90:9:1, by vol). The elution volume was 1 ml/min and the fractions were collected every 1 min. Bovine insulin 250 µg (Ciba-Geigy) was used as an internal marker (dotted lines).

All calcitonins so far described immunologically or structurally fall into 3 groups: primate-rodent, artiodactyl and teleost[18]. The evidence presented in this study would suggest that amphibian CT ought to be placed in the 1st group.

The finding of a human calcitonin-like molecule in amphibia is of great evolutionary interest. We have previously reported that a human CT-like molecule is present in the nervous system of protochordates[11], and have suggested that human CT is the parent molecule from which other CTs are derived during evolution. The finding that amphibian CT is related to human CT supports the above suggestion and presumably the gene specifying a human CT molecule must be present in fish, which are intermediary between protochordates and amphibians and possibly also in other vertebrates throughout the evolutionary tree between amphibians and man.

Amphibians come in evolution between fish and reptiles, whose calcitonins are known to be SCT-like[19, 20]. However, we could not detect any SCT-like molecules in amphibian's UBG. This can be explained by the possibility that the gene specifying SCT is not expressed in amphibia.

The purification and sequencing of amphibian CT will be an important step in clarifying the evolution of the CT molecule.

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Radioimmunoassay of cortisol in blood of buffaloes *(Bubalus bubalis)* during the oestrous cycle[1]

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Summary. Changes in the concentration of cortisol were measured by radioimmunoassay in the blood plasma of buffaloes *(Bubalus bubalis).* There were minor fluctuations in the level during the oestrous cycle, but the differences between the days were not significant. The study revealed that cortisol under normal conditions does not appear to be involved in the regulation of the cycle.

The response of domestic animals to various physiological, environmental and management-related stresses is characterized by activation of the hypothalamic pituitary adrenal axis. Determination of circulating cortisol is a useful parameter for assessing adrenocortical activity. The radioimmunoassay of cortisol in human blood plasma has been reported[3-5], but no attempt has been made to quantify this hormone in the blood plasma of buffaloes. The present study deals with the radioimmunoassay of cortisol in the blood plasma of buffaloes during the oestrous cycle.

Materials and methods. Animals and blood collection: 6 Murrah buffaloes with normal cycles were selected from the Institute herd, and blood samples were collected on alternate days between 10.00-12.00 h from the day of oestrus to the next oestrus. Blood was collected rapidly in tubes packed in ice and centrifuged at 4 °C; plasma was stored at −20 °C pending hormonal analysis. Maximum care was taken while collecting blood to avoid any possible stress which might influence the hormone level.

Radioimmunoassay: Duplicate (1 ml) plasma samples were vortexed vigorously for 1 min with 10 ml distilled dichloromethane. Following brief centrifugation, the upper aqueous plasma layer was removed and the lower organic layer was transferred and evaporated to dryness under nitrogen in a 40 °C water bath. The residue was dissolved in 200 µl of 0.1 M phosphate buffered saline, by keeping the tubes at 45 °C for 15 min and vortexing for 30 sec. The remaining procedure was same as reported earlier from this laboratory by Arora et al.[6]. The validity of the assay was determined by adding known amounts of cortisol in charcoal-treated (hormone free) plasma. Recoveries of 0.5, 1.0, 2.0 and 4.0 ng of cortisol added to 1 ml of charcoal-treated plasma were 0.54±0.08, 1.12±0.07, 2.08±0.03 and 4.08±0.08 (mean±SEM) respectively. The sensitivity of the assay was 0.075 ng. The coefficients of intra- and inter-assay variations were 11.8 and 13.9%. The antiserum cross-reacted with 30% deoxycortisone and 4.1% corticosterone; cross-reaction was very low with other steroids.

Results and discussion. The changes in the concentration of cortisol during the oestrous cycle are shown in table 1. The level on the day of oestrus was 14.00±0.53 ng/ml, and fluctuated between 8.36 and 15.03 ng/ml during the oestrous cycle.

Our observations revealed minor fluctuations in the levels of plasma cortisol during the oestrous cycle, and the differences between the days were not significant (table 2). This pattern for cortisol level during the oestrous cycle was similar to that observed in the ewe[7] and in dairy cows[8-11]. On the other hand, Swanson et al.[12] and Gimenez et al.[13], for heifers, and Bhattacharya et al.[14], for goats, have described a higher level of glucocorticoids at oestrus compared with the level observed during the rest of the cycle. Sprague et al.[15] showed that adrenal release of corticoids under stress conditions may influence the oestrous cycle. Since in our study maximum care was taken to avoid stress while collecting blood, differences in cortisol levels between the days of the cycle cannot be attributed to stress. This implies that cortisol under normal conditions does not

Table 1. Cortisol concentration (ng/ml) in blood plasma of cycling buffaloes

Days	No. of observations	Concentration ± SE
0	6	14.00±0.53
1	6	9.70±0.52
3	5	9.97±1.98
5	6	8.36±1.47
7	6	10.53±1.71
9	6	11.28±1.65
11	6	11.88±2.66
13	6	11.34±2.44
15	5	12.14±1.49
17	6	11.44±1.51
19	5	15.03±2.01
−4	6	10.87±1.12
−2	5	11.70±1.13
0	4	14.37±0.59

Table 2. ANOVA

Source of variation	df	Mean squares	F
Between days	13	20.373	1.448*
Within days (error)	67	14.065	

* Non-significant.