be accelerated in hypophysectomized animals in comparison to intact ones (Table II). Controls in which the hypothalamic region of the brain is exposed with the pituitary remaining intact respond like unoperated animals to background changes.

Darkness: In darkness the lower dermal melanophores of white- and black-adapted hypophysectomized catfish equilibrate at a mean MI 2.76 in about 6 h, suggesting that the contribution of the pituitary to equilibrium in darkness is not appreciable.

4. *Injection of pituitary extract.* Intraperitoneal injection of 0.3 ml of crude pituitary extract (2 glands from long white-adapted fish in 4 ml Ringer) into white-adapted hypophysectomized fish of the same size induces dispersion to MI 4.00 (mean of 3 animals) in 2 h whereas the same injection causes only slight dispersion (MI 2.20) in unoperated white-adapted controls.

Discussion. Comparison of the time relations of the chromatic changes of other teleosts shows that *I. melas* fills the gap between the species in which these changes are entirely or primarily neurally coordinated (e.g. *Macropodus*[4], *Fundulus*[5], *Gasterosteus*[6], *Lebistes*[7], and *Phoxinus*[8]) and those in which their control is mainly hormonal (e.g. *Anguilla*[9]).

The pituitary appears to be essential for full dispersion of melanophores in black background adaptation[10]. Of all the layers the epidermal melanophores appear to be most influenced by pituitary hormones. Furthermore, adaptation of hypophysectomized fish to a white background shows no statistically significant difference from that of the normal fish, and therefore indicates that no pigment aggregating hormone is playing a part in the normal colour changes of *I. melas*.

Zusammenfassung. Beim Zwergwels *Ictalurus melas* liegen die Melanophoren im Ektoderm sowie im oberen und tieferen Entoderm und zeigen ein schichttypisches Verhalten. Nach Hypophysektomie bleibt das unterschiedliche Verhalten bestehen. Postoperativ wird der Fisch auf schwarzem Untergrund weniger dunkel als das normale Tier, während die Anpassung auf weissem Untergrund unverändert bleibt.

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[4] K. UMRATH and H. WALCHER, Z. vergl. Physiol. *33*, 129 (1951).
[5] G. H. PARKER, *Animal Colour Changes and their Neurohumors* (Cambridge University Press, 1948).
[6] L. HOGBEN and F. W. LANDGREBE, Proc. R. Soc. *B 128*, 317 (1940).
[7] R. M. NEILL, J. exp. Biol. *17*, 74 (1940).
[8] E. G. HEALEY, J. exp. Biol. *28*, 297 (1951).
[9] R. M. NEILL, J. exp. Biol. *17*, 74 (1940).
[10] G. H. PARKER, *Animal Colour Changes and their Neurohumors* (Cambridge University Press, 1948).

An Unusual Trabecular Thyroid Cancer Producing Calcitonin

We have established that thyroid medullary carcinoma with amyloid stroma[1,2] is a differentiated tumour secreting calcitonin (CT)[3,4], the hypocalcemic hypophosphatemic hormone[5]. In the course of a systematic study of the histochemistry and biochemistry of thyroid carcinomas, we had the opportunity to observe a case of trabecular thyroid cancer lacking amyloid stroma and producing large amounts of CT.

The patient was a 60-year-old female. The left lobe of the thyroid increased in size within 9 months, was hard to palpation and the scan demonstrated a lack in ^{131}I uptake. The patient had no signs of diarrhoea, flush or cutaneous neurofibromatosis. Serum calcium levels fluctuated between 9.5 and 7.0 mg/100 ml; serum phosphate levels were 4.0 mg/100 ml. Total ablation of the thyroid was performed (April 1970): the left lobe (35 g) was the seat of a hard, white mass with a hemorrhagic center. Lymphnodes had a normal appearance and were left in situ. Different samples of the tumour were either extracted for CT or fixed for microscopic examination.

Methods. CT-bioassay: Tumour fragments were defatted with ether and extracted with butanol-acetic acid water[6]; the hormone content of the extracts and of fresh serum were assayed for hypocalcemic and hypophosphatemic[3] activity using a 4 point bioassay in the rat with a M.R.C. reference as a standard[18]. The results are expressed in mU/mg of tissue dry weight.

