the method employed. The results obtained are in agreement with our previous findings that, in animals exposed to 650 R, there was a clear tendency towards replenishment of noradrenaline and adrenaline stores in the heart and the brain 7 days following irradiation; but no such tendency was observed during the same period in animals exposed to 850 R[11-14]. It was also found earlier that irradiation with 900 R caused a significant depletion of noradrenaline content in whole brain 23 h after irradiation[18]. Changes in the catecholamine stores following irradiation have been found in other species as well. For example, a lethal dose of gamma-irradiation reduced catecholamine content in adrenal medulla and hypothalamus of monkey and these changes were observed during the first 24 h after irradiation[10]. In contrast, Johnsson et al.[19] found no significant changes in the amount of noradrenaline in the heart, vas deferens and brain 24 and 48 h after whole-body X-irradiation of rats with 850 R.

The results presented indicate that, during the time interval from 24 h to 5 days irradiation, there is no significant difference between the animals exposed to 650 R and those exposed to 850 R of X-rays in respect to the absolute decrease of dopamine content in the corpus striatum. However, the 2 groups of animals differ substantially in respect to the duration of the decrease of dopamine stores. Thus, in animals which received 650 R, reestablishment of physiological values of dopamine stores in the corpus striatum occured, indicating that the said dose of X-rays does not impair permanently the biosynthetic processes. On the other hand, replenishment of dopamine stores in the investigated organ was not observed in animals with 850 R, suggesting that high doses of X-rays produce irreversible defects in synthesis of catecholamines. In order the better to understand the extent of damage produced by ionizing radiation on the mechanisms controlling the biosynthesis and metabolism of catecholamines, it would be necessary to study the catecholamine turnover in the central nervous system using labelled DOPA, dopamine or noradrenaline.

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Immunochemical studies on rabbit calcitonin

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Summary. Cross reaction studies using radioimmunoassays specific for human and porcine calcitonin showed that rabbit calcitonin is structurally more closely related to human than to porcine calcitonin.

Calcitonin in mammals has been considered as an example of unusual biochemical evolution[1] due to the large variations encountered in the 10-31 sequence of the molecule. For example, human (HCT) and porcine (PCT) calcitonin share only 6 residues in common in this region. This indicates a high degree of mutation in the CT molecule during evolution.

We have shown that murine calcitonin (RCT) showed total cross reaction with human calcitonin using antibodies directed against HCT[2]. We concluded from these studies that RCT should have a high degree of similarity in its tertiary structure and probably in its primary amino acid sequence with HCT. Recently elucidation of the amino acid sequence of RCT[3] has strikingly confirmed these predictions based on immunological studies, as the 2 molecules differ by only 2 amino acids. Thus CT in primates and rodentia are structurally quite similar but very different from CT in artiodactyls.

We have undertaken an immunochemical study of rabbit calcitonin (lagomorphs) to establish whether in this species CT was related to the artiodactyl group or to the primate rodentia group. Such a study within the limits of immunological methods would be helpful in establishing whether CT in this order evolved separately or shared a common ancestor with either one of these 2 groups.

Material and methods. Thyroids from young (1 kg) male rabbits were extracted with cold 0.1 N HCl and freeze dried. CT content of the extract was estimated by a 4 point bioassay using synthetic HCT[4] (specific activity 100 MRC units/mg as a standard).

Aliquots were dissolved in the appropriate radioimmunoassay (RIA) buffer. Several RIA specific for either HCT or PCT were used in the study. The RIA were specific for each species as no cross reaction was present. 4 antisera specific for PCT and 3 antisera specific for HCT were used.

The antibodies specific for HCT were directed either towards mid (As 15-As 16) or carboxy terminal (As 36) portions of the human molecule. The detailed procedures for the RIA's used have already been published[2,5]. Histological localization of rabbit C cells was performed by an indirect immunofluorescent technique using antibodies specific for HCT according to the procedure used for RCT[2].

Results. The extract used in the study contained the equivalent of 230 ng of HCT/mg, as estimated by bioassay; this corresponded to 23 munits MRC of calcitonin. Rabbit thyroidal extracts displaced labelled HCT in the 3 RIA used (figure 1). In each system, rabbit extract and HCT displaced the human tracer in an identical fashion. No displacement of labelled PCT was observed when increas-

