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原　　著

Histochemical Elucidation of Hamster Pancreatic Carcinogenesis Induced by N-nitroso-bis (2-hydroxypropyl) amine

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Summary

Hamster pancreatic carcinogenesis induced by N-nitroso-bis(2-hydroxypropyl)amine was observed pathologically and histopathologically. Five weeks later, hyperplasia of the intralobular ductules, interlobular ductules and main pancreatic ducts, intrainsular glandular structures and small adenocarcinomas appeared. Twenty-one weeks later, hypertrophic epithelial multiplication increased. After 22 weeks, adenocarcinomas appeared. Macroscopical tumor nodules were also seen. And, they were examined histochemically. The distribution and intensity of alkaline phosphatase, succinate dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and NADH tetrazolium reductase in control pancreases and adenocarcinomas were examined. All their activities were proved at the epithelium of adenocarcinomas. The difference of ^{3}H-thymidine uptake among each tissue component of control pancreases was clarified. ^{3}H-thymidine uptake of duct system and acinar cells increased in accordance with the increase of treatment weeks. ^{3}H-thymidine uptake of the epithelium of adenocarcinomas was very large.

Introduction

Now, pancreatic cancer is increasing[17], but few facts about it are known[6]. By the way, a good experimental pancreatic cancer model is induced by N-nitroso-bis(2-hydroxypropyl)amine which was first synthesized by POUR et al. at 1974[13]. The carcinogenesis of it was analized

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pathologically, histopathologically and histochemically[8]. Data obtained must contribute to elucidation of human pancreatic carcinogenesis.

Materials and Methods

Twenty outbred 8-week old Syrian golden hamsters were kept under standardized condition (room temperature, 24±2°C, humidity, 60±10%) and received solid diet for breeding F-2 (Funahashi farm) and water *ad libitum*. Five males and 5 females received weekly subcutaneous injection of 500 mg/kg body weight N-nitroso-bis(2-hydroxypropyl)amine (DHPN) in 1 ml of olive oil. A similar number of males and females received the vehicle only. One hour prior to sacrifice, a male at 21 weeks and a female at 22 weeks of DHPN administered group and a male at 24 weeks and a female at 23 weeks of control group were injected intraperitoneally 3 mCi/kg body weight ^{3}H-thymidine. Males at 5, 17, 21, 23 and 42 weeks and females at 5, 14, 22, 25 and 27 weeks of DHPN administered group and males at 5, 24 and 44 weeks and females at 2, 5, 23 and 44 weeks of control group were sacrificed by 0.5 ml of Somnopentyl i.p. injection. Routine autopsies were performed on all dead animals and observed pathologically.

(*Enzyme histochemistry*)

About 4 mm^{3} specimens were picked up from pancreatic tumor regions of females at 22 and 25 weeks of DHPN administered group and not tumor regions of males or females of both groups, and fixed in cold 2% glutaraldehyde in 0.1 M cacodylate-Na buffer pH 7.4 or not fixed. These specimens were embedded in O.C.T. compounds and cut into 10μ thick sections by a cryostat. Fixed specimens were stained with lead citrate method (OGAWA et al 1967, MAYAHARA et al 1967) for the proof of alkaline phosphatase activity (ALPase).

Not fixed specimens were stained with NACHLAS, TSOU, SOUZA, CHANG, SELIGMAN's (1957) Nitro-BT method for succinate dehydrogenase activity (SDH), NACHLAS, WALKER, SELIGMAN's method (1959) for lactate dehydrogenase activity (LDH), RUDOLPH, KLEIN's method (1964) for glucose-6-phosphate dehydrogenase activity (G-6-PD) and BURSTONE's method (1962) for NADH tetrazolium reductase activity (NADH-TR)[8].

(*Histopathology*)

The rest of pancreases was fixed in 10% buffered formalin, cut into 10 step sections and stained with hematoxylin and eosin.

(*Autoradiography*)

Two micron thick sections of pancreases fixed in 10% buffered formalin were dipped in nuclear emulsion Sakura NR M2, exposed for one month at 4°C and developped. Labeled nuclei among 1000～2000 nuclei of each tissue component were counted and labeling indexes were calculated[8].