Histochemical stains: Periodic acid-Schiff (PAS), alcian blue, thioflavin T, Congo red, crystal violet, toluidine blue, lead hematoxyline before and after acid hydrolysis[7,8], and Daventport's silver impregnation.

Results. 1. CT: The hypocalcemic activity of the tumour is 137.2 mU/mg (85–214, $P < 0.05$), the hypophosphatemic activity is 126 mU/mg (94–158, $P < 0.05$). Hypocalcemic activity of the serum: 0.2 mU/ml.

2. Pathology: The tumour crosses the capsule of the thyroid, invades blood vessels and in particular a thyroidal vein, forms a paraisthmic metastatic nodule 3 mm in diameter. The right lobe of the thyroid is uninvolved and shows evidence of thyroiditis. The tumour is composed of closely packed neoplastic lobules, separated by thin zones of regressive parenchyma. The lobules are formed of trabeculi containing 5–50 cells, which are surrounded by a thin fibrous sheet and separated by voluminous capillaries. Microscopic appearance: the cells are often large, polyhedric or fusiform, the cytoplasm is clear and distinctly eosinophilic. Numerous atypical forms exist. The nuclei are polymorphic. Some intracellular eosinophilic deposits are found. All staining

[1] J. B. HAZARD, W. A. HAWK and G. CRILE JR., J. clin. Endocr. Metab. *19*, 152 (1959).
[2] E. D. WILLIAMS, C. L. BROWN and I. DONIACH, J. clin. Path. *19*, 103 (1966).
[3] G. MILHAUD, M. TUBIANA, C. PARMENTIER and G. COUTRIS, C. r. Acad. Sci., Paris *266*, Série D, 608 (1968).
[4] M. TUBIANA, G. MILHAUD, G. COUTRIS, J. LACOUR, C. PARMENTIER and B. BOK, Br. med. J. *4*, 87 (1968).
[5] P. F. HIRSCH, G. F. GAUTHIER and P. L. MUNSON, Endocrinology *73*, 244 (1963).
[6] R. NEHER and F. W. KAHNT, in *Calcitonin* (Ed. S. TAYLOR; W. Heinemann Medical Books Ltd., London 1968), p. 57.
[7] E. SOLCIA, G. VASSALLO and C. CAPELLA, Stain Technol. *43*, 257 (1968).
[8] E. SOLCIA, C. CAPELLA and G. VASSALO, Histochemie *20*, 116 (1969).

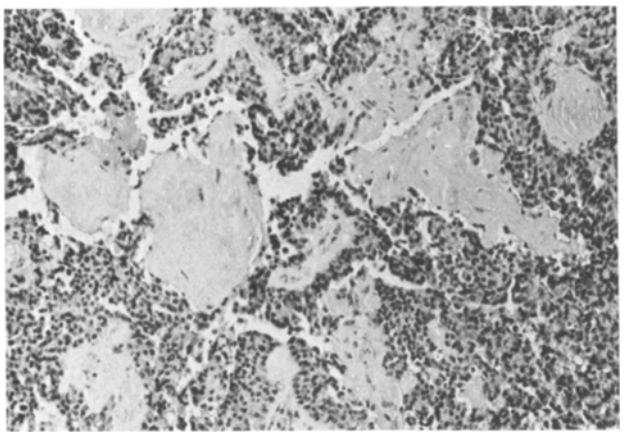

Fig. 1. Medullary carcinoma: amyloid deposits. Alkaline Congo Red. ×140.

