Gastroenterologia Japonica
Copyright © 1976 by The Japanese Society of Gastroenterology

Vol. 11, No. 3
Printed in Japan

—Original Article—

FLUORESCENCE HISTOCHEMICAL STUDY OF THE PANCREAS IN THE CAT

Masakuni ONDA, M.D.

The Third Department of Surgery, School of Medicine, Toho University
2-17-6, Ohashi, Meguro-ku, Tokyo, Japan
(Director: Prof. Kiyohiko Turumi)

Summary

The exocrine and endocrine pancreas was investigated according to the fluorescence histochemical method of Falck and Hillarp. 1) Green fluorescent adrenergic fibers were regularly seen associated with arteries and arterioles in the exocrine pancreas. 2) Cholinergic fibers as shown by cholinesterase activity, were also found in the parenchyma of pancreas. 3) Yellow fluorescent cells scattered in the exocrine parenchyma and localized to a population of pancreatic islet cells with a characteristic distribution at the islet periphery were found. 4) By the fluorescence microscopic observation, inter-or intralobular pancreatic ducts, involving the zymogen granules, can also be seen after treatment with HCL vapor. 5) Yellow fluorescent cells, β-cells containing insulin, remained at the Islet periphery. At present, the above mentioned yellow fluorescent cells are identified as containing HPP (Human pancreatic polypeptide) according to the immunofluorescence technique.

With the use of the Falck and Hillarp histochemical technique ethionine induced pancreatitis in cats has been investigated. 1) After seven days of ethionine (5 mg/kg BW oral ad.) treatment, pancreas showed histochemical changes such as hemorrhage, fat necrosis, destruction of acinar cells and degranulation of zymogen from the parenchyma of pancreas. 2) Oral administration of ethionine for ten days induced severe degranulation, rupture of vessels, especially of veins and venules and later influenced arteries or arterioles. 3) Necrosis and fibrosis began to appear in the spaces between the cellular debris and marked pancreatic atrophy could be found. 4) The destruction of Islets of Langerhans can be found in the ethionine induced pancreatic parenchyma. On the other hand, an increased number of Islets of Langerhans was also observed in the site of lobule. 5) The presented finding may also suggest that the duration of administration of ethionine is more important factor than graded doses of ethionine in the production of ethionine induced pancreatitis in cats.

Key Words: *Inter-or Intralobular pancreatic ducts, HPP. (Human pancreatic polypeptide), Ethionine induced pancreatitis, Zymogen granules in the ruptured blood vessels, Degranulation and necrosis of acinar cells*

Introduction

Pancreatic surgery today is a vast field for the treatment of pancreatic diseases such as acute hemorrhagic pancreatitis, chronic pancreatitis and cancer of pancreas. Although pancreatitis has been extensively studied, still many problems concerning this disease remain unsolved. This is mainly due to the fact that pancreatitis rather seldom exists as a distinct entity but is offen superimposed on and overshadowed by other significant disorders such as gallbladder disease, diabetes mellitus,

fibrocystic disease of pancreas and pancreatic tumor, benign and malignant. A profound knowledge of all pancreatic diseases is, however, necessary since almost all these above mentioned disorders are difficult to diagnose because of the hidden position and large reserve function of the pancreas and because they appear in a wide variety of clinical forms ranging from a catastrophic "acute abdomen" to a silent growth or advance of a malignancy. In addition, the pancreas has many functions and furthermore consists of two anatomically and functionally separate units, i.e. an exocrine portion consisting of the pancreatic acinar cells and ductal system, involving the major ducts of Wirsung and Santorini and also another unit, the endocrine portion consisting of the islets of Langerhans. The islets are built up by different types of cells which produce at least two hormones, insulin from the β-cells and glucagon from the α_2-cells.

The functional and physiological significance of these are discussed elsewhere. Of the above mentioned details, the purpose of the present study is to identify or to define more clearly the changes in the function of the islet cells, and the acinar cells respectively, and of the ductal system in the pathogenesis of the clinical manifestation of acute pancreatitis in human through experiences gained from the experimental pancreatitis induced by ethionine in animal. Moreover, it is also the purpose to assist the clinical diagnosis of the pancreatic disorders in human by a basic study of the pancreatic adrenergic and cholinergic innervation, the type of endocrine monoamines, and the exocrine and endocrine functions according to the fluorescence histochemical methods of Falck and Hillarp[1,2] (1961) (Flack and Torp[3] 1961; Falck 1962; Falck et al. 1962; Corrodi[4] and Hillarp 1963, 1964).

Part I

"Fluorescence Histochemical Study of the Pancreas, Especially, Innervation, the Exocrine and Endocrine in the Cat"

Basal Experiments:

Material and Methods.

Thirty healthy adult cats were used for the fluorescence histochemical experiments. Food and water was freely available up to the time of sacrifice. Each cat was anesthetized by intraperitoneal injection of sodium pentobarbital in a dose of 30 mg/kg BW. Specimens from various regions (head, body and tail) of the pancreas were dissected. These small pieces of the pancreas were immediately frozen to the temperature of liquid nitrogen in a mixture of propane and propylene or acetone-dryice solution.

After freeze drying, the specimens were treated with formaldehyde gas according to the procedure of Falck and Hillarp, embedded in paraffin wax and sectioned at 6–10 μ thickness. Also, tissue specimens were taken from non-treated cats (as control) in the way described above, and from L-DOPA and/or L-5HTP pre-treated cats who were sacrificed 30–60 min after intraperitoneal injection of the drug.

The specimens taken were then processed (see Falck and Owman[5] 1965) for fluorescence microscopic demonstration of certain aryethylamines according to the formaldehyde condensation technique of Falck and Hillarp, i.e., the sections were mounted in Entellan (Merck) or xylene and examined in a fluorescence microscope.

The sections or examined sections were then exposed for 1–15 min to HCL-vapour generated from 2 ml concentrate acid in a closed vessel (about 70–80 ml vol.) and re-examined for acinar cells and fluorescent cells. The borohydride reaction[6,7] was applied to formaldehyde-treated sections before and after

HCL exposure as a specificity test and then underwent histochemical staining for identification of some specific endocrine cells or monoamines in the cat pancreas.

Chemistry.

The formaldehyde condensation method is superior to all other histochemical techniques in this field because of its high specificity and extreme sensitivity.