(*Serum RNAase and amylase*)

Serum RNAase activity of a male at 42 weeks and a female at 22 weeks of DHPN administered group and males at 44 weeks and females at 44 weeks of control group was determined

by REDDI et al's method[3]. Serum amylase activity of males at 5, 17 and 21 weeks and females at 5, 14 and 22 weeks of DHPN administered group and males at 24 and 44 weeks and females at 23 and 44 weeks of control group was determined.

Results

(*Pathological and histopathological observation*)

Five weeks later, a male, 19% of the intralobular ductules showed hyperplasia (Fig. 1). Two per cent of them were flat epithelial multiplication and 3% of them showed hypertrophic epithelial multiplication. Eighty-four per cent of the interlobular ductules showed hyperplasia (Fig. 2). Pancreatic ducts showed also hyperplasia. Lesions with desmoplastic reaction appeared. A female, 2% of the intralobular ductules showed hyperplasia. Twenty per cent of them showed flat epithelial multiplication. Two per cent of them showed hypertrophic epithelial multiplication (Fig. 3). Thirty-three per cent of the interlobular ductules showed hyperplasia. A small area of a pancreatic duct was an intraductal carcinoma (Fig. 4). A few of islets showed intrainsular glandular structures (Fig. 5). Small adenocarcinomas appeared (Fig. 6).

Fourteen weeks later, a female, 27% of the interlobular ductules showed multilayer hyperplasia. A small area of a pancreatic duct showed multilayer hyperplasia.

Seventeen weeks later, a male, 23% of the intralobular ductules showed flat epithelial multiplication.

Twenty-one weeks later, a male, 2% of the intralobular ductules showed unilayer hyperplasia. Two per cent of them showed flat epithelial multiplication. The percentage of hypertrophic epithelial multiplication increased obviously and became 23%. Twenty-four per cent of the interlobular ductules showed unilayer hyperplasia.

Twenty-two weeks later, a female, adenocarcinomas appeared. Macroscopical tumor nodules were seen at the head and tail of splenic lobe of the pancreas (Fig. 7).

Twenty-three weeks later, a male, 11% of the intralobular ductules showed unilayer hyperplasia. Seven per cent of them showed flat epithelial multiplication. Seventeen per cent of them showed hypertrophic epithelial multiplication. Fourty-two per cent of the interlobular ductules showed multilayer hyperplasia. Lesions with desmoplastic reaction were seen.

Twenty-five weeks later, a female, adenocarcinomas were seen. Macroscopical tumor nodules were seen at the heads of splenic lobe and gastric lobe.

Twenty-seven weeks later, a female, adenocarcinomas (Fig. 8) and adenocarcinomas with cystic structures appeared. Macroscopical tumor nodules were seen at the heads of splenic lobe and gastric lobe.

Forty-two weeks later, a male, adenocarcinomas were seen.

(*Enzyme histochemistry*)

The distribution and intensity of the enzyme activity is listed on Table 1. In control group, ALPase activity was proved at the epithelium of intralobular ductules and interlobular ductules (Fig. 9) but was not proved at the islets or acinar cells. SDH activity was proved at

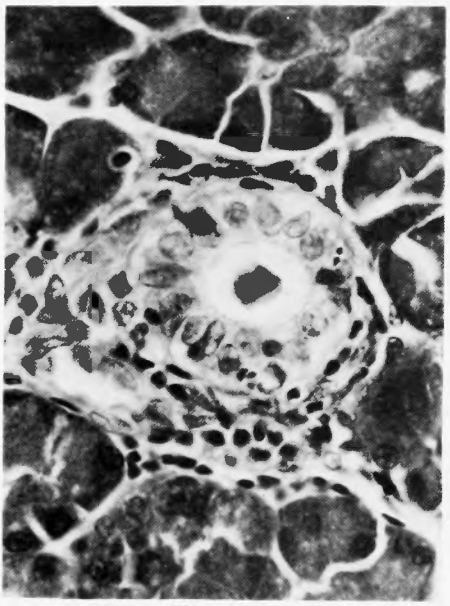


Fig. 1.