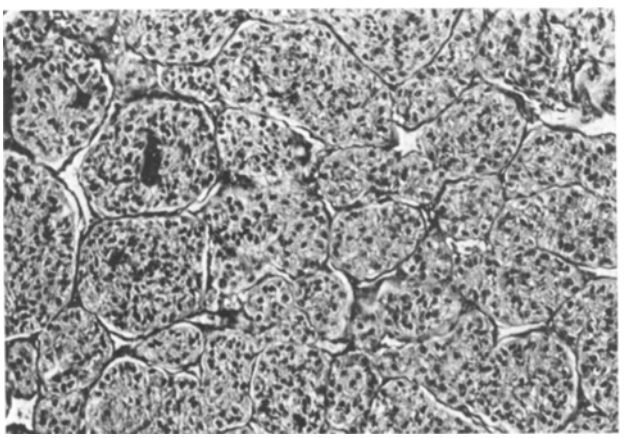

Fig. 2. Trabecular carcinoma: Intercellular deposits. Alcian Blue. ×140.

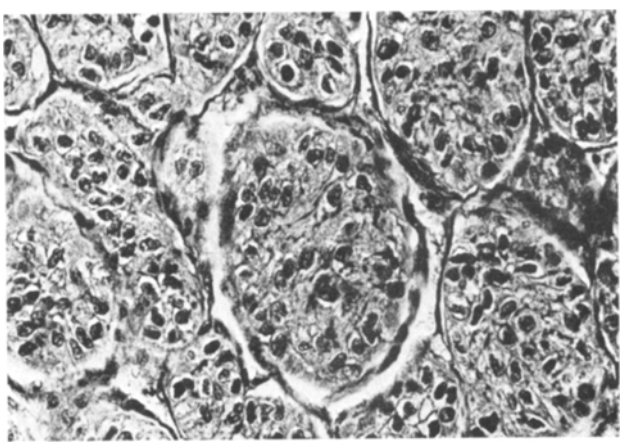

Fig. 3. Trabecular carcinoma: Acid mucopolysaccharides intracellular deposit. Alcian Blue. ×340.

reactions for amyloid stroma (congo red, crystal violet and thioflavine T) are negative in all the fragments studied. Strong staining with alcian blue is noted in the granulations of many cells as well as in the majority of intracellular deposits. Selective stains for C cells: Toluidine blue, lead hematoxylin after acid hydrolysis and silver impregnation by Davenport's method are positive in large sometimes multinucleated cells. Alkaline diazo stain and lead hematoxylin without hydrolysis give negative results. A few cells contained PAS-positive material.

Discussion. The hypocalcemic and hypophosphatemic activities found in tumour extracts are likely to be due to the presence of CT: the extraction and purification procedures used are those which demonstrate the presence of CT in normal human thyroid gland; the responses of the extracts in the 4-point bioassay are parallel to the M.R.C. standard for CT as well as to human CT; the ratio of the hypocalcemic and hypophosphatemic activity is 1.09, close to 1, i.e. to the responses of human CT itself. Furthermore the tumour contains cells with the staining characteristics of C Cells. Studies are being carried out on the tumour extract to ascertain possible identity or differences with CT produced by medullary carcinoma.

Fluctuation of plasma calcium level is one of the biological signs of CT excess syndrom[9]; such fluctuations, noted in this patient, are in agreement with increased levels of circulating calcitonin. After the operation, the CT content of the serum was normal.

As to the classification of this tumour, 2 types of thyroid carcinoma are known to secrete CT: primary medullary carcinoma with amyloid stroma[3, 10–12] and thyroid metastases of bronchial carcinoid[13]. The case reported here cannot be classified in either category. It differs from the 30 cases of medullary cancer (Figure 1) we have collected by several features: lack of amyloid stroma in the tumour, in the veins and in the paraisthmic metastases; its trabecular structure (Figure 2) with reduced amount of stroma, the rather larger size of the cells, the presence of acid mucopolysaccharides in the cells and in the deposits (Figures 2 and 3), finally the rapid rate of growth of this tumour. It differs as well from a thyroid carcinoid metastases by its morphology, the absence of staining with lead hematoxylin[8, 13] before acid hydrolysis and the high content of CT (137 mU/mg) as compared to carcinoid: thyroid metastases (1.6 mU/mg)[13], bronchial carcinoid tumour (0.1 mU/mg) and caecal carcinoid tumour (0.3 mU/mg)[14].

