Morphological Characteristics of the Secretory Granules in Pancreatic β Cells from Species with Identical Primary Structures of Insulin

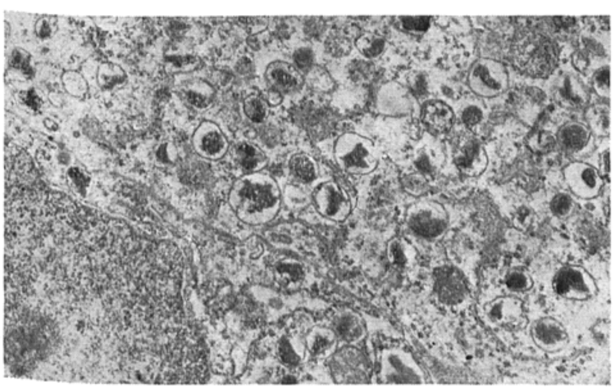
It is generally accepted that the secretory granules of the pancreatic β cells represent the storage form of insulin[1–3]. The shape of the β granules varies in different species, indicating a significant difference in their internal structure. While they are spherical in the mouse and the rat[4,5], they appear as multiple irregular elements in the dog[4,6], pig[6] and man[7,8]. The reason for the variations in the shape of the β granules is unknown. The possibility that they may simply reflect the primary structures of their insulins must be considered. This means, as has been recently emphasized by GRODSKY and FORSHAM[9], that it would be of interest to compare the morphology of the β granules in those species which have identical primary structures of insulin. The results of such analyses, using pieces of pancreas from dogs and pigs, are reported in the present communication.

Material and methods. Pancreatic material from 3 dogs and 3 pigs were studied. Small cubes of tissue were fixed in glutaraldehyde[10], followed by osmium tetroxide, and processed for electron microscopy. The histological treatment of the material was as uniform as possible. For the identification of the islets, large, 1-μ-thick sections were cut, mounted on slides and stained with buffered toluidine blue. Thin sections from selected parts of the blocks containing islets were then examined in an electron microscope (Siemens Elmiskop 1) at 60 kV. 6 islets from each species were systematically analysed by measurements on electron micrographs prepared at a final magnification of × 20,000. The greatest diameter and the diameter at right angles to it were measured in the first 50 clearly identifiable granules and surrounding sacs found in each islet. In addition, the number of granular elements in each sac was noted.

Results. In both the pig and the dog the secretory granules of the β cells appeared as multiple irregular elements (Figure). The results of the quantitative analyses can be seen in the Table. While no significant differences were recorded for the sizes of the secretory granules, they were more elongated in the dog ($t = 2.72$; $P \sim 0.02$). Furthermore, the sacs tended to be larger in the dogs; the percentage of them being occupied by granules was 28% in this animal as compared with 36% in the pig. Similar values were noted in the 2 types of animals for the number of granular elements in each sac.

Discussion. The insulin extracted from the pancreatic glands of the dog and the pig has an identical primary

1 A. W. LINDALL, G. E. BAUER, P. K. DIXIT and A. LAZAROW, J. Cell Biol. *19*, 317 (1963).
2 S. HJERTEN, S. HÖGLUND, and B. HELLMAN, in *The Structure and Metabolism of the Pancreatic Islets* (Eds. S. BROLIN, B. HELLMAN and H. KNUTSON; Pergamon Press, Oxford 1964), p. 223.
3 D. KVISTBERG, G. LESTER and A. LAZAROW, J. Histochem. Cytochem. *14*, 609 (1966).
4 P. E. LACY, Diabetes *6*, 498 (1957).
5 N. BJÖRKMAN, C. HELLERSTRÖM and B. HELLMAN, Z. Zellforsch. mikrosk. Anat. *58*, 803 (1963).
6 T. SATO, L. HERMAN and P. J. FITZGERALD, Gen. and Comp. Endocr. *7*, 132 (1966).
7 P. E. LACY, Coll. Endocr. *15*, 75 (1964).
8 N. BJÖRKMAN, C. HELLERSTRÖM, B. HELLMAN and B. PETERSSON, Z. Zellforsch. mikrosk. Anat. *72*, 425 (1966).
9 G. M. GRODSKY and P. H. FORSHAM, A. Rev. Physiol. *28*, 347 (1966).
10 D. D. SABATINI, K. BENSCH and R. J. BARRNETT, J. Cell Biol. *17*, 19 (1963).



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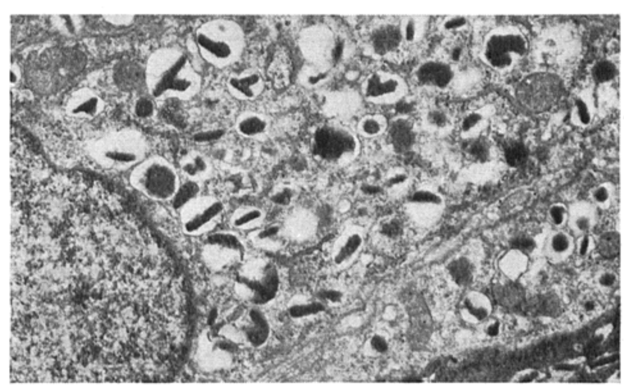

b

Electron micrograph showing part of a β cell in the pig (a) and the dog (b). In both species a similar pattern of secretory granules with irregular, cuboidal or polyhedral profiles can be seen. × 20,000.