The formaldehyde condensation method of Falck and Hillarp for the fluorescence histochemical demonstration of catecholamins and indolamines has found widespread use. The reaction sequence and the specificity of formaldehyde as a histochemical reagent has been analyzed in protein droplet models. (Corrodi and Jonsson, 1967)

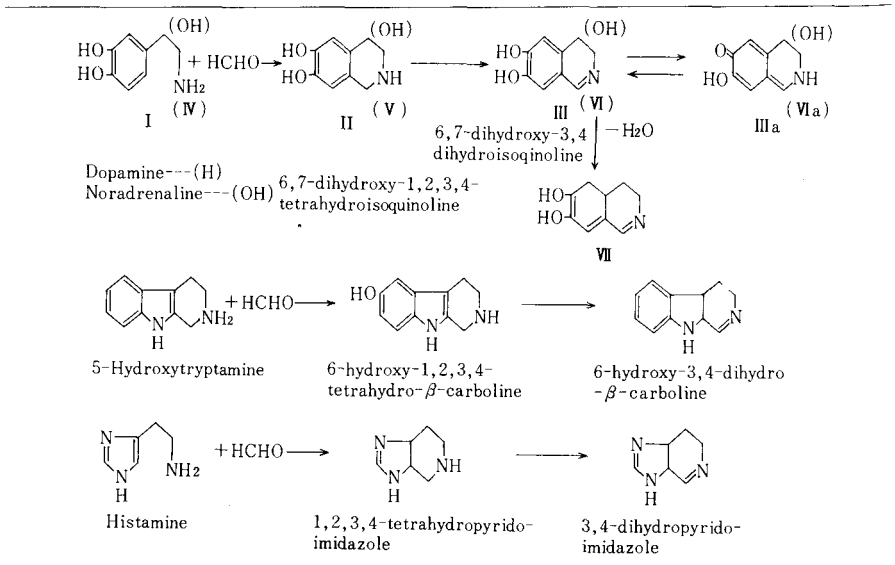
This method permits the histochemical demonstration at cellular level of the biogenic monoamines DA, NA, A, 5-HT (DA, dopamine; NA, noradrenaline; A, adrenaline; 5-HT, 5-hydroxytryptamine). Also the immediate amine precursors, e.g., DOPA, 5-HTP, highly fluorescent products with formaldehyde (DOPA, 3,4-dihydroxyphenylalanine; 5-HTP, 5-hydroxytryptophan).

The histochemical reaction are as follows (Table 1.). For example, the histochemical reaction between catechol amines, e.g., DA or NA and formaldehyde are first condensed with formaldehyde to form 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines and then dehydrogenated to form highly fluorescent 6,7-dihydroxy-3,4-dihydroisoquinolines.

Microspectrofluorometry.

For microspectrofluorometric analysis, the formaldehyde-induced fluorophore of the pancreatic endocrine cells was carried on quartz slides, mounted in xylene and covered by a conventional histological cover slip. The analysis for fluorescence was performed with a modified Farand microspectrograph. A xenon high pressure lamp (oslam BO 150) was used for recording the excitation spectra. The emission spectra were obtained with a high pressure mercury lamp as the light source.

Table 1. Proposed reaction scheme for the condensation of Dopamine, Noradrenaline, Serotonin and Histamine with formaldehyde and for the subsequent dehydrogenation of the condensation products.



The curves were registered with an X-Y recorder and corrected in the way described by Björklund et al.[8,9] (1968) the values expressed as relative quanta versus wavelength (**Table 2, 3**).

Histological and Histochemical Identification.

For histological and histochemical identification of endocrine monoamines, α-cells and β-cells in the Islets of Langerhans of the pancreas, the section of the pancreas was first identified and photographed in the fluorescence microscope (Tiyoda Ind. Co., Tokyo, Model FM 200). The cover slip was then removed by xylene and the borohydride reaction was applied to the section. After treatment, the section was stained histochemically and re-photographed. Glucagon (α_2-cells and α_1-cells) was demonstrated by staining by the Grimellius[10] or Lead-Haematoxylin[11] method, and to include insulin (β-cells) the section was also staind by the aldehyde-fuchsin method[12,13] to demonstrate the β-cells in the pancreas.

The argentaffin reaction in enterochromaffin cells was demonstrated by staining with the Masson-Hamperl method[14] (Hamperl 1927).

The collagen fibers in the tissue was stained according to the Orcein Van Gieson method[15]. By the Karnovsky technique[16] cholinesterase as cholinergic fiber, was also demonstrated after the fresh specimens were either cut directly or in a cryostat (−30°C −25°C) in

Table 2. The spectral characteristics of the OPT-induced fluorophores reported are summarized in the Table

Source	Exc. Max. (mμ)	Em. Max. (mμ)
Gastric endocrine cell (RAT)	370	425
Pancreatic A-cell (RAT)	420	490
Glucagon (1 μg)**	415	490
Secretin, VIP (1 μg)**	410–430	460–480–490

**The emission spectra were recorded with those mercury lines of the Hg-lamp closest to the value of Max. excitation for each fluorophore.
By C.H. Owman et al.

Table 3. Fluorescence microscopic techniques for the cellular localization and characterization of biogenic amines in pancreas
(Falck and Hillarp Method)

Source	Exc. Max. (mμ)	Em. Max. (mμ)
Catecholamines Paraformaldehyde gas treatment	320, 410	470—480
Serotonin Paraformaldehyde gas treatment	385, 415	520—530
Tryptamine Paraformaldehyde-ozone treatment	360—370	490—500
Dopamine Paraformaldehyde-HCl Vapour treatment	320, 365	490—500
Histamine O-Phthaldialdehyde treatment	450	540

sections of 10–20 μ thickness. All above mentioned sections from freeze dried specimens and cryostat sections were identified by staining with the Haematoxylin-Eosin method. (Afterwards, the sections were exposed for HCL treatment.)

Drugs.

Drugs were used for the fluorescence microscopy investigation of the L-5HTP and L-DOPA turnover in the exocrine and endocrine pancreas of the cat.

L-5HTP and L-DOPA was used separately. Each drug was dissolved in 0.9% saline solution. L-5HTP in a dose of 25 mg/kg and L-DOPA in a dose of 40 mg/kg was injected intraperitoneally[17-19].

Results

Green fluorescent adrenergic nerve fibers were regularly seen associated with the large vessels in the exocrine pancreas of the control cats (**Fig. 1**). Fine varicose green fluorescent adrenergic terminals could be seen among the exocrine acinar glands but could not always be demonstrated because these terminals generally were scarce and appeared very thin. Adrenergic fibers were also distributed to the Islet of Langerhans (**Fig. 2**). It is noteworthy that the fine adrenergic terminals among the exocrine acini can be seen only under optimum technical condition. These findings are similar to those of Cegrell[20] (1968). Fine intrapancreatic ganglia were also found in the cat pancreas. These ganglia were usually located in the ductal area and between exocrine acini and ducts (**Fig. 3**).

After pre-treatment with L-DOPA these intrapancreatic ganglia had taken up the L-DOPA and the intensity of fluorescence increased (**Fig. 4**).

After the cats had been pre-treated with L-DOPA or L-5HTP, the fluorescence increased in all adrenergic nerve fibers among the acinar tissue but noradrenaline containing adrenergic nerves in the Islets of Langerhans did not exhibit a dense network of varicoes fibers with the exception of the golden hamster[21]. It would seem that only occasionally fibers displaying the intensity of adrenergic nerve fibers are to be found in the cat. After pre-treatment with L-5HTP, the pancreas revealed more intensely fluorescent adrenergic fibers than after L-DOPA pre-treatment. Adrenergic varicose fibers were actually associated with vessels forming a plexus among them. These plexuses distribute fibers to the ducts and to the intrapancreatic ganglia. Adrenergic fibers also surround the pancreatic ducts regularly.