Finally, it can be distinguished from thyroid hyalin carcinoma[15] rich in acid mucopolysaccharides[16] by the absence of hyalin changes and of amyloid. The weight

[9] G. MILHAUD, G. COUTRIS and M. TUBIANA, Presse méd. *77*, 2129 (1969).
[10] J. S. MEYER and W. ABDEL BARI, New Engl. J. Med. *278*, 523 (1968).
[11] W. J. CUNLIFFE, M. M. BLACK, R. HALL, I. D. A. JOHNSTON, P. HUDGSON, S. SHUSTER, T. V. GUDMUNDSSON, G. F. JOPLIN, E. D. WILLIAMS, N. J. Y. WOODHOUSE, L. GALANTE and I. MACINTYRE, Lancet *2*, 63 (1968).
[12] A. H. TASHJIAN and K. E. W. MELVIN, New Engl. J. Med. *279*, 279 (1968).
[13] G. MILHAUD, C. CALMETTES, J. P. RAYMOND, J. BIGNON and M. S. MOUKHTAR, C. Acad. Sci., Série D, *270*, 2195 (1970).
[14] Unpublished data.
[15] J. LASKOWSKI, Nowotwory *7*, 23 (1957).
[16] M. BESKID, Folia histochem. cytochem. *2*, 261 (1964).

of the evidence presented favors the recognition of a new class of thyroid carcinoma producing CT, though the possibility of the formation of amyloid by eventual metastases cannot be actually rejected [2, 17].

In summary, the investigation of CT-production should be extended to trabecular tumours of the thyroid and not restricted to medullary cancer with amyloid[18].

Résumé. Un cancer trabéculaire de la thyroïde, riche en mucopolysaccharides et dépourvu de stroma amyloïde, produit 1000 fois plus de calcitonine que le tissu thyroïdien normal. Il semble s'agir d'une entité anatomopathologique nouvelle, correspondant à une tumeur différenciée sécrétant de la calcitonine.

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[17] R. Peraldi, Thèse Fac. Med. Montpellier (1969).
[18] M. R. C. - Medical Research Council standard for thyroid calcitonin.

Chromosomes of Some Asian and South American Squirrels (Rodentia: Sciuridae)

North American squirrels are characterized by differing degrees of chromosomal variation; cytological mechanisms postulated to account for karyotype evolution vary in different taxa. Striking diversity, due mainly to Robertsonian changes, occurs in terrestrial squirrels, Marmotini, of the genera *Spermophilus* ($2n = 30$–46)[1–4] and *Marmota* ($2n = 36$–42)[5, 6]; species of *Cynomys* ($2n = 40$; $2n = 50$) are differentiated by both Robertsonian mechanisms and pericentric inversions[7]. *Ammospermophilus* ($2n = 32$)[8] constitutes a monomorphic genus. Taxa within the genus *Tamias* ($2n = 38$)[9–12] share similar diploid numbers but differ due to pericentric inversions. In another ground squirrel tribe, Xerini, *Spermophilopsis* has a $2n = 38$[12, 13].

In contrast, tree squirrels, Sciurini, exhibit remarkable chromosomal homogeneity examplified by $2n = 40$ and similar karyotypes in 4 species of *Sciurus*[14]. Two species of *Tamiasciurus*[15], Tamiasciurini, have $2n = 46$. Comparison of karyotypes from the above taxa usually provides a clear appraisal of the number and kinds of chromosomal rearrangements associated with their evolution, but direction of evolution is difficult to evaluate, because of inability to distinguish centric fusions from fissions. This difficulty is compounded by lack of knowledge concerning the probable basic or 'ancestral' $2n$ of Sciuridae.

