tion (PIF), it would be expected that the transplanted pituitary would be more receptive to the DES-induced tumorigenic stimulus, a phenomenon which clearly did not occur in this study.

The existence of a hypothalamic prolactin releasing factor (PRF) in birds has been well established[11] and recent studies suggest that this factor may exist in mammals as well[12]. The results of the present study support this concept and provide evidence that this factor is influenced by estrogen administration. Estrogens have been reported to be actively concentrated by the hypothalamus as well as other areas of the limbic system[13] and to act directly on the pituitary tissue[9]. Although this study suggests that the hypothalamus plays an important role in pituitary tumorigenesis, it has not yet been determined whether the tumorigenic effects of DES are exerted primarily at the hypothalamic or pituitary level. The possibility cannot be ruled out that growth of the transplanted pituitary is not as vigorous as the in situ pituitary because of an inferior blood supply. This seems unlikely as all grafted pituitaries appeared to have a rich blood supply, as illustrated in Figure 2, and the subcapsular bed of the kidney is considered to be one of the best sites for effective revascularization of grafted tissue[15].

Résumé. Les résultats de cette étude démontrent nettement que la glande pituitaire du rat transplantée répond au stimulus tumeurigénique du diéthylstilboestrol, mais dans beaucoup plus faible mesure que la glande pituitaire in situ.

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Inactivation Studies of Angiotensin II by Purified Enzymes

This communication describes the effect of three highly purified, hydrolytic enzymes from pig brain on Ile^5-angiotensin II. The enzymes selected for this study belong to the following categories: intracellular acid proteinases, aminopeptidases and arylamidases. While the acid proteinase and aminopeptidase cause no significant inactivation of Ile^5-angiotensin II, arylamidase degrades this peptide as shown by bioassay as well as by quantitative determination of the end products by amino acid analysis.

Brain acid proteinase (or Cathepsin D)[1], aminopeptidase (determined by the specific cleavage of Leu from Leu-Gly-Gly)[2] and arylamidase (determined by the hydrolysis of Arg. βNA)[3] were prepared from pig brain and assayed as described. Ile^5-angiotensin II was obtained from Calbiochem., L.A., Calif. One aliquot of each hormone-enzyme incubate, together with appropriate controls, was subjected to bioassay on the isolated rat uterus according to the method of HOLTON[4] as modified by MUNSICK[5] with the use of Mg^{2+}-free van Dyke-Hastings solution as the bathing fluid. Another aliquot of the digest was directly applied to amino acid analysis on a Technicon analyzer. Amino acids released were identified and quantified according to the short-column procedure of CATRAVAS[6] with the following modification: a 0.6 × 60 cm column packed with Amminex A-4 (Biorad., Palo Alto, Calif.) was used and was eluted at 60 °C with a 9-chamber Varigrad containing sodium citrate buffers at pH 2.75, 2.88, 3.80 and 6.10[2]. Aminopeptidase and arylamidase were incubated with hormone for set periods of time at 37 °C. Enzyme-substrate ratios (on a molar basis) were 1:150 to 1:400 and 1:75 to 1:150, respectively, in a total volume of 0.4 to 0.6 ml of 40 *mM tris*-HCl (pH 7.6) containing 0.1 *mM* dithiothreitol. Acid proteinase-substrate mixtures (enzyme to substrate ratios 1:5 and 1:10) were incubated in a total volume of 0.4 to 0.6 ml of 50 *mM* citrate buffer, pH 3.2. Reaction mixtures not directly utilized were stored frozen at -20 °C.

Bioassay revealed full retention of the rat uterotonic activity of Ile^5-angiotensin II during a 24-h.-incubation with aminopeptidase or acid proteinase. Moreover, no free amino acids resulted from the incubation of Ile^5-angiotensin II with these 2 enzymes. Contrary, the incubation of 100–200 µg of substrate with purified arylamidase led to the rapid appearance of the first 5 N-terminal amino acids (Figure). Quantitative determination of the individual amino acids after a 20-min digestion showed approximately equimolar quantities of aspartic acid, arginine, valine, tyrosine and isoleucine. After a 24-h incubation period 35% of the hormone had been degraded. The existence of peptide intermediates in the breakdown of Ile^5-antiotensin II is indicated by an initially high ratio (amino acid released/aspartic acid released) for material eluting with histidine (at 20 min). Undegraded Ile^5-angiotensin II was retained on the column under these experimental conditions.