The results of the quantitative analyses of the β granules and their surrounding sacs in electron micrographs from the pancreatic islets of pigs and dogs. The size of the granules has been expressed as the value obtained by multiplying the greatest diameter (in mm) with that at right angles to it. The eccentricity denotes the percentage by which the former diameter exceeds the latter. The figures represent the mean values ± S.E.M. for the islets studied

Animals	Granules			Sacs		
	Size	Eccentricity	No. of elements	Size	Eccentricity	Part occupied by granules (%)
Pig	27.3 ± 3.1	85 ± 14	1.23 ± 0.06	78.7 ± 9.2	26 ± 4	36 ± 2
Dog	30.6 ± 1.6	140 ± 14	1.16 ± 0.02	113.5 ± 9.9	36 ± 4	28 ± 2

structure[11,12]. It emerged from the present study that the β granules in the pig displayed a similar picture of multiple irregular granules to that previously described in the dog[4,6]. More careful quantitative analyses revealed, however, that the granules in the dog were more elongated than those in the pig. This indicates that the morphological appearance of the β granules is influenced also by other factors than the primary structure of the insulin. Whether an identical composition and order of the amino acids in the insulin molecule may also be compatible with the more striking species differences which have been encountered in the β granules remains to be settled. In the further exploration of this matter it would, for example, be of interest to extend the quantitative electron-microscopic analyses also to other species (sperm and fin whale) with identical primary structures of insulin[13].

Zusammenfassung. Die Primärstruktur des Insulins ist bei Hund und Schwein identisch. Elektronenmikroskopisch wurde festgestellt, dass die sekretorischen Granulae der pankreatischen β-Zellen bei beiden Arten eine unregelmässige Form haben. Eine quantitative Analyse ergab jedoch, dass die β-Granulae des Hundes länger sind als die des Schweines.

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[11] F. G. YOUNG, Br. Med. J. *2*, 1449 (1961).
[12] L. F. SMITH, Am. J. Med. *40*, 662, (1966).
[13] Supported by the United States Public Health Service (Grant No. 5 R05 TW00083-03) and the Swedish Medical Research Council (Grant No. K67-12X-562-03).

Riboflavin in the Blood Serum, the Skin and the Venom of some Snakes of Burma

Some Brazilian snakes (*Bothrops jararaca, Eudryas bifossatus*) have been reported to contain significant amounts of riboflavin in the blood plasma[1,2]. However, other species like the rattlesnake (*Crotalus terrificus terrificus*) and the non-venomous *Xenodon merrimii*, lack flavin in their blood and possess only a blue fluorescent pigment related to pteridines[3]. It is interesting to note that the venom of the *Bothrops* is also rich in riboflavin whereas that of the *Crotalus* is devoid of it[4]. The flavins detected in the plasma are free or combined with proteins and microbiologically active[3].

It seemed therefore worthwhile to study whether some of the common snakes of Burma, India and South-East Asia, of different species not found in South America, contain flavin in their blood serum. The venomous species which we worked with were the cobra, the viper and the krait; the non-venomous were the python and two other smaller species (*Oligodon cyclurus* and *Cercerus rynchops*). Riboflavin was found in measurable amount in only two venomous snakes (krait and viper) in the blood serum as well as in the skin; the python showed the largest riboflavin content. This flavin was not found in the venom of the cobra or the viper and only in low concentration (38 μg/g dry weight) in the venom of the krait (*Bungarus fasciatus*). The skin is generally richer in all the species studied, except the cobra and *Oligodon cyclurus* which do not contain a measurable amount. It is interesting to note that the skin of the krait contains flavin only in the yellow band. It has been reported by BLAIR and GRAHAM that the skin of some African green snakes (*Philothammus semi-variegatus* and *Disphòlidus typus*) also contain riboflavin[5]. The Table summarizes our results.

Riboflavin in the blood serum and skin of some Burmese snakes

Snakes	Flavin as riboflavin in μg/100 ml serum[a]	Flavin in μg/g dry weight skin
Naja naja (cobra)	–	–
Bungarus coeruleus (viper)	18 (12, 15, 17, 22, 27)	17
Bungarus fasciatus (banded krait)	50 (22, 37, 82, 86, 120)	210
Python molurus	257 (250, 285)	315
Oligon cyclurus	–	–
Cerberus rynchops	25.5 (21, 24, 27, 28, 28)	42

[a] Average of pooling sera and of some individual determinations.

The blood was collected in large test tubes immediately after decapitation of the animal and the serum separated by centrifugation. In a few cases (python) the determinations were performed with cold blood plasma 1 h after the decapitation of the snake. The technique of VILLELA and PRADO was followed for the extraction and the flavin was estimated visually by comparing the intensity of the green fluorescence of the clear extracts with riboflavin standard solutions[1]. An UV-lamp, adapted to a special device in which the light path hits the solution through a filter at an angle of 45°, was used as comparator[6]. The hydrosulphite test was performed according to NAJJAR[6].

Zusammenfassung. Es wurden sowohl giftige als auch ungiftige Schlangen aus Burma auf den Gehalt an Flavinen in Blut und Haut untersucht.

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Department of Physiology, Institute of Medicine I, Rangoon (Burma), 14th March 1967.

[1] G. G. VILLELA and J. L. PRADO, J. biol. Chem. *157*, 693 (1945).
[2] G. G. VILLELA and J. L. PRADO, Revta bras. Biol. *4*, 469 (1944).
[3] G. G. VILLELA, Revta bras. Biol. *5*, 113 (1945).
[4] A. TABORDA and L. C. TABORDA, Mem. Inst. Butantan *15*, 47 (1941).
[5] J. A. BLAIR and J. GRAHAM, Biochem. J. *56*, 286 (1954).
[6] S. UDENFRIEND, *Fluorescence Assay* (Academic Press, New York 1962), p. 239.
[7] Present address: Biochemical Laboratory, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.