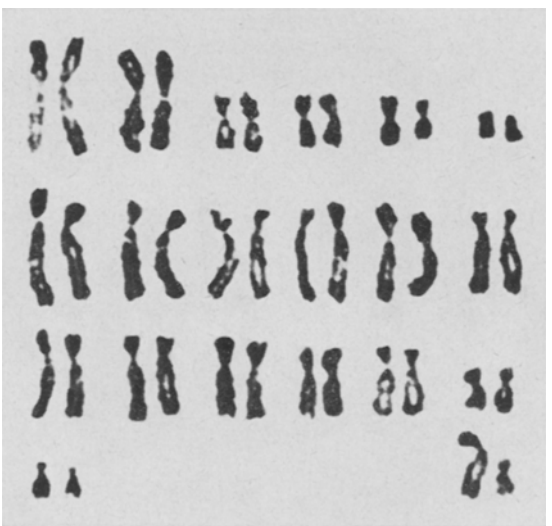

Fig. 1. Karyotype of a male *Sciurus anomalus* ($2n = 40$) $\times 3000$. The sex chromosomes are at the right of the bottom row.

The present investigation describes the chromosomes of several species of South American and Asian squirrels of the tribes Sciurini and Callosciurini. Chromosomal data from these and previously reported species provide a broadened perspective that allows a preliminary appraisal of trends in karyotype evolution in the subfamily Sciurinae.

Materials and methods. Chromosomes from the following specimens were studied: *Sciurus anomalus* Güldenstaedt, Iran, Zagros Mountains, 1 male; *Menetes berdmorei* (Blyth), South Vietnam, Calu, 1 male; *Dremomys rufigenis* (Blanford), South Vietnam, Mt. Sontra, 1 male; *Callosciurus flavimanus* (I. Geoffroy), South Vietnam, Vanderift Combat Base, Quan Tri, 1 male; *Sciurus granatensis* Humbolt, South America, unknown locality, 1 male; and Venezuela, Los Venados, Monte Avila, D.F., 1 female. The number of autosome arms were counted to determine the fundamental number (FN)[16].

Results. The $2n$ of *Sciurus anomalus* is 40 (FN 76) and the karyotype contains 18 pairs of metacentric and submetacentric autosomes and 1 pair of subtelocentric autosomes; an unpaired medium sized submetacentric and a second small submetacentric probably represent the X and Y respectively (Figure 1).

Sciurus (or *Guerlinguetus*) *granatensis* has a $2n = 42$ (FN 78) and a karyotype comprised of 19 pairs of metacentric and submetacentric autosomes and 1 pair of acrocentric autosomes, a large submetacentric X and a medium sized submetacentric Y chromosome (Figure 2).

[1] C. F. Nadler, J. Mammalogy *47*, 579 (1966).
[2] C. F. Nadler, Syst. Zool. *15*, 199 (1966).
[3] C. F. Nadler, Cytogenetics *7*, 144 (1968).
[4] N. N. Vorontsov and E. A. Lyapunova, Mlekopitayushchie (2nd All-Union Mammalogy Conference, Novosibirsk, 1969), p. 41.
[5] R. S. Hoffmann and C. F. Nadler, Experientia *24*, 740 (1968).
[6] E. A. Lyapunova and N. N. Vorontsov, Mlekopitayushchie (2nd All-Union Mammalogy Conference, Novosibirsk 1969), p. 36.
[7] R. S. Hoffmann and C. F. Nadler, in press (1970).
[8] C. F. Nadler and D. A. Sutton, Proc. Soc. exp. Biol. Med. *110*, 36 (1962).
[9] C. F. Nadler, Am. Midl. Nat. *72*, 298 (1964).
[10] D. A. Sutton and C. F. Nadler, J. Mammalogy *50*, 524 (1969).
[11] C. F. Nadler, R. S. Hoffmann and D. M. Lay, Experientia *25*, 868 (1969).
[12] E. A. Lyapunova and E. I. Zholnerovskaya, Mlekopitayushchie (2nd All-Union Mammalogy Conference, Novosibirsk 1969), p. 57.
[13] C. F. Nadler, D. M. Lay and J. D. Hassinger, Experientia *25*, 774 (1969).
[14] C. F. Nadler and D. A. Sutton, Experientia *23*, 249 (1967).
[15] T. C. Hsu, Mammal. Chromosomes Newsl. *19*, 22 (1966).
[16] R. Matthey, Experientia *1*, 50, 78 (1945).