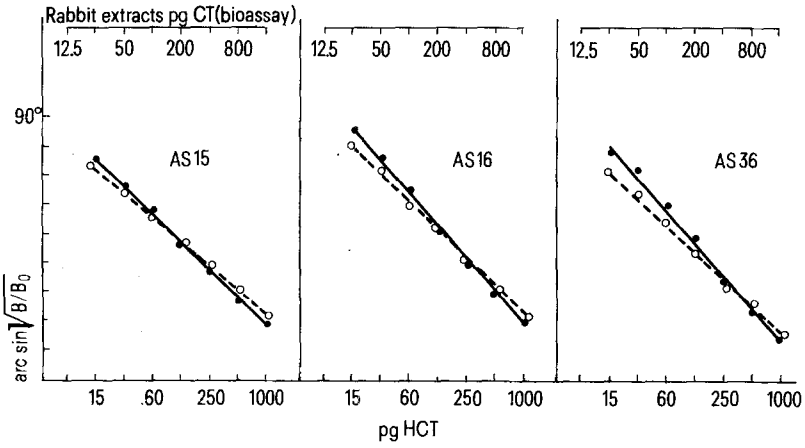

Fig. 1. Immunological cross reaction of rabbit calcitonin with human calcitonin. Inhibition of specific binding of ^{125}I HCT to anti HCT antibodies by synthetic human calcitonin (—) and rabbit extractive CT (---).

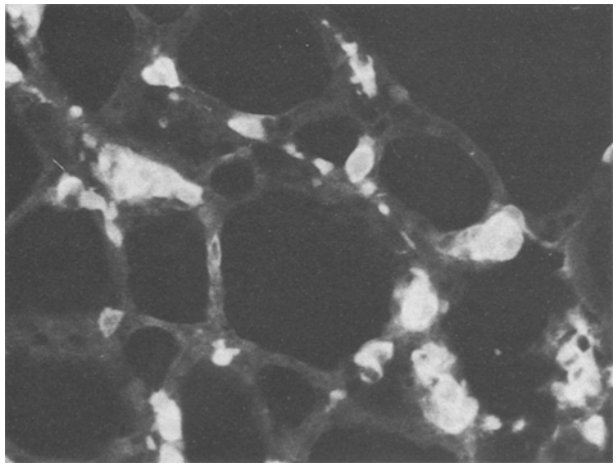

Fig. 2. C cells in rabbit thyroid. Indirect immunofluorescence (×450).

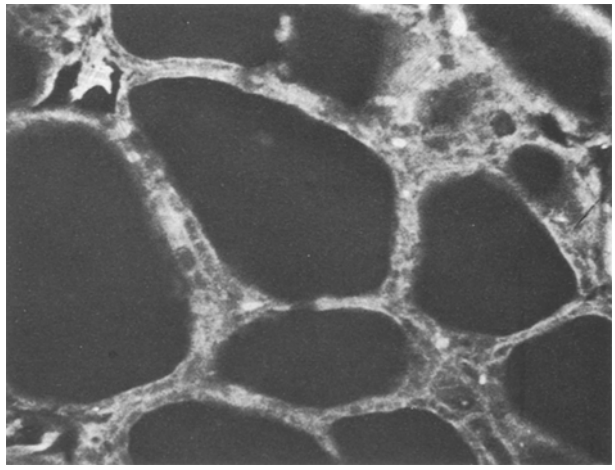

Fig. 3. Control reaction. Absence of specific staining. Antibody saturated with HCT (×450).

ing amounts of the rabbit extract were added in the 4 RIA specific for this molecule.

From 1.6 to 1.2 times more rabbit CT than HCT were required to provoke a 50% decrease in bound tracer. Sections stained by the indirect immunofluorescent technique using an antibody specific for HCT (As 14) showed intense staining in certain cells either present in clusters or in a parafollicular position (figure 2). The stain is specific for CT as no fluorescence was observed if the specific antibody was omitted or saturated with synthetic HCT (figure 3).

Discussion. The high cross reaction of rabbit calcitonin with HCT in all the systems used, and the reverse finding of no cross reaction with PCT, indicate that rabbit calcitonin is immunochemically similar to HCT and not to PCT. RIA for human CT and probably murine CT may be used for the estimation of the hormone in this species, and specific localization of CT in rabbit C cells may be achieved using antibodies raised against HCT, using either the immunofluorescent[2] or the immunoperoxydase[6] technique. The immunochemical studies presented here show that rabbit calcitonin has a tertiary configuration similar to HCT and not to PCT; and this probably reflects a higher degree of homology between the amino acid sequences of human and rabbit calcitonin. Of course, only elucidation of the sequence of rabbit calcitonin will establish the exact degree of similarity. Mammalian calcitonins can thus for the moment be separated into 2 groups on the basis of their structure and their immunochemical reactions: 1. Human, murine and rabbit, 2. Bovine, porcine, ovine and caprine. Differences are minimal within groups and maximal between groups. The studies reported here show that rabbit calcitonin potentially belongs to group 1. Preliminary studies using the same approach indicate that bat (chiroptera) and hedgehog (insectivora) probably also belong to this group. Thus in the course of evolution from a common ancestor, at least 2 major types of CT have evolved in mammalia. This may reflect important changes in physiological role of the hormone in different mammalian species.

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