On the other hand, cholinergic fibers (cholinesterase) innervate the parenchyma of the exocrine pancreas (**Fig. 5**). This cholinesterase activity is seen in the reticular fibers of the acinar glands. It may be thought that all exocrine parenchyma is surrounded by many capillary sized vessels.

The studies of monoamines in the pancreas were all made in the cat. The enterochromaffin cells are pointed out by the flask-like appearance with the yellow fluorescence characteristic for 5-HT. These endocrine cells were more clearly seen after treatment with L-5HTP. In some tissue, they can be seen in a large number in the lobe and lie scattered in the epithelium of the large or largest pancreatic ducts. Yellow fluorescent cells were seen in the Islets of Langerhans. They were usually scattered in the periphery of the Islets of Langerhans. They may contain insulin, i.e., they may be β-cells. In general, the "small intensely fluorescent cells" are located in the intrapancreatic ganglia (animals without L-DOPA pre-treatment), and they contain the dopamine of the sympathetic ganglia.

In physiological studies of the pancreatic

secretion, injection of dopamine will induce secretion of pancreatic juice more rapidly than will secretin. This may suggest that a dopamine receptor may exist in the ducts which increase the sensitivity of the central acinar cells of the ducts.

After the injection of L-DOPA and/or L-5HTP, the pancreatic acinar cells have shown both uptake of L-aromatic amino acids and decarboxylation of the zymogen of acinar cells, then stored in the zymogen granules. These fluorescent granules were identical with the zymogen granules. Highly fluorescent zymogen granules were more clearly seen after exposure to the HCL-vapour for about 5 min. L-5HTP was also taken up by the exocrine pancreas as well as was L-DOPA in the cat. After uptake both L-DOPA and L-5HTP was localized to the same portion of the zymogen granules of the apical part of the exocrine cells. After careful exposure to the HCL-vapour, green fluorescent zymogen granules were seen in the intralobular or interlobular ducts (**Fig. 6**).

In the Islets of Langerhans, most of the cells showed a specific fluorescence. Fluorescent cells in the bright to green to yellow range were also found in the islets of the cat (**Fig. 7**). After histochemical staining the green fluorescent cells were identified as glucagon containing cell (**Fig. 8, 9**). It is interesting to note that the green fluorescent cells are scattered among the exocrine acini. These cells display the same spectral characteristics as catecholamines (dopamine). According to the Grimelius staining these cells are endocrine cells. They contain glucagon but aldehyde-fuchsin staining for β-cells (insulin) was negative.

On the other hand, the specific yellow fluorescent cells in the Islets of Langerhans were shown to be β-cells containing insulin, according to the subsequent aldehyde-fuchin staining (**Fig. 10**). With L-5HTP pretreatment, a storage of monoamines has been shown in the Islets of Langerhans. Yellow fluorescent cells increased in number and showed the Intensity of yellow fluorophores. Yellow fluorescent cells were also found located in the lobe of the pancreas (**Fig. 11**). These cells were localized to a population of pancreatic islet cells with a characteristic distribution at the islet periphery (**Fig. 12**).

In the human pancreas, human pancreatic polypeptide (HPP) cells were mainly localized at the periphery of the islets. At present, HPP cells have been identified by a immunohistochemical procedure. Cat pancreatic polypeptide, however, has not been identified.

Part II

"Fluorescence Histochemical Study of Experimental Pancreatitis Induced by Ethionine in the Cat"

This report is concerned with the production of pancreatic damage (as experimental pancreatitis) in cat following oral administration of ethionine. Fluorescence histochemical investigation was then performed in various stages of the experimental pancreatitis.

Material and Methods.

Ethionine, (α-amino-ethylthiolbutyric acid), the ethly analogue of metionine and presumed antagonist of metionine, was used for the production of experimental pancreatitis. Prior to the main experiments, investigation of ethionine induced pancreatitis was made. Doses of Ethionine administered were as follows: 2 mg/kg BW, 5 mg/kg BW, 10 mg/kg BW, 20 mg/kg BW, 30 mg/kg BW respectivelly.

The drugs were administered orally to a total of 50 cats for 10 or 20 days. On basis of the data arrived at a dose of 5 mg/kg of ethionine was chosen for the main experiment.

Thirty cats were used and divided into 6

groups. The cats in each group, consisting of 5 cats, were each given 5 mg/kg BW of ethionine orally. The schedule was as follows.

	Dose	Duration
Control	—	—
Group I	5 mg/kg BW	3 days
Group II	5 mg/kg BW	5 days
Group III	5 mg/kg BW	7 days
Group IV	5 mg/kg BW	10 days
Group V	5 mg/kg BW	20 days

After the experiments, the cats were sacrified by the administration of sodium pentobarbital in a dose of 30 mg/kg BW i.p. The pancreas was rapidly dissected, frozen in liquid nitrogen cooled propane or dry ice-acetone solution, freeze-dried, treated with formaldehyde of standardized humidity, embedded in paraffin wax in vacuo, sectioned and investigated in a fluorescence microscope according to the method of Falck and Hillarp.

Histochemical and histological technique was also applied in this experimental section. The Lead-Haematoxylin method and Grimelius method for staining α-cells in the pancreas (the Islet of Langerhans) was used.

Cholinesterase activity was also studied using the method of Karnovsky.

Results

General Symptoms:

Administration of ethionine to the cat was usually followed by loss of apetite and of body weight. After 2 or 3 days the animals began to vomit and showed decreased activity, lethargy and weakness. Ethionine in high doses was followed by quiet disturbance and difficulty in keeping the body upright and the animals developed bloody diarrhea and hemorrhage from the gums. After the observation period the cats were sacrificed at various intervals for study of fluorescence histochemical changes induced by ethionine administration. The results obtained by fluorescence microscopy were as follows (**Table 4**). Histochemical findings in ethionine induced pancreatitis:

In group I (5 mg/kg BW of ethionine orally for 3 days), histochemical changes were not observed. After 7 days of ethionine treatment, however, the pancreas showed histological changes such as hemorrhage, fat necrosis and degranulation of zymogen from the acinar cells. Fluorescence histochemical findings:

These findings are accounted for in **Table 4.** Oral administration of 5 mg/kg BW of ethionine induced hemorrhagic and cytological changes in the pancreas (**Fig. 13**). The acinar cells showed loss of zymogen granules (degranulation) (**Fig. 14**).

The initial parenchymal changes were seen in acinar cells and central acini. The hemorrhage was localized to a small area where it was surrounding the pancreatic ducts. These degenerative changes were followed by pro-

Table 4. Fluorescence histopathological changes of cat pancreas following ethionine administration for varying periods

Finding / Duration	Hemorrhage	Degranulation	Fibrosis	Necrosis
Three days	+	+	+	+
Five days	++	++	++	++
Seven days	+++	++	+++	+++
Ten days	++++	+++	+++	+++
Twenty days	+	++	++++	++++

Note: + Slight ++ Moderate +++ Marked ++++ Severe.

gressive loss of the normal architecture of the pancreas (**Fig. 15**). Histological examination of the degenerative area showed it to be completely fibrotic with degranulation of acinar cells.