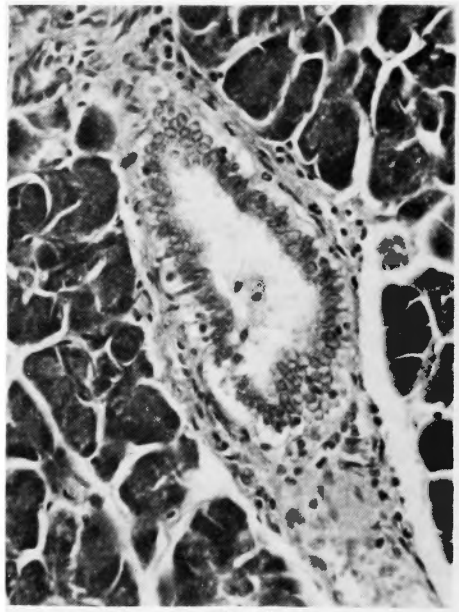
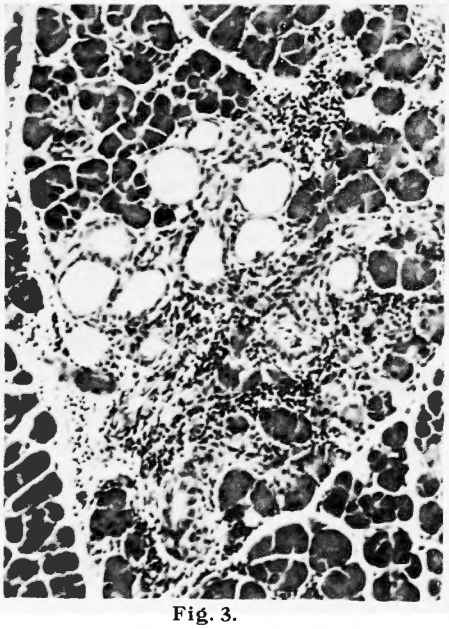


Fig. 2.



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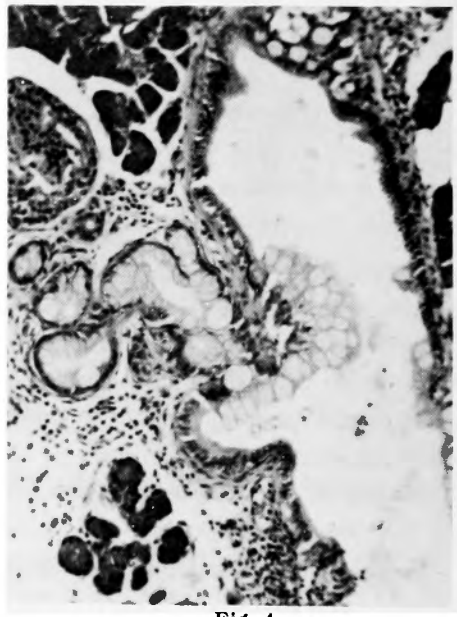

Fig. 4.

Fig. 1. Multilayer hyperplasia of an intralobular ductule. The cells are hypertrophic and atypical and the nuclei are enlarged and atypical. It is surrounded with a small number of lymphocyte infiltration. Male, 5 weeks ×200.

Fig. 2. Multilayer hyperplasia of an interlobular ductule. It is surrounded by relatively thick interlobular connective tissue. The lower region is a vessel. Male, 5 weeks ×100.

Fig. 3. Hypertrophic epithelial multiplication of intralobular ductules is surrounded by a plenty of lymphocytes infiltration. Female, 5 weeks ×50.

Fig. 4. Intraductal carcinoma of a main pancreatic duct breaks basement membrane and spreads to the surrounding tissue on the left side. Female, 5 weeks ×50.

