activité puisse être augmentée. Puisque l'I.M. de nos cellules est nul, l'important pourcentage de cellules tétraploïdes que nous trouvons dans nos histogrammes (cellules mésothéliales moyennes) ne peut pas exprimer une intense prolifération cellulaire par mitose. Nous pensons donc qu'il s'agit là d'un blocage des cellules en post-synthèse ou G_2.

Au point de vue de l'ADN, nos résultats nous permettent de penser qu'au moment du prélèvement les petites cellules mésothéliales se trouvent en période de «repos» (R_1, diploïdes en ADN). Les cellules mésothéliales moyennes doublent leur ADN (synthèse d'ADN) mais ne peuvent pas entrer en mitose. Certaines d'entre elles stationnent en période de «repos» (R_2, tétraploïdes en ADN) pendant un laps de temps plus ou moins long; d'autres peuvent de nouveau entrer en synthèse pour former des cellules hypertétraploïdes (voir histogrammes, figure 1). Pour les grandes cellules mésothéliales, un pourcentage assez important subit des processus dégénératifs (picnose nucléaire, vacuolisation du cytoplasme) et se situe dans l'histogramme à gauche de la valeur diploïde comme il fallait s'y attendre. Il existe, également pour ces cellules, un pourcentage qui peut doubler son ADN (incorporation de la Th-^{3}H) et se trouver en post-synthèse ou G_2. Comme les moyennes, ces cellules ne peuvent pas entrer en mitose et stationnent, peut-être pendant une période moins longue, en période de «repos» (R_2, tétraploïdes en ADN). Les histogrammes du contenu en protéines (figure 2) des cellules moyennes et grandes ont la même allure: ils présentent en effet 2 pics distincts correspondant à des valeurs simples (2p) et doubles (4p). Les petites cellules mésothéliales sont toutes groupées autour d'un pic. Ces résultats s'accordent avec ceux obtenus pour le contenu en ADN des mêmes cellules, puisque là aussi nous avons pour les cellules moyennes et grandes des valeurs diploïdes et tétraploïdes et pour les petites uniquement des valeurs diploïdes.

Si l'on juge l'histogramme (figure 3) d'après la répartition du contenu en histones des cellules mésothéliales moyennes; on s'aperçoit que (comme il a été démontré pour d'autres cellules[10]) ce contenu est en rapport avec les teneurs en ADN et en protéines totales des mêmes cellules. En effet il existe un pourcentage de cellules avec un contenu en histones double, et plus, de celui des cellules du premier pic du même histogramme. Par conséquent on peut admettre que les cellules tétraploïdes en ADN contiennent le double d'histones par rapport aux cellules diploïdes.

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Demonstration of a tryptaminergic mechanism in the rat β-cell[1]

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Summary. Yellow 5-HT fluorescence has been histochemically demonstrated in rat pancreatic islet cells in animals injected with L-5-HTP with or without pretreatment with the monoaminoxidase inhibitor nialamide. This fluorescence was not observed after inhibition of the aromatic amino acid decarboxylase. The results strongly suggest the presence of a tryptaminergic mechanism in the rat islet cells.

The presence of some of the biogenic amines within pancreatic islet cells in several species is now well established[2–4]. However, great species differences appear to exist regarding the nature, location and relative concentration of these amines as well as with regard to the methodology required for their demonstration[3,4]. Serotonin (5-HT) has been demonstrated in pancreatic islets of mice both by histochemistry and autoradiography after the administration of L-5-hydroxytryptophan (L-5-HTP)[2,5–7]. In the rat, however, the presence of tryptaminergic mechanism in the pancreatic islets is an unsettled question. Cegrell[3] has mentioned that after treatment with L-5-HTP, serotonin can be demonstrated histochemically in this species. In contrast, Gagliardino et al.[8] were unable to find any histochemically demonstrable 5-HT in pancreatic islets of rats after administration of tryptophan by gastric tube. From this study, the above authors concluded that rat islet cells do not contain tryptaminergic mechanism. In the present work, we have investigated the question of whether or not serotonin is present in rat pancreatic islet as revealed by fluorescence histochemistry.