The Islets of Langerhans and ductal cells showed no characteristic changes. After extention of the duration of ethionine administration, however, pancreatic tissue necrosis with surrounding inflammatory reaction appeared and complete degranulation was seen in the exocrine pancreas.

The grade of hemorrhage was increased by increasing doses of ethionine and so was the destruction of vessels, especially, of venules among the acini. Loss of zymogen granules (degranulation) from the parenchyma of pancreas and degranulation of the Islets of Langerhans was found by the fluorescence microscopic technique. The necrotic acinar cells and specific cells of Islets of Langerhans disintegrated and fibrous tissue began to appear in the spaces between the cellular debris and the proliferation of connective tissue occurred throughout the pancreas. Marked pancreatic atrophy and fat necrosis was prominent and increased Group IV and Group V.

Degranulation of the exocrine pancreas is found more frequently in the tissue by the fluorescence microscopic technique. It is interesting to note that the zymogen granules from the acinar cells penetrate into the vessels (**Fig. 16**). In the pancreatic tissue, the number of Islets of Langerhans seemed to be increased and the Islets cells showed lytic phenomena in Groups IV and V (**Fig. 17**). The cholinergic fibers were not changed if compared to normal pancreatic tissue (**Fig. 18**).

Only mast cells increased among the acinar cells. However, vessel wall destruction was seen in veins and venules, developed into fibrosis. Replacement of acinar cells was not observed at this time.

Discussion

Many authors have investigated the experimentally induced disease of the pancreas[22-25]. Even if such studies cannot give complete and unequivocal answers to the problems of the spontaneously occurring pancreatitis in man, they will nevertheless give valuable information of the mechanisms behind the disease. Farber and Popper[26], Goldberg, Chaikoff and Dodge[27] introduced the ethyl analog of methionine as a means to produce experimental pancreatitis in the animal. De Almedia and Grossman[28] have also investigated the ethionine induced pancreatic damage in animals. Ethionine acts as a metabolic blocking agent to methionine. It is, however, still a question whether it is possible or not that nutritional deficiencies or the block of metabolism may play a role in the pathogenesis of pancreatitis in the human. Up to date there is no evidence to corroborate such a causation for pancreatic disorders such as, acute pancreatitis, chronic pancreatitis and cancer of pancreas. The pancreas is one of the most active organs in the protein biosynthesis. Nishizaki[29] has suggested that the effect of alternation in dietary levels of pancreatic enzymes in animals, that is, low protein—high fat diet can produce morphological and biochemical changes in the pancreas of experimental animals.

In addition, Goldberg et al. observed that feeding a high fat—low protein diet to rats resulted in zymogen degranulation of the pancreatic acinar tissue. They also found that pre-treatment of methionine prevented its occurrence. If, however, the ethyl analogue of methionine, ethionine, was given to the experimental animals, fatty infiltration of the liver and destruction of the acinar cells of the

pancreas could be produced.

For this reason, the purpose of the present study has been observed the fluorescence histochemical response of the cat to ethionine administered orally in doses generally lower than those used by previous authors. From the results, the author has observed many new phenomena in the present experiments. The study in Part I suggests that green fluorescent adrenergic nerve fibers among the pancreatic acinar glands can be seen but are very difficult to observe in most specimens. Most of the green fluorescent adrenergic nerve fibers throughout the exocrine pancreas were associated with arterioles and formed a dense interconnecting paravascular plexus[30-32]. The fibers from the plexus extended to the pancreatic ducts where they surrounded the arterioles. Several green adrenergic fibers are found closely connected to veins[33,34].

The exocrine pancreas has both an adrenergic and a cholinergic innervation, but few enterochromaffin cells that occur in the large and largest pancreatic ducts and small intensely fluorescent catecholamine containing cells, "so called SIF-CELLS". All pancreatic parenchyma cells can take up and decarboxylate L-DOPA and L-5HTP to their corresponding amines (i.e. dopamine and 5 HT).

In addition, a number of 5 HT containing cells, also scattered in the lobes of the pancreas, and dopamine cells can be seen in the duct epithelium but dopamine usually was histochemically localized in the SIF-cells. The acinar cells take up and decarboxylate L-Histidine to its corresponding histamine, but the localization of uptake of L-Histidine may exist in a different part as compared to L-DOPA and L-5HTP.

Pancreas has been shown to possess a high capacity for uptake of L-DOPA and L-5HTP. The high uptake is presumed from the rapid turnover of the precursors of these amines and it also suggests the existence of decarboxylase in these cells. The author observed that after the administration of L-DOPA or L-5HTP, dilatation of intra or interlobular ducts can be seen in the main part of the pancreas. These substances can be found by way of zymogen granules in these ducts. The substances are in paticular accumulated in the parenchyma of pancreas after administration of L-5HTP.

Whether 5-HT is synthetized in the pancreas or taken up from the blood is not known. The pancreas has been regarded to have the greatest protein synthetizing capacity of all organs. Likewise, the rapid pancreatic uptake and turnover or metabolism of L-aromatic amino acids—L-DOPA and L-5HTP, is a specialized or specific function which is associated to the synthesis of pancreatic enzymes or serves to decompose L-DOPA and L-5HTP. Moreover, another function of pancreas may be take up amino acids and select the essential from the non-essential amino acids. On the other hand, endocrine pancreas, especially, the Islets of Langerhans has an adrenergic innervation rather than a cholinergic one. Staining for cholinestrase activity, however, did not reveal any cholinergic innervation of islet tissue. The green fluorescent adrenergic fibers innervating the Islets of Langerhans emanate from the plexuses around the arteries or the arterioles[35].

Many green fluorescent cells are found in the islets and scattered in the exocrine parenchyma showing emission curves varying between those of catecholamines and 5-HT. (when tissues were fixed at a temperature lower than the ordinary). There are two main possibilities to consider: either these cells contain 5-HT or dopamine in varying proportions, or which is a more interesting assumption, they contain an hitherto unknown substance (hormone) or polypeptide capable

of condensing after treatment with paraformaldehyde to a fluorescent derivative.

At present, 5 HT is store in specifically yellow fluorescent cells occurring in the Islets of Langerhans. Håkansson and et al.[36] suggested that the elevated level of pancreatic insulin-like activity and 5HT might be the result of a direct effect of a decrease in the proposed cytotropic action of glucagon on the β-cell or administered need of circulating insulin following the destruction of the glucagon cells (as green fluorescent cells in the islets).

On the other hand, the green fluorescent cells containing glucagon are found scattered among the exocrine parenchyma of pancreatic lobes. L-I, Larsson et al. in 1974 and 1975[37-39], using a specific immunofluorescence method, reported on the cells scattered in the exocrine parenchyma of all four pancreatic lobes and postulated the existence of a new hormone. Avian Pancreatic Polypeptide (APP) or Human Pancreatic Polypeptide (HPP).

