(b) chitin is absent; and (c) there is no differentiation into epi- and procuticle. In these respects, they recall ootheca of the cockroach, *Blatella orientalis*[8] and spore walls of the fungus, *Aspergillus* sp.[9].

Reference to previous literature shows that the papillary cone of reptiles and pecten of birds are homologous organs. Though the role of the former is not known, the latter is reported to increase the efficiency of visual powers of birds. It has been suggested that the shadows of the ridges of pecten, falling on the retina, act as a grille, and that small or distant moving objects are more readily discerned as their images pass from one component to another on this grille[10].

The presence of sclerotin in the vertebrates is very unusual. Among the vertebrates, as reported earlier, the egg case of selachians (Pisces) is sclerotized[7]. The inner lining of the gizard of birds (Aves) is said to be partially tanned[11]. Among the Prochordata, walls of the stolon of the Pterobranch, *Rhabdopleura* sp. has been found to be sclerotized[12]. Since the primary mode of hardening of the integument and its derivatives of the vertebrates is by keratinization and calcification[13], the significance of the occurrence of sclerotin in the papillary cone and pecten seems to be an enigma. A point of interest to be mentioned in this connection is that the rhabdomeres of the compound eyes of the housefly, *Musca vicina* Macquart also are derivatives of polyphenols[14,15].

Résumé. Pour la première fois on rapporte la présence de sclérotine dans la papille conicale du gecko, *Hemidactylus turicus turicus* (Reptilia) et dans la pectine du pétrel, *Fregetta grallaria* (Aves).

A. SANNASI[16]

Department of Entomology, University of Georgia, Athens (Georgia 30601, USA), 6 January 1969.

[8] M. G. M. PRYOR, Proc. R. Soc. London *B 128*, 378 (1940).
[9] A. SANNASI, Mycopath. Mycol. Appl., in press (1969).
[10] A. S. ROMER, *The Vertebrate Body*, 2nd Edn (Saunders Co., London 1955), p. 504.
[11] G. SUNDARA RAJULU, personal communication.
[12] A. SANNASI, unpublished observations.
[13] L. PICKEN, *The Organisation of Cells and Other Organisms* (Oxford University Press, 1960).
[14] K. SUZUKI, Nature *195*, 994 (1962).
[15] Acknowledgments. I am thankful to Dr. M. S. BLUM for his continued interest in my work.
[16] Present address: Biology Department, Georgia Southern College, Statesboro.

Dopa-Decarboxylase and Monoamine Oxidase Activities in a Transplantable Islet Cell Tumour of the Golden Hamster

A storage of biogenic monoamines in the endocrine pancreas has been demonstrated in many mammalian species by means of the histotechnical fluorescence technique of FALCK and HILLARP in combination with chemical analyses. The adult golden hamster is remarkable, however, because its islets contain a well-developed plexus of adrenergic nerve terminals, whereas the endocrine cells do not store any histochemically demonstrable monoamines. On the other hand, a transplantable, insulin-producing islet cell tumour of the golden hamster, originally described by KIRKMAN[1], has recently been found to contain dopa, dopamine, and 5-hydroxytryptamine, and also an unidentified, possibly monoamine-like, substance[2] which condensed with formaldehyde in tissues and on chromatograms to an intensely fluorescent derivative. The presence of dopamine and 5-hydroxytryptamine in the tumour is of particular interest since these are the 2 amines commonly found in mammalian islets[3]. These findings have prompted an analysis of the tumour cell enzymes involved in the synthesis and breakdown of monoamines. The present study reports the dopa-decarboxylase and monoamine oxidase (MAO) activities in the tumour.

