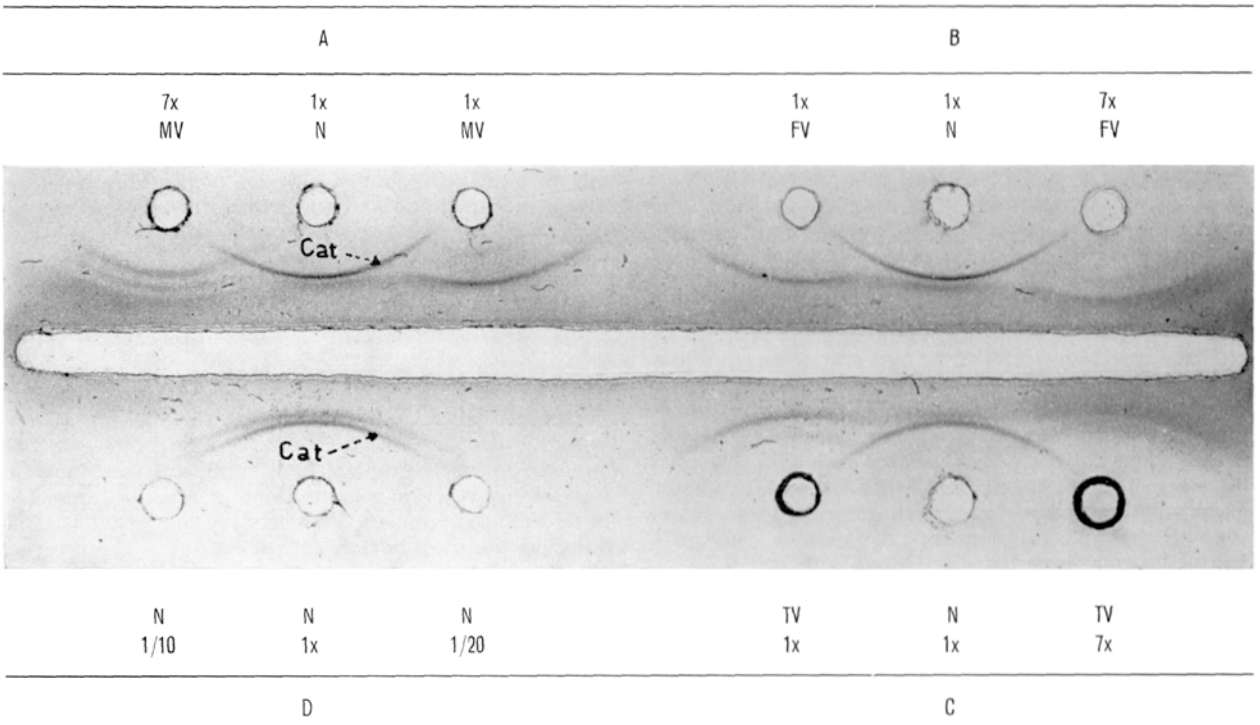

Fig. 2. Diffusion double en gélose. Gouttière centrale: *Immunsérum* de lapin No. VI anti hémolysat humain normal. *Antigènes: A* Hémolysat du cas M.V. chargé 1 fois et 7 fois, comparé à un hémolysat normal N. *B* Hémolysat du cas F.V. comparé dans les mêmes conditions à l'hémolysat normal. *C* Hémolysat du cas T.V. comparé dans les mêmes conditions à l'hémolysat normal. *D* Hémolysat normal non dilué (1 ×) et dilué $^1/_{10}$ et $^1/_{20}$. – L'immunprécipité de la catalase déterminé à partir de l'hémolysat normal (N 1 ×) est bien net dans chacun des segments A, B, C et D. Il est dévié par les faibles quantités de catalase contenues dans l'hémolysat dilué $^1/_{10}$ et $^1/_{80}$ en C. Il n'est dévié, par contre, par aucun des hémolysats d'acatalasémiques M.V., F.V. et T.V., même lorsque ceux-ci sont concentrés 7 fois.