In this experiment, the author has tried to identify the character of those yellow fluorescent cells which have been found in the pancreatic lobes by the ordinary use of the method of Falck and Hillarp, i.e. the scattered yellow fluorescent cells and extrainsular cells ultrastructurally similar to the APP or HPP cells that have previously been described by Braun-Blanquet[40].

Finally, the author wishes to discuss the experimentally induced pancreatitis in the cat. In general, histopathological observation in acute pancreatitis, both in the experimental animal and in man, will show edema and inflammatory cellular infiltration of the interstitial spaces, necrosis and disintergration of acinar cells, intrapancreatic and extrapancreatic fat necrosis and hemorrhage from necrotic vessels. All these pathologic features may be found in both the experimental and in the human disease, as pointed out by several authors. Fluorescence histochemical investigation of the experimental pancreatitis induced by ethionine has not yet been performed in the animal. In the Group IV and Group V animals who ungerwent ten days of ethionine administration, fluorescence microscopy revealed an increasing severity of the pancreatitic process.

Fat necrosis and a peculiar variety of vascular necrosis with hemorrhage are distinctive and unique features of animal pancreatitis. The fat necrosis on may be due to the action of pancreatic lipase on the triglyceride of fat storage cells. The vascular destruction or hemorrhagic necrosis is produced by the action of the proteolytic of the pancreas. The hemorrhagic necrosis may be revealed by fluorescence microscopy. Degranulation of acinar cells can be seen in the tissue of pancreas. The zymogen granules seen to enter or penetrate into the vessel walls as suggested by the authors observation of zymogen granules in side the vessel walls. These zymogen granules may cause rupture or destruction of pancreatic vessels or parenchyma.

Rich and Duff[41] demonstrated that subcutaneous injection of a solution of crystalline trypsin or chymotrypsin will locally reproduce the same vascular lesion, just it occurs in pancreatitis as well as author's results. Grossman[42] suggested that under normal conditions, trypsinogen and chymotrypsinogen would be expected to enter the blood in the same manner as amylase and lipase. The presence of these protease precursors in the blood has not, however, been demonstrated. In the present study this would have been shown by the fluorescence microscopic method. On the other hand, Koboth and De Almeida and Grossman, have also employed the physiological study of the effect of ethionine on the secretion of serum enzymes. That is, the subsequent decrease in plasma amylase

noted in their experiments could result from depletion of the supply of this enzyme in the damaged pancreas, as suggested by the loss of cytoplasmatic granules in the cells of the damaged acini. They may have found the correct answer to physiological sequence, because the proteolytic enzymes cannot pass through the ruptured or destructed pancreatic ducts normally. From the findings at fluorescence microscopy, it seems that a large amount of zymogen granules escaped from the lesion of acinar cells into the abdominal cavity and that they also cause damage to pancreatic tissue. Of particular interest in the present study is the completely different effects produced by graded doses of ethionine as compared to the findings of other investigators. Cats given ethionine orally in doses of only 5 mg/kg BW per day developed a severe damage of the pancreas.

It is assumed by the author that ethionine in graded doses not markedly influences the development of experimental pancreatitis. It would seem that duration of administration rather than dosage is the important factor in the development of ethionine induced pancreatitis. The hypothetical mechanism of ethionine induced pancreatitis, however, can at least partly be understood if ethionine is considered to be an anti-metabolite of its structural analogue, methionine. Dietary imbalance involving protein deficiency can lead to abnormality in pancreatic function and to histopathological changes in the pancreas both experimentally and clinically.

Ethionine consequently induces the pancreatic dysfunction which can lead to the production of pancreatic damage in animal. In addition, Kalser and Grossman[43] have reported that the ratio of trypsin inhibitor to total protease is lowered in both pancreatic tissue and pancreatic juice and the occurrence and concentration of active protease in the duct juice is increased in the presence of ethionine pancreatitis. Administration of ethionine for ten days, may increase the number of Islets of Langerhans in the pancreatic tissue.

Also many cells which stain by the Grimelius method increase in number and these usually show the yellow fluorescence. As those cells can also be seen in the Islets of Langerhans, L.-I. Larson et al. suggested that APP (Avian pancreatic polypeptide revealed by immunofluorescence) is present in the chicken pancreas as it is in the human pancreas. APP and HPP (Human Pancreatic Polypeptide) like-cells were found in the parenchyma and in the Islets of Langerhans by the method of Falck and Hillarp.

At present the author is trying to identify these cells immunohistochemically. Kimmel et al.[44] isolated a peptide contaminant of chicken insulin. The HPP cells were mainly localized at the periphery of the islets. The author has also found these cells in the periphery of the islets by way of fluorescence microscopy. HPP as well as APP is a powerful gastric secretagogue, stimulating HCL as well as pepsin secrection (Hazelwood, Turner, Kimmel and Pollock[45], 1973).

Also accellerates hepatic glycogenolysis without altering blood glucose levels. It has been suggested that APP and HPP, if in fact the same substance, represent a new pancreatic hormone, the author, attempts to identify the polypeptide hormone from the point of view of fluorescence microscopical analysis.

I have reported a part of the thesis in the 62nd General Meeting of the Japanese Society of Gastroenterology in 1976.

Acknowledgement

The author would like to express cordial appreciation to Prof. K. Turumi, and also author are indebted to Dr. H. Nishizaki for

his skiful technical assistance and his sincere guidance and encouragement through this study.