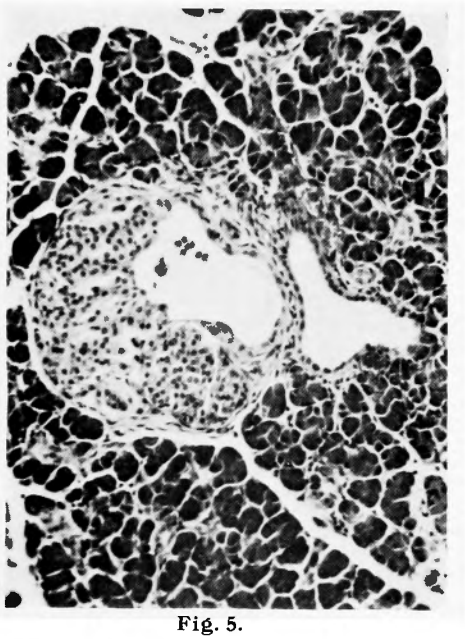

Fig. 5.

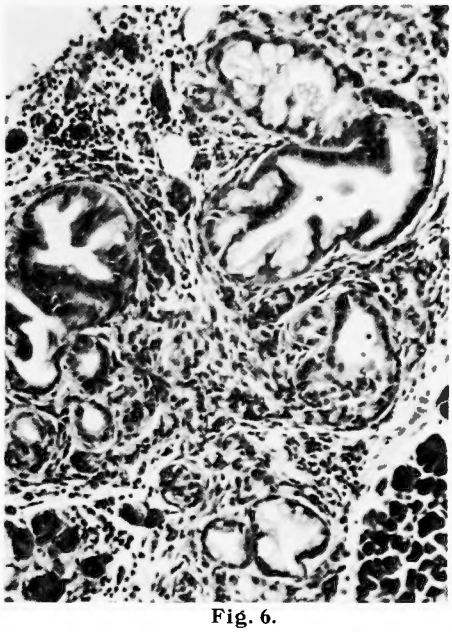

Fig. 6.

Fig. 5. An intrainsular glandular structure. Eosinophilic substance in it indicates its communication to the surrounding intralobular ductules. This is one of the most remarkable structures seen in islets by DHPN administration. Female, 5 weeks ×50.

Fig. 6. A small adenocarcinoma seen at the peripheral region of a lobule. Yet, stromal proliferation is not accomplished. Female, 5 weeks ×50.

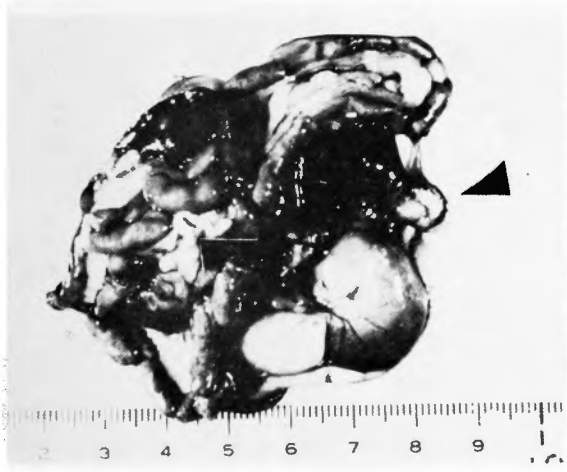

Fig. 7.

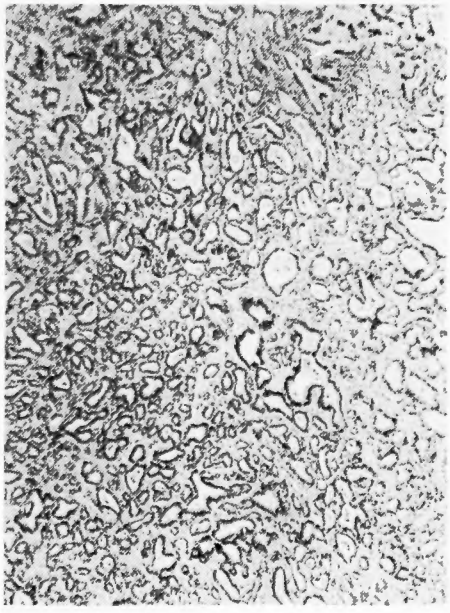

Fig. 8.

Fig. 7. There are macroscopical pancreatic tumor nodules at the head and tail of splenic lobe lying along lesser curvature of the stomach. Arrows indicate tumors. Female, 22 weeks.

Fig. 8. Well differentiated adenocarcinoma. Numerous glands are surrounded by stromal proliferation. Female, 27 weeks ×20.