The vasoactive hormone angiotensin II, which appears to elicit its dipsogenic response via receptors located in certain regions of the brain[7], is known to be cleaved by a large number of exo- and endopeptidases[8]. Recently,

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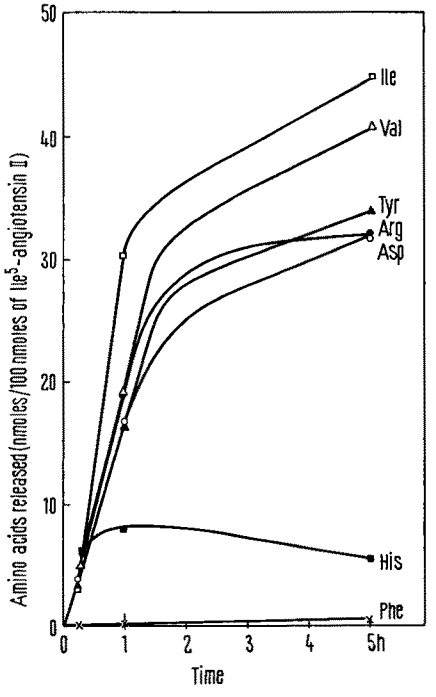
MATSUNGA et al[9]. reported the inactivation of Val[5]-angiotensin II by an enzymic activity, present in crude brain extracts, with properties similar to a neutral proteinase. The highly purified brain enzyme used in the present study, which possessed a marked specificity only for neutral and basic arylamides (Leu. βNA, Arg. βNA), rapidly hydrolyzed Ile[5]-angiotensin II. Thus this enzyme must differ from that described by MATSUNGA et al.[9] as well as from the Ca^{++}-dependent peptide hydrolase present in plasma, which is specific for acidic arylamides (α-Glu. βNA, α-Asp. βNA)[10]. Our finding that acid proteinase is unable to inactivate angiotensin is in accord with the known specificity of this enzyme for phenylalanine and leucine residues[8]. Inactivation by N-terminal cleavage is considered to be caused by aminopeptidases, yet interestingly the purified aminopeptidase from pig brain used in this study failed to release any free amino acids from Ile[5]-angiotensin II. The resistance of angiotensin II to this aminopeptidase may be associated with the presence of an aspartic acid residue adjacent to an arginine residue.

The arylamidase requires further study from the point of view of its possible physiological importance in the inactivation of peptide hormones in brain.



Release of amino acids as a function of time from Ile[5]-angiotensin II upon incubation with pig brain arylamidase. The values for histidine and isoleucine include peptide intermediates eluted at the same buffer volume as the given amino acids.

Zusammenfassung. Die hochgereinigte Arylamidase vom Schweinehirn spaltet, im Gegensatz zur ebenfalls isolierten Säureprotease und Aminopeptidase, Ile[5]-Angiotensin II, wobei die ersten 5 N-terminalen Aminosäuren von hydrolytischen Enzymen freigesetzt werden.

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Somatic Chromosomes of the Flat-Headed Bats (*Tylonycteris* spp)

The Malayan flat-headed bats, genus *Tylonycteris* Peters (Chiroptera, Vespertilionidae), have recently been reviewed[1]. The two species, *T. pachypus* (Temminck) and *T. robustula* Thomas, occurring in Malaya, are sympatric[2]. They appear to occupy very similar ecological niches but do not voluntarily associate at roost[3]. These closely related sympatric animals are therefore ideal for studying the isolating mechanisms which keep them distinct despite extensive overlap of ecological niche. The present communication deals with the somatic chromosomes of these flat-headed bats.

Materials and methods. Both species of *Tylonycteris* were collected from the University of Malaya Field Studies Centre, 16 mi Ulu Gombak, Selangor, Malaya. The bats were injected i.p. with 0.01 ml/g body weight of 0.04% colchicine solution. After 1.5 h, chromosome preparations were made from bone marrow by the usual hypotonic pretreatment, acetic-alcohol fixation and air-drying technique.

Results. The Table summarizes the number of specimens studied and the karyotypes of *Tylonycteris pachypus* and *T. robustula*.

Tylonycteris pachypus (Figure 1). 2 pairs of the meta/submeta-centric autosomes, as well as the *Y* chromosome, are distinctly smaller than the rest and constitute the smallest chromosomes in the complement. The acrocentric chromosomes together with the *X* chromosome are difficult to distinguish individually. All the larger chromosomes are also similar in size.

Tylonycteris robustula (Figure 2). As in *T. pachypus* the karyotype is characterized by 2 pairs of distinctive minute meta/submeta-centric autosomes as well as a minute *Y* chromosome. Unlike *pachypus*, there are 7 extra pairs of meta/submetacentric autosomes with a corresponding decrease of 14 pairs of acrocentrics. These meta/submetacentrics are larger than, or as large as, the largest acrocentrics and subacrocentrics. The acrocentric *X* chromosome is probably smaller than the largest acrocentric autosome. The larger meta/submeta-centric autosomes are also difficult to identify individually.

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