References

1) Falck, B. and Hillarp, N.: Fluorescence of catechol amines and related compounds condensed with formaldehyde. J. Histochem. Cytochem., 10: 348, 1962.
2) Falck, B.: Observation on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta. Physiol. Scand., 56. Suppl.: 1, 1962.
3) Falck, B. and Torp, A.: A fluorescence method for histochemical demonstration of noradrenaline in the adrenal medulla. Med. Exp., 5: 429, 1961.
4) Corrodi, H.: Fluoreszenz methoden zur histochemischen sicht barmachung von monoaminen. 1. identifizierung der fluoreszierenden produkte aus modellversuchen mit 6, 7-dimethoxyiso chinolinolinderivaten und formaldehyd. Helvetica. Chemica. Acta., 267: 2425, 1963.
5) Falck, B. and Owman, C.H.: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta. Univ. Lundensis, Sectio II., 7: 1, 1965.
6) Corrodi, H., Hillarp, N.-A. and Jonsson, G.: Fluorescence methods for the histochemical demonstration of monoamines. 3. Sodium borohydride reduction of the fluorescent compounds as a specificity test. J. Histochem. Cytochem., 12: 243, 1968.
7) Geyer, G.: Histochemische reduktion von aldehyden mit natrium borohydrid. Acta. Histochem., 15: 1, 1963.
8) Björklung, A., et al.: A method for differentiating dopamine from noradrenaline in tissue sections by microspectrofluorometry. J. Histochem. Cytochem., 16: 263, 1968.
9) Björklund, A., et al.: Histochemical demonstration of tryptamine properties of the formaledhyde-induced fluorophores of tryptamine and related indole compounds in models. Acta. Physiol. Scand., Suppl. 318: 1, 1968.
10) Grimelius, L.: A silver nitrat stain for α_2-cells in human pancreatic islets. Acta. Soc. Med., 73: 243, 1968.
11) Solcia, E., et al.: Lead-Haematoxylin as a stain for endocrine cells: Significance of staining and comparison with other selective methods. Histochemie., 20: 116, 1969.
12) Scott, H.D.: Rapid staining of beta cell granules. Stain Technol., 72: 267, 1952.
13) Jenning, B.M.: Aldehyde-fuchsin staining applied to frozen section for demonstrating pituitary and pancreatic beta cells. J. Histochem. cytochem., 13: 328, 1965.
14) Singh, I.: A modification of the Masson-Hamperl method for staining of argentaffin cells. Anat. Anz. Bd., Suppl.: 81, 1964.
15) Mcmanus, J.F.A.: Methods of general utility for routine study of tissues, Harper & Row, Staining methods, p. 62, John Weatherhill, New York, 1960.
16) Karnovsky, M.J., et al.: A direct-coloring thiocholine method for cholinesterase. J. Histochem. Cytochem., 12: 219, 1964.
17) Alm, P., et al.: L-dopa turnover in the mouse pancreas. Life. Sciences., 6: 913, 1967.
18) Alm, P.: Fluorescence microscopy of the 5-HTP turnover in the exocrine pancreas of mice and rats. Z. Zellforsch., 96: 212, 1969.
19) Alm, P., et al.: Effects of pilocarpine on L-dopa turnover in the exocrine rat pancreas. Acta. Physiol. Scand., 83: 269, 1971.
20) Cegrell, L.: The occurrence of biogenic monoamines in the mammalian endocrine pancreas. Acta. Physiol. Scand., Suppl. 314: 1, 1968.
21) Cegrell, L., et al.: Catechol and indol derivation in a transplantable islet cell tumour of the golden hamster. Acta. Physiol. Scand., 77: 23, 1969.
22) Rich, A.R., et al.: Experimental and pathological studies on the pathogenesis of acute hemorrhagic pancreatitis. Bull. Johns Hopkins Hosp., 58: 212, 1936.
23) Frank, J., et al.: Some effects of ethionine in the dog with particular reference to external pancreatic secretion. Gastroenterology, 27: 743, 1954.
24) Alvin, M., et al.: Experimental hemorrhagic pancreatitis produced by staphylococcal toxin. Surgery., 47: 587, 1960.
25) Lester, F., et al.: Natural variation in experimental hemorrhagic pancreatitis. Surg. Gynecol. Obstet., 124: 531, 1967.
26) Farber, E. and Popper, H.: Production of acute pancreatitis with ethionine and its prevention by methionine. Proc. Soc. Exp. Biol. Med., 74: 838, 1950.
27) Goldberg, R.C., Chaikoff, I.L. and Dodge, A.H.: Destruction of pancreatic acinar tissue by dl-ethionine. Proc. Soc. Exp. Biol. Med., 74: 869, 1950.
28) De Almeida, A.L. and Grossman, M.I.: Experimental production of pancreatitis with ethionine. Gastroenterology., 20: 554, 1952.
29) Nishizaki, H.: The effect of alternation in dietary levels on pancreatic enzymes in rats. Folia. Endocrinol. Jpn., 35: 929, 1959.

30) Hamberger, B., et al.: Evidence for adrenergic nerve terminals and synapses in sympathetic ganglia. Int. J. Neuropharmacol., 2: 279, 1964.
31) Alm, P., et al.: Remarkable adrenergic nerves in the exocrine pancreas. Z. Zellforsch., 83: 178, 1967.
32) Spriggs, T.L.B., et al.: Controlled formaldehyde catecholamine condensation in cryostat sections to show adrenergic nerves by fluorescence. Stain Technology., 41: 323, 1966.
33) Fuxe, K., et al.: The distribution of adrenergic nerve fibers to the blood vessels in skeletal muscle. Acta Physiol. Scand., 64: 75, 1965.
34) Lever, J.D., et al.: Paravascular nervous distribution in the pancreas. J. Anat., 101: 189, 1967.
35) Falck, B., et al.: Evidence for the presence of biogenic amines in pancreatic islets. Experientia., 19: 139, 1963.
36) Håkansson, R., et al.: Elevated levels of insulin-like activity and 5-hydroxytryptamine in guinea pig pancreas following $CoCl_2$ treatment. Endocrinology, 94: 324, 1974.
37) Larsson, L.-I., et al.: Localization of APP, a postulated new hormon, to a pancreatic endocrine cell type. Histochemistry., 42: 377, 1974.
38) Larsson, L.-I., et al.: Immunohistochemical localization of human pancreatic polypeptide (HPP) to a population of islet cells. Cell Tissue Res., 156: 167, 1975.
39) Langslow, D.R.: Studies of the distribution of a new avian pancreatic polypeptide and insulin among bird, reptiles, amphibians and mammals. Endocrinology., 93: 558, 1973.
40) Braun-Blanquet, M.: Examen du pancréas de canard normal au microscope électronique précédé de son observation macroscopique et microscopique. 1. glande exocrine. Acta. Anat. (Basel). 72: 161, 1969.
41) Rich, A.R. and Duff, G.L.: Experimental and pathological studies on the pathogensis of acute hemorrhagic pancreatitis. Bull. Johns. Hopkins., 58: 212, 1936.
42) Grossman, M.I.: Experimental pancreatitis. Arch. Int. Med., 96: 298, 1955.
43) Kalser, M.H. and Grossman, M.I.: Secretion of trypsin inhibitor in pancreatic juice. Gastroenteorlogy., 29: 35, 1955.
44) Kimmel, J.R., et al.: Isolation and characterization of chicken insulin. Endocrinology., 83: 1323, 1968.
45) Hazelwood, R.L., Turner, S.D., Kimmel, J.R. and Pollock, H.G.: Spectrum effects of a new polypeptide (third hormon?) isolated from the chicken pancreas. Gen. Comp. Endocrinol., 21: 485, 1973.

Received June 10, 1976
Accepted June 14, 1976
Address requests for reprints to: Dr. Masakuni Onda, M.D., The Third Department of Surgery, Toho University School of Medicine, 2-17-6, Ohashi, Meguro-ku, Tokyo, 153 Japan.

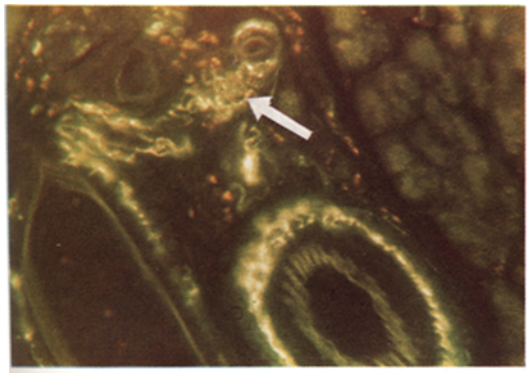


Fig. 1. Fluorescence photograph showing the dense plexus of adrenergic nerve fibers surrounding the artery and arteriole and also innervation of the pancreatic duct. ×200.