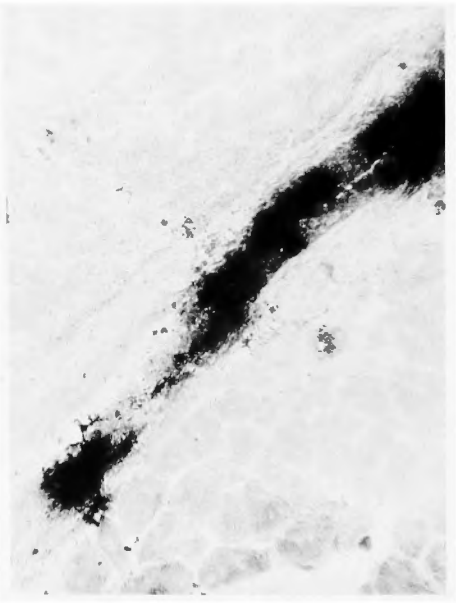

Fig. 9.

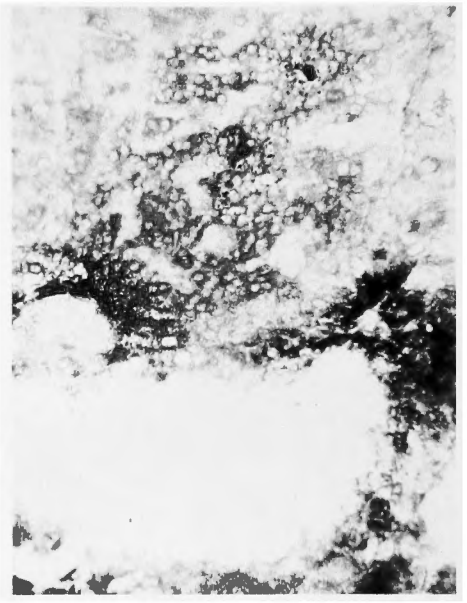

Fig. 10.

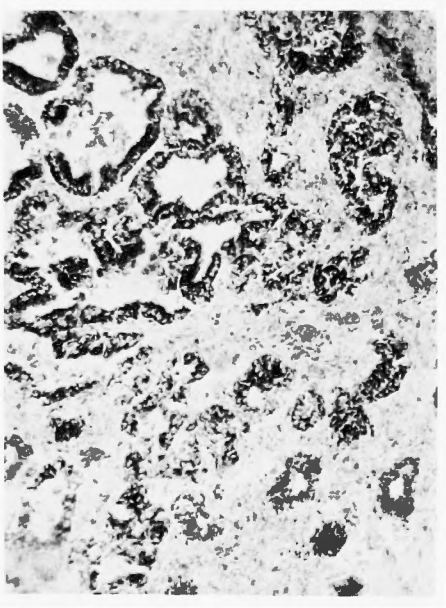

Fig. 11.

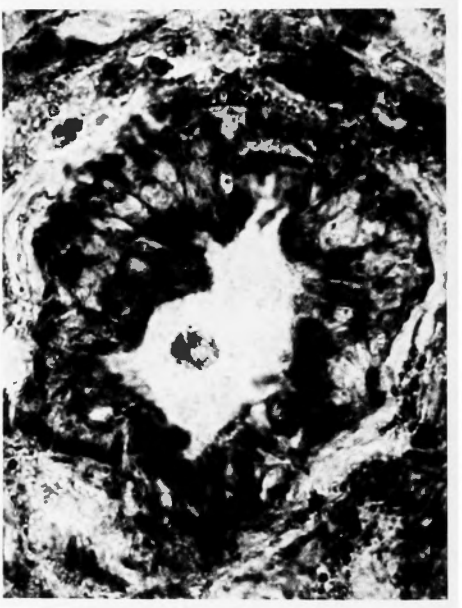

Fig. 12.

Fig. 9. Alkaline phosphatase activity at the cytoplasm of lining epithelium of an interlobular ductule is stained brownish black. But, it is not stained at the surrounding acinar cells at all. Male, 17 weeks ×200.