The tumour was transplanted subcutaneously[4] to 16 golden hamsters and was allowed to grow for 4–10 weeks. 5 tumours were used for determination of dopa-decarboxylase and monoamine-oxidase activities, and the contents of dopa and dopamine were estimated fluorimetrically[5] in 7 tumours. 4 animals were pretreated with the dopa-decarboxylase inhibitor NSD 1015 (m-hydroxybenzyl hydrazine; Smith and Nephew through Ferrosan AB, Sweden) 100 mg/kg, $1^1/_2$ h before killing, and the dopa-decarboxylase activity and the contents of dopa and dopamine in the tumours were then determined[5]. The identity of dopa was further established with the use of paper chromatography (phenol: 0.1 NHCl; 85:15)[6]. The dopa-decarboxylase activity was estimated by means of the formation of dopamine from L-dopa[6], and the monoamine oxidase activity by means of the formation of indoleacetic acid (IAA)[7]. Small tissue specimens from

	Tumours of non-treated animals M ± S.E.M. (*n*)	Tumours of NSD 1015 treated animals M ± S.E.M. (*n*)	
Dopa-decarboxylase activity µmol formed per g tissue/h	70 ± 3.7 (5)	16 ± 0.9 (4)	$p < 0.001$
MAO activity µmol IAA formed per g tissue/h	0.74 ± 0.05 (5)		
Dopa µg/g	0.20 ± 0.06 (7)	3.8 ± 0.21 (4)	$p < 0.001$
Dopamine µg/g	3.0 ± 0.27 (7)	2.2 ± 0.14 (4)	$p > 0.05$

[1] H. A. KIRKMAN, Stanford med. Bull. *20*, 163 (1962).
[2] L. CEGRELL, B. FALCK and A. M. ROSENGREN, Acta physiol. scand., in press (1969).
[3] L. CEGRELL, Acta physiol. scand. suppl. *314* (1968).
[4] T. A. I. GRILLO, A. J. WHITTY, H. KIRKMAN, P. P. FOA and S. D. KOBERNICK, Diabetes *16*, 409 (1967).
[5] A. ANTON and D. SAYRE, J. Pharm. exp. Ther. *145*, 326 (1964).
[6] Å. BERTLER and E. ROSENGREN, Acta physiol. scand. *47*, 350 (1959).
[7] W. LOVENBERG, R. J. LEVINE and A. SJOERDSMA, J. Pharmac. exp. Ther. *135*, 7 (1962).

each tumour were processed for fluorescence microscopy according to the method of FALCK and HILLARP[8].

Histochemically, the tumour cells were found to emit a formaldehyde-induced green fluorescence which was confined to the cytoplasm. The intensity was moderate to strong. No other structures displayed formaldehyde-induced fluorescence. No difference between the tumour cells of NSD 1015 treated and non-treated animals could be seen in the fluorescence microscope. The observations completely agreed with those previously obtained[2]; thus, judged by the fluorescence microscopy, the continued transfers of the tumour had not brought about any changes in the cellular fluorigenic substances.

The recorded dopa-decarboxylase and MAO activities and the concentrations of dopa and dopamine in the tumours are summerized in the Table. The concentrations of dopa and dopamine found in this study agree well with those earlier reported[2].

The concomitant occurrence of dopa and a relatively high dopa-decarboxylase activity strongly suggests that the dopamine stored in the tumour cells is formed within them. In fact, the rate of formation may well be high, since inhibition of the dopa-decarbocylase resulted in a dramatic increase of dopa in the tumour as was evident from both the fluorimetric and paper chromatographic analyses. The finding of high amounts of dopamine after dopa-decarboxylase inhibition need not be contradictory to this view. As can be seen from the Table a complete inhibition was not obtained with NSD 1015, and the remaining enzyme activity in combination with the highly increased amounts of the substrate may well account for a maintenance of the dopamine level. It should be noted that the inhibition of dopa-decarboxylase may have been more effective in vivo than the recorded figure denotes owing to sources of error in the determination procedure. Thus, e.g. the dilution of the homogenized tissue in the incubation mixture and the presence of excessive amounts of pyridoxal-5-phosphate may to a certain extent reverse the effect of NSD 1015.

As 5-hydroxytryptophan in other tissues is also a good substrate[9,10] for dopa-decarboxylase, the 5-hydroxytryptamine that has recently been demonstrated to occur in the tumour[2] may also be synthesized by the tumour cells. However, it remains to be evaluated whether the second pre-requisite for the monoamine synthesis, the hydroxylating enzyme, is also present in the tumour.