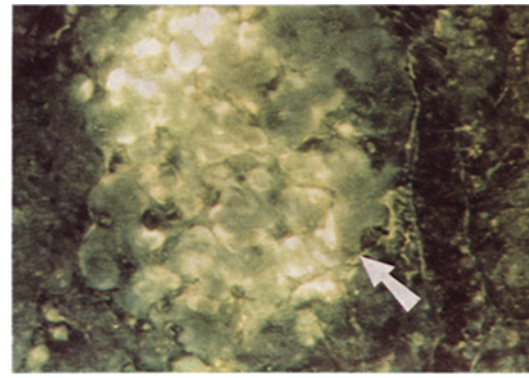


Fig. 2. Adrenergic fibers distributed to the Islets of Langerhans. ×400.

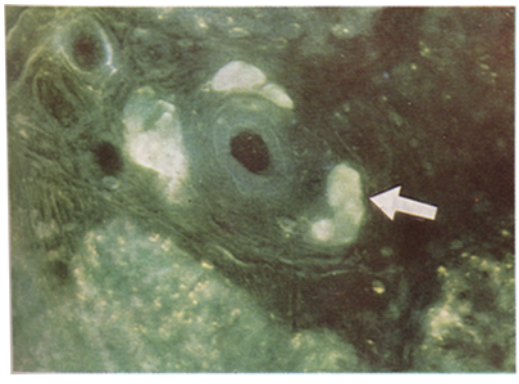


Fig. 3. The intrapancreatic ganglions which located at the pancreatic ducts. ×200.

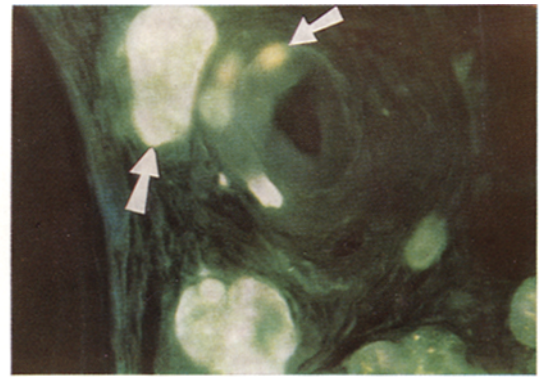


Fig. 4. The intrapancreatic ganglions. A network of fluorescent nerve fibers surrounds some of the nerve cell bodies and duct. Yellow fluorescent cell located in the epitherial region of pancreatic duct. ×200.

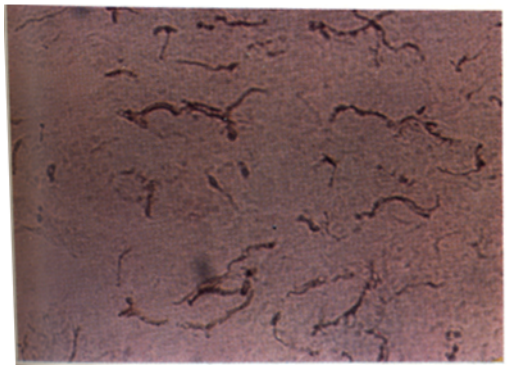


Fig. 5. Cholinergic fibers of the pancreas. (stained by Karnovsky). ×100.

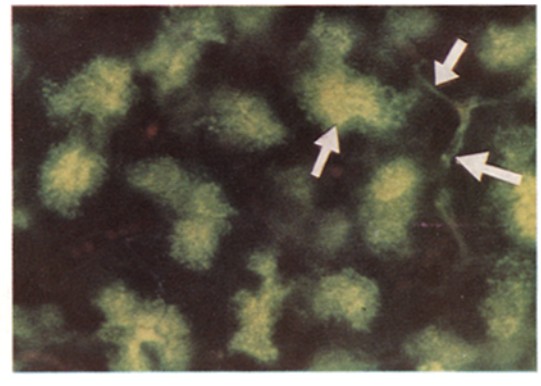


Fig. 6. The acinar cells. There is close correspond ence between a coarse fluorescent zymogen granulum and inter or intralobular ducts. ×400.

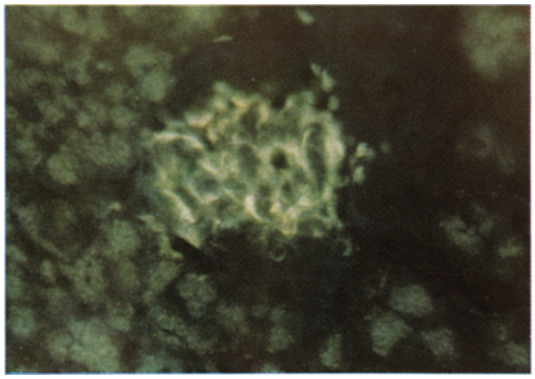


Fig. 7. Green fluorescent cells in the islets of Langerhans. (as glucagon). ×100.

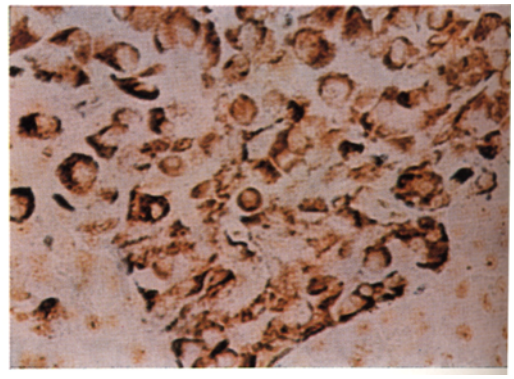


Fig. 8. Histochemical staining (Grimelius method). Green fluorescent cells were identified as glucagon followed by the Grimelius method. ×400.

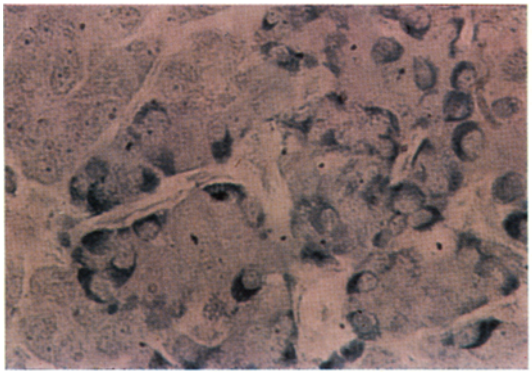


Fig. 9. Histochemical staining (Lead-Haematoxylin.) Green fluorescent cells were identified as glucagon followed by the Lead-Haematoxylin. ×400.