Materials and methods. Sprague-Dawley male albino rats, weighing approximately 400 g were employed. The animals were divided into 5 experimental groups of 6 rats each; the different substances used were administered intraperitoneally. The time intervals between injections in all groups were the same as described below for the first group and the doses for each given substance were constant.

Group 1: Received nialamide (5 mg/kg BW) a monoaminooxidase inhibitor, followed at 60 min by L-5-HTP (100 mg/kg BW).
Group 2: Saline and L-5-HTP.
Group 3: Nialamide and saline.
Group 4: As group 1 but prior to nialamide, the animals received RO 4-4602 (100 mg/kg BW) an aromatic amino acid decarboxylase inhibitor.
Group 5: RO 4-4602 and L-5-HTP.

For each of the groups above 2 animals were injected with saline alone following the same schedules used for the experimental groups. 2 hours after the first injection all animals were anesthetized with Na amytal® (100 mg/kg BW) and pieces of pancreas were taken and frozen in isopentane/liquid nitrogen, freeze-dried, and treated according to the formaldehyde condensation technique for the demonstration of 5-HT[9]. Pancreas from treated and control animals were processed at the same time and studied using a fluorescence microscope equipped with an exciting filter BG3-S405 and barrier filter K460. This combination of filters gives a blue fluorescence on catecholamines and yellow fluorescence for 5-HT.

Chemicals. L-5-HTP and nialamide were obtained from Sigma Chemical Company, St. Louis, Mo. 63178, USA, and RO 4-4602 was a gift from Hoffmann-La Roche Limitee, Vaudrevil, Quebec, Canada.

Results. Pancreatic tissue from saline injected animals and from those which received nialamide alone failed to show any fluorescence within the islet cells or the acinar pancreas. Treatment with both L-5-HTP or nialamide and

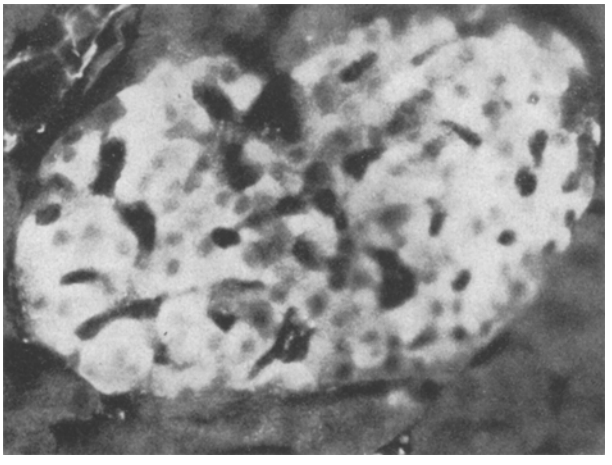

Fig. 1. Fluorescent pancreatic islet cells from a rat killed 1 h after L-5-HTP injection, without pretreatment with nialamide. × 410.

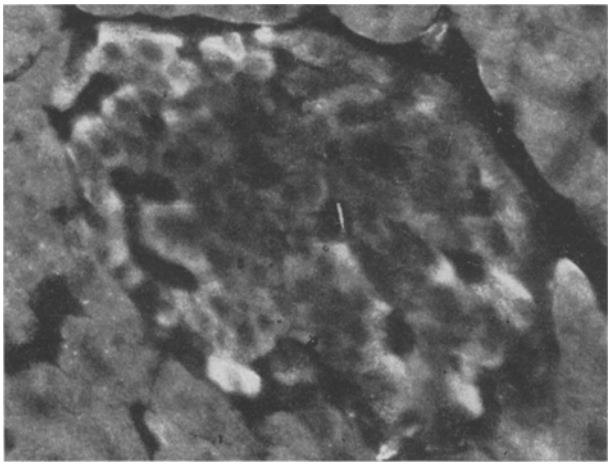

Fig. 2. Intense cytoplasmic fluorescence displayed by islet cells from a rat pretreated with nialamide and killed 1 h after L-5-HTP injection. × 410.