ment et immunologiquement la présence de la «catalase protein», chez les individus atteints d'acatalasémie au Japon. TAKAHARA et al., pour expliquer la persistance, dans l'hémolysat des acatalasémiques, d'un «pic rapide» à l'électrophorèse simple, postulent trois alternatives: (1) ce pic pourrait être dû à une protéine non identifiée accompagnant normalement la catalase à l'électrophorèse; (2) il pourrait s'agir de catalase altérée et inactive; (3) enfin ces deux premières conditions pourraient être réunies en une seule. Or nous voyons à l'analyse immuno-électrophorétique de l'hémolysat (Figure 1) que deux lignes au moins se forment au voisinage immédiat de celle de la catalase, même dans les cas d'acatalasémie. Le «pic rapide» est donc formé de «protéines non identifiées» lorsque manque la catalase.

En revanche, l'absence de ligne de précipitation de catalase dans ces cas pathologiques nous permet tout au plus de dire que la molécule de catalase, si elle est présente, est assez modifiée pour ne plus précipiter avec l'anticorps correspondant.

Summary. The hemolysates of the red blood cells from 3 acatalasemic and 2 hypocatalasemic subjects from the same family were investigated by double diffusion in agar and immunoelectrophoretic analysis. It was shown that the loss of catalase activity is accompanied by a loss of specific immunoprecipitation.

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Evidence for the Presence of Biogenic Amines in Pancreatic Islets

The introduction of a very specific and sensitive method for the demonstration of certain catecholamines and tryptamines at the cellular level[1] has prompted a survey of the occurrence of these compounds in various tissues. The present report deals with observations made in the pancreas by means of this procedure.

Methods. Adult pancreatic tissue from rat, mouse, guinea-pig, cat, dog, horse, and duck was used. Ducks were included on account of the characteristically localized distribution of the three types of islet cells in this species. The tissue pieces were freeze-dried and then treated in dry formaldehyde gas at 80°C for 1 h. The catecholamines and 5-hydroxytryptamine then condense with the formaldehyde to form intensely fluorescent products[1]. Serial sections (5–10 μ) were examined in the fluorescence microscope and then stained with haematoxylin-eosin or in some cases impregnated with silver[2].

Results. A specific fluorescence developed in the enterochromaffin cells, in the adrenergic nerves and in some of

[1] B. FALCK, Acta physiol. scand. *56*, Suppl. 197 (1962).
[2] B. HELLMAN and C. HELLERSTRÖM, Z. Zellforsch. *52*, 278 (1960).

the islet cells. Since the sympathetic nervous system may directly influence the functional activity of the islet cells, the distribution of adrenergic nerves in the pancreas will be briefly summarized.

In the species investigated the terminal adrenergic fibres were sparsely distributed in the exocrine parenchyma, being most often found in connection with the vessels. Only occasionally were singular varicose fibres seen within the islets of the rat, mouse, guinea-pig and horse. The α-cell islets in the duck contained a comparatively large number of nerves, probably in association with the prominent vascular system. In the islets of the cat and dog, however, the adrenergic nerve pattern was different and consisted of a network of fine varicose fibres, which surrounded small lobules of islet cells. It has as yet not been possible to determine whether these axons represent vascular or true parenchymatous innervation. Scattered in the cat pancreas there were groups of non-fluorescent ganglion cells, on which varicose adrenergic fibres were superimposed, an arrangement suggestive of a synaptic system[3]. No adrenergic nerves were found passing to the pancreatic lamellar bodies in the cat.