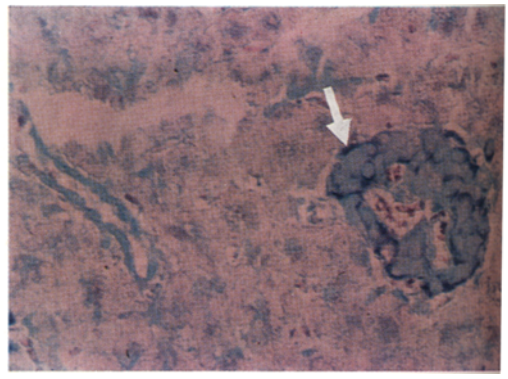


Fig. 10. B-cell in the islets of Langerhans were staind with Gomori's aldehyde-fuchin. ×100.

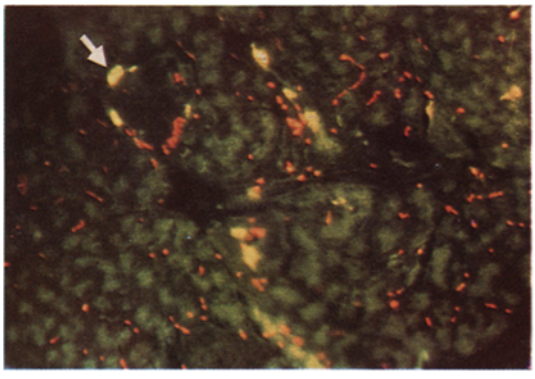


Fig. 11. Fluorescence photograph showing the yellow cells in the parenchyma of the pancreas. ×100.

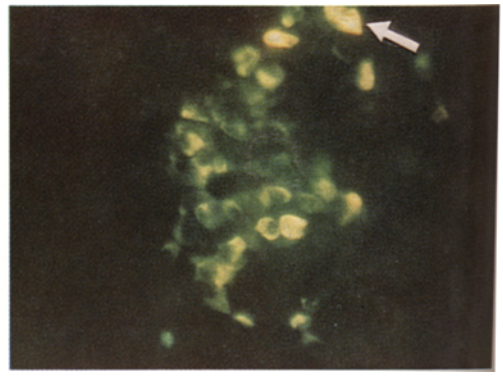


Fig. 12. Yellow fluorescent cells in the islets of Langerhans. It may be exhibited the HPP-like cells. ×200.

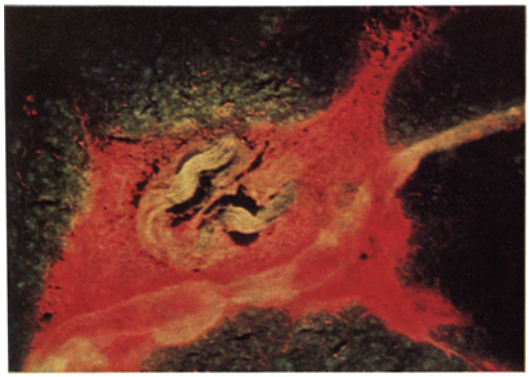


Fig. 13. Ethionine induced pancreatitis. Fluorescence photograph showing the severe hemorrhage in the parenchyma of pancreas. ×100.

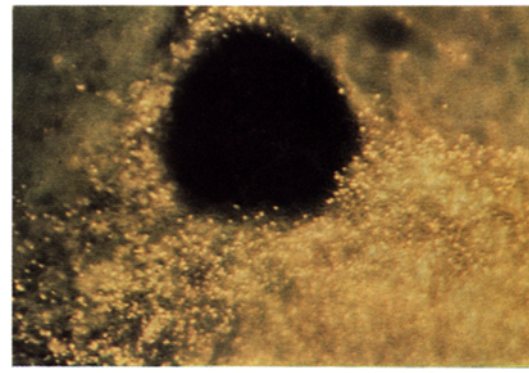


Fig. 14. Fluorescence photograph showing the degranulation from the acinar cells which was induced by ethionine. × 400.

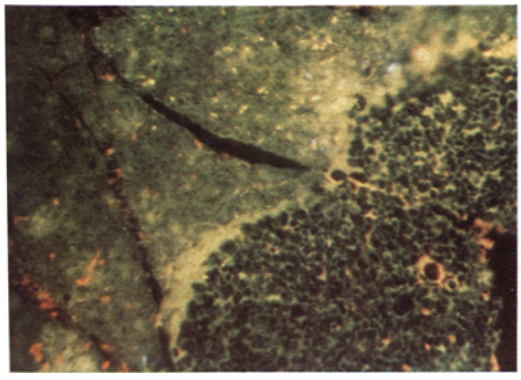


Fig. 15. Ethionine induced pancreatitis. This is a representative picture of diffuse and severe necrosis of acinar cells with complete loss of the normal architecture of the acini in an animal which had received ethionine 5 mg/kg for 10 days. ×100.

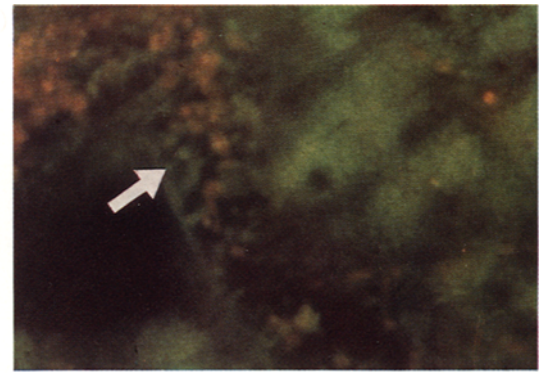


Fig. 16. Fluorescent zymogen granules can be seen in the ruptured blood vessels. ×400.

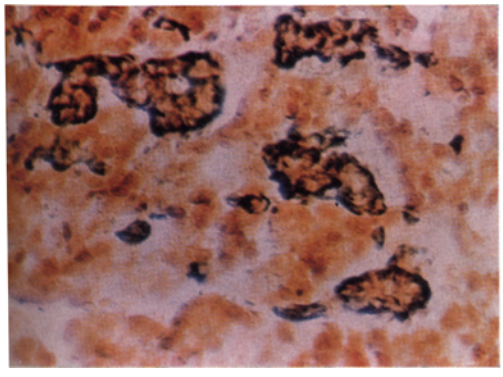


Fig. 17. In the pancreatic tissue, the number of islets of Langerhans seemed to be increased in Groups IV and V. (by the Grimelius method). ×50.

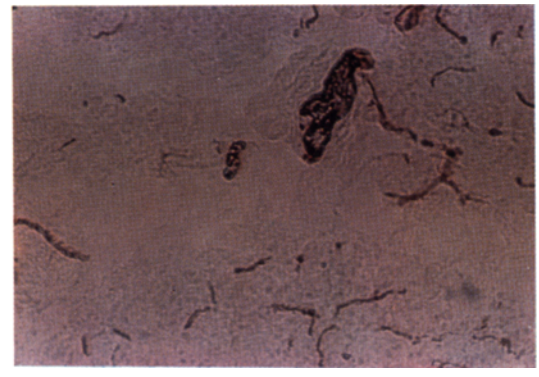


Fig. 18. Cholinergic fibers of the pancreatic tissue with ethionine induced pancreatitis. (stained by Karnovsky) The cholinergic fibers were not changed if compared to the normal pancreatic tissue (Fig. 5). ×100.