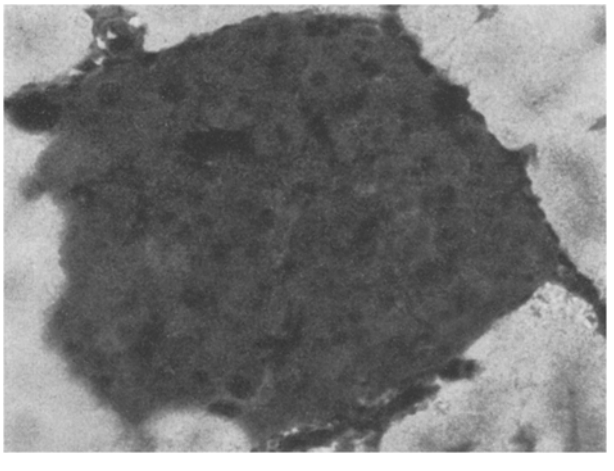

Fig. 3. Non-fluorescent rat pancreatic islet cells from an animal pretreated with RO 4-4602 and nialamide, killed 1 h after L-5-HTP injection. × 410.

L-5-HTP resulted in the appearance of a yellow fluorescence within the islet cells and a yellow-green fluorescence within the acinar pancreas (figures 1 and 2). However, islet cells from rats treated with nialamide before injection of L-5-HTP showed a much higher and more uniform distribution of the yellow fluorescence as compared to animals injected with L-5-HTP alone. This yellow fluorescence disappeared from all islets, without exception, when RO 4-4602, an aromatic amino acid decarboxylase inhibitor was injected before treatment with either L-5-HTP or nialamide and L-5-HTP (figure 3). Under these conditions, the acinar pancreas showed a stronger yellow-green fluorescence.

Discussion. The yellow fluorescence observed in the rat pancreatic islet cells from animals pretreated with L-5-HTP with or without pretreatment with nialamide appeared to be clearly due to an increased concentration of intracellular 5-HT, since this fluorescence within islet cells disappeared after inhibition of the aromatic amino acid decarboxylase. These results strongly suggest that the rat islet cells possess a tryptaminergic mechanism.

The significance of the increase in fluorescence observed in the acinar pancreas after administration of an inhibitor of the aromatic amino acid decarboxylase in animals treated with L-5-HTP is not clear at present. Further investigations will be necessary before advancing views on this finding. The results presented above do not support the view of Gagliardino et al.[8] regarding the absence of a tryptaminergic mechanism in rat pancreatic islet cells. This apparent discrepancy may be related to the fact that these authors used tryptophan in an attempt to increase the intracellular concentration of serotonin. Gylfe et al.[10] have demonstrated by incubation of microdissected islets with labelled tryptophan that islet cells lack the ability to transform tryptophan to serotonin.

The ability of rat pancreatic islet cells to take up 5-HTP to decarboxilate this amino acid to produce 5-HT and to store the latter amine, as demonstrated in the present studies, shows that these cells share properties in common with cells of the APUD (Amine content, Amine Precursor Uptake and Decarboxilation) series of cells producing polypeptide hormones[11,12].

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It has been generally accepted that most of APUD cells are derived from the neural crest. However, there exists evidence contrary to a neural crest origin for the pancreatic islet cells[13,14] although their possible neuroectodermal origin has not been precluded and data supporting the latter hypothesis has been reported[15].
Our findings, while not providing evidence to support either of the above views, indicate that the rat pancreatic islets do not differ from pancreatic islets in other species in terms of their ability to take up, decarboxylate and store amines.