A specific fluorescence also developed in some of the islet cells in the duck, guinea-pig, cat, dog and horse (Figure 1). The identity of these fluorescent cells was assessed in the duck, where there are two types of islets, the 'light' or α-cell islets and the 'dark' or β-cell islets. The α-cell islets are easily differentiated from the β-cell islets; they are very large and mainly concentrated to the splenic lobe in contrast to the small β-cell islets, which have a more even distribution throughout the pancreas[2]. Whereas no fluorescence was noted in the β-cells, the α-cells exhibited a fluorescence of moderate intensity, which sharply outlined the islets against the dark background of surrounding exocrine parenchyma. Both types of α-cells participated in the reaction, which was evident from the fact that no non-fluorescent cells could be seen in the cell strands known to be composed of α_1- and α_2-cells[2]. Some of the α-cells showed a slightly more pronounced fluorescence and were often seen to have a short process. However, the fluorescent intensity could not be correlated with a particular type of α-cell, as confirmed by a subsequent silver impregnation, which method selectively demonstrates the α_1-cells[2]. In ducks which were injected with reserpine (5 mg/kg) subcutaneously and killed 24 h after the injection, no fluorescence developed in nerves and islets.

No specific fluorescence was observed in the islets of rat and mouse. In the guinea-pig, cat, dog, and horse, however, a moderate and sometimes rather strong fluorescence developed in some of the islet cells. Guinea-pigs, which were given two subcutaneous injections of 2 mg/kg reserpine at an interval of 24 h and killed 7 h after the second injection, showed only a weak fluorescence in the islets. The number of fluorescent cells was also reduced.

Discussion. The results obtained in extensive studies of model systems and tissues from a large number of vertebrates and invertebrates show that the present fluorescence method has a high specificity for certain catecholamines and tryptamines[1]. This, and the fact that the fluorescence reaction was inhibited or greatly diminished in reserpinized animals, supports the view that the fluorescence observed in the islet cells is due to the presence of such amines.

The identity of the fluorescent cells has not yet been established in the mammalian species used in the present investigation. The fluorescent material was, however, definitely localized to both the α_1- and α_2-cells in the duck. Some adrenergic and/or tryptaminergic mechanism may thus be involved in the functional activity of these cells. This is rather puzzling as regards the α_2-cells, which are very probably the site of glucagon production[4–6]. At present it is merely a matter for speculation as to whether the amine in question is secreted by the cells or involved in the production of glucagon or some other intracellular metabolic process. The findings may also throw some light on the hitherto unknown function of the α_1-cells. It has recently been suggested that in addition to glucagon other hyperglycemic substances such as serotonin or a catecholamine are present in the pancreato-duodenal blood of dogs[7,8].

Zusammenfassung. Mit neuer empfindlicher Fluoreszenzmethode wird das Vorhandensein biogener Amine in Langerhansschen Inselzellen von Ente, Meerschweinchen, Katze, Hund und Pferd sehr wahrscheinlich gemacht. Bei der Ente konnten die fluoreszierenden Inselzellen als α_1- und α_2-Zellen identifiziert werden.

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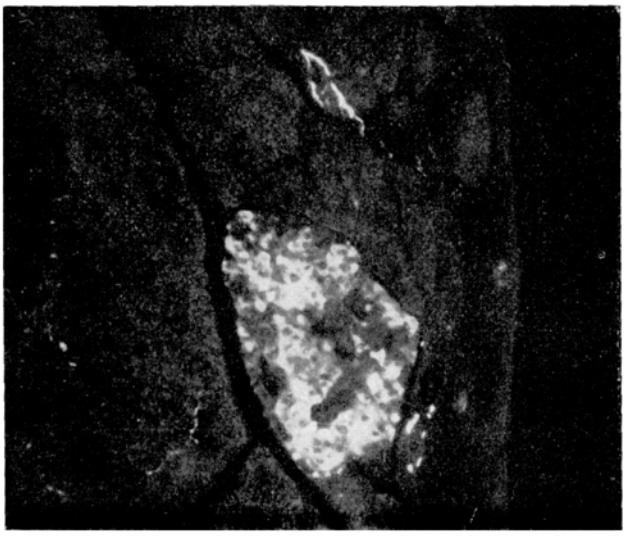

Islet of Langerhans from a guinea-pig showing fluorescence in the majority of the cells. The fluorescent structures outside the islets are adrenergic nerve fibres. ×140.

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[8] Supported by research grants A–5759 and B–2854 from United States Public Health Service and from the Swedish Medical Research Council.