The estimated activity of the MAO seems to be consistent with the idea that the tumour cells possess the ability for oxidative deamination of their monoamines in agreement with recent findings by GRILLO[11].

The presence of the 2 enzymes is a feature which the insulin-producing tumour cells and normal endocrine pancreatic cells share in common. Thus, evidence was produced that exogenous L-dopa is taken up into islet cells of the mouse and subsequently decarboxylated to dopamine, which in turn is exposed to MAO within the cells[3]. MAO in islet cells of many mammalian species has also been demonstrated by means of the tetrazolium method[12].

Zusammenfassung. In einem transplantierbaren Inselzelltumor des Goldhamsters, der neben 5-Hydroxytryptamin, Dopa und Dopamin eine bisher unidentifizierte, vermutlich monoaminähnliche Substanz enthält, wurde eine hohe Dopadecarboxylase- und Monoaminooxydase-aktivität gefunden. Nach Dopa-decarboxylasehemmung wurde eine starke Anhäufung von Dopa im Tumorgewebe beobachtet; dieser Befund weist auf eine schnelle Monoaminsynthese und einen schnellen Monoaminumsatz im Tumorgewebe hin.

L. CEGRELL, B. FALCK
and A. M. ROSENGREN[13]

Department of Pharmacology, Institute of Anatomy and Histology and Department of Biochemistry, University of Lund (Sweden), 21 May 1969.

[8] B. FALCK and CH. OWMAN, Acta univ. lund. II *7*, 1 (1965).
[9] E. ROSENGREN, Acta physiol. scand. *49*, 364 (1960).
[10] T. L. SOURKES, Pharm. Rev. *18*, 53 (1966).
[11] T. A. I. GRILLO, Reported at the Symposium on The Structure and Metabolism of the Pancreatic Islets (Umeå, Sweden 1969).
[12] P. PETKOV, Ann. Histochim. *10*, 17 (1965).
[13] This work was supported by grants from the Swedish Cancer Society (67–111) and was carried out within a research organization sponsored by the Swedish Medical Research Council (Projects No. B69-14X-56-05C and B69-14X-712-04C).

The Growth of Allogeneic Tumour Cells in Wasting, Cyclophosphamide-Treated Rats

We have previously found that rats, treated with a single dose of 110 mg cyclophosphamide/kg body weight, after the initial weight loss during the first 2–4 days and a subsequent, apparently normal weight gain, began to lose weight again and died in a wasting disease after one to several months[1,2]. In the terminal stage, the rats showed the impaired hair growth, hunched posture and high-stepping gait, described as characteristic of runting. It was considered to be of interest to study the cyclophosphamide-treated, wasting rats in greater detail. As a part of such an investigation, the present paper will give a brief report on the growth of allogeneic tumour cells in cyclophosphamide-treated rats.

Experiments. One hundred 3-week-old, male, white, non-inbred rats of the Sprague-Dawley strain (AB Anticimex, Stockholm, Sweden) were given a s.c. injection of either 110 mg cyclophosphamide ('Sendoxan', kindly supplied by AB Pharmacia, Sweden) per kilogram body weight, or the equivalent amount of distilled water. On the following day, or on the 55th day after these treatments, when the cyclophosphamide-treated rats were entering the wasting stage (see the Figure), the rats were injected s.c. into the back near the head with viable tumour cells in the numbers denoted in the Table. The tumour cells were from the in vitro tumour cell line, previously used and described[3], obtained from an SV 40 rat sarcoma[4]. The in vitro passages employed were 348 and 371

[1] U. STENRAM, R. BERG and E. NILSSON, Z. Krebsforsch. *72*, 119 (1969).
[2] U. STENRAM and H. NORDLINDER, Nature *219*, 1154 (1968).
[3] R. BERG and U. STENRAM, Acta path. microbiol. scand. *73*, 305 (1968).
[4] H. DIDERHOLM, R. BERG and T. WESSLÉN, Int. J. Cancer *1*, 139 (1966).