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So-called annular gap junctions in bone cells of normal mice

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Summary. Spherical bodies consisting of a granular matrix and a pentalaminar limiting membrane were found in cells from the proximal tibial metaphysis of normal mice. The structures measured about 280–570 nm in diameter and were located mainly in the cytoplasm of osteoblasts and occasionally in preosteoblasts. The granules within the bodies resembled ribosomes. The multi-layered composition of the limiting body membrane was identical with that of intercellular gap junctions.

Peculiar spherical structures characterized by a pentalaminar limiting membrane have been observed in cells from a variety of tissues of different species[2–8]. The bodies have been termed 'sphaerae occlusae'[3], 'bulb gap junctions'[4], 'gap junction-bounded vesicles'[5,7], 'annular nexuses'[6], or 'annular gap junctions'[6]. Recently, such intracytoplasmic structures were found in cells from the periosteum of normal and lathyritic rats[8]. Electron microscopic study of cells from the metaphyseal trabecular bone tissue of the tibia of normal mice has revealed the presence of similar configurations as described below.
Materials and methods. Six normal female NMRI mice and 3 normal $(C3H \times 101)F_1$ hybrid mice, 2 males and 1 female, were used. The randomly outbred NMRI mice weighing 19–25 g were 3–4 weeks old. The inbred hybrid mice with a weight of 11 g were 3 weeks old. Immediately after sacrifice of the apparently healthy mice, the proximal part of the right tibia of each animal was rapidly removed and split longitudinally. Both halves of each tibial metaphysis were cut into cubes of about 1 mm^3. The specimens from 3 NMRI mice were fixed in phosphate-buffered 1% osmium tetroxide and those from the other 3 NMRI mice in phosphate-buffered 6.25% glutaraldehyde. The specimens from the 3 $(C3H \times 101)F_1$ mice were fixed in cacodylate-buffered 3% glutaraldehyde. The glutaraldehyde-fixed tissue cubes from the NMRI mice were postfixed in phosphate-buffered 1% osmium tetroxide and those from the hybrid mice in chrome-osmium[9]. Following dehydration through a graded series of ethanols to propylene oxide, the specimens were embedded in Epon 812. Thin sections were cut with a diamond knife on a Reichert Om U3 microtome and stained with uranyl acetate and lead citrate. They were examined in an AEI EM6B electron microscope.
Results. Many bone cells which were randomly examined in the undecalcified specimens from the proximal tibial metaphysis of the young normal mice contained peculiar membrane-bound structures (figures 1–4). These intracytoplasmic bodies which were more frequently seen in the NMRI than in the $(C3H \times 101)F_1$ mice were spherical and varied in size. The outer diameters of the inclusion bodies ranged from about 280 to 570 nm. The bodies consisted of a matrix being surrounded by a multi-layered limiting membrane. The matrix, similar in appearance to the cellular cytoplasm, contained diffusely scattered granules which resembled ribosomes (figures 1–4). Occasionally, an electron-lucent halo was interposed between the granular core of a body and the annular limiting membrane (figure 4).
The unusual membrane limiting the bodies had a pentalaminar appearance which was not modified in its preservation by the various fixatives used. The membrane consisted of an innermost and outermost electron-dense layer, both similar to the outer leaflets of a unit membrane. Between these 2 layers, an intermediate punctuated one was to be seen. This leaflet seemed to consist of periodically arranged subunit particles appearing in cross-section as a chain of electron-dense dots. It was separated from the innermost and outermost electron-dense leaflet by an electron-lucent layer on either side. The five-layered membrane was approximately 20 nm thick. Its multi-layered composition was identical with that of intercellular gap junctions[10] ('tight junctions'[11,12]) occasionally observed between cells from the trabecular bone tissue examined (figure 3).
The innermost leaflet of the limiting membrane of a few inclusion bodies was continuous with a saccular structure measuring up to about 150 nm in size (figures 2 and 3). Such a saccule was formed by a protrusion of the innermost layer of the pentalaminar membrane into the matrix of a body. In one body, an intrasaccular dense, knob-like

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