Fig. 10. Succinate dehydrogenase activity at islet cells located at the center and acinar cells is stained reddish purple deeply. It at epithelium of an interlobular ductule located at the lower is stained reddish purple faintly. Male, 17 weeks ×50.

Fig. 11. Succinate dehydrogenase activity at the epithelium of an adenocarcinoma and spreading cancer cells is stained greenish blue deeply. Female, 25 weeks ×50.

Fig. 12. NADH tetrazolium reductase activity at the epithelium of an adenocarcinoma and spreading cancer cells is stained dark blue very deeply. Female, 22 weeks ×200.

Table 1. Distribution and intensity of enzyme activity

		ALPase	SDH	LDH	G-6-PD	NADH-TR
Normal tissue	islet	−	++	+	−	−
	acinar cell	−	++	++	++	+++
	intralobular ductule	+++	++	+	+	+++
	interlobular ductule	+++	++	+	+	+++
Adenocarcinoma	epithelium	+++	+++	++	+	+++
	spreading cancer cell	+++	++	+	+	++
	stroma	+	+	+	−	+++

Positively stained +, moderately deeply stained ++, very deeply stained +++.

all tissue components and its relatively strong activity at the islets was a distinctive feature (Fig. 10). LDH activity was proved weakly at all tissue components. G-6-PD activity was proved weakly at the intralobular ductules, interlobular ductules and acinar cells but was not proved at the islets. NADH-TR activity was proved strongly at the intralobular ductules, interlobular ductules and acinar cells but was not proved at the islets. In adenocarcinomas, strong activities of ALPase, SDH (Fig. 11), LDH, G-6-PD and NADH-TR (Fig. 12), moderate activity of LDH and weak activity of G-6-PD were proved at the epithelium of them.

(*Autoradiography*)

Silver particles of labeled nuclei were developped black (Fig. 13). Labeling indexes of each tissue component of pancreases of control group, them of DHPN administered group and adeno-

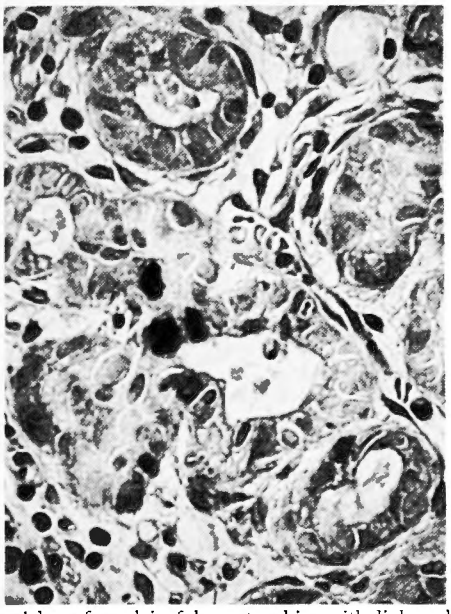

Fig. 13. Silver particles of nuclei of hypertrophic epithelial multiplication uptaked ^{3}H-thymidine located at center are developped black.
Male, 21 weeks ×200.

Table 2. Labeling index of tissue components

	Male wks	Tissue component	L.I./1000 nuclei	Female wks	L.I./1000 nuclei
Control group	24	islet	3.70±3.21	23	2.70±2.68
		acinar cell	9.45±5.75		3.78±1.43
		intralobular ductule	6.88±5.19		9.67±5.00
		interlobular ductule	6.50±4.20		3.92±3.14
		pancreatic duct	—		7.12±5.56
DHPN administered group	21	islet	5.78±3.80	22	—
		acinar cell	24.49±6.22		—
		intralobular ductule	19.62±7.90		—
		flat epithelial multiplication	16.63±6.42		—
		hypertrophic epithelial multiplication	27.22±2.66		65.63±6.02
		interlobular ductule	15.49±10.88		—
		multilayer hyperplasia	20.13±7.64		—
		pancreatic duct	19.11±6.92		—
		epithelium of adenocarcinoma	—		124.82±0.15
		spreading cancer cell	144.14±0.47		54.69±5.27
		papillary cystadenocarcinoma	—		79.85±0.16

Mean±S. E.
About 1000～2000 nuclei of each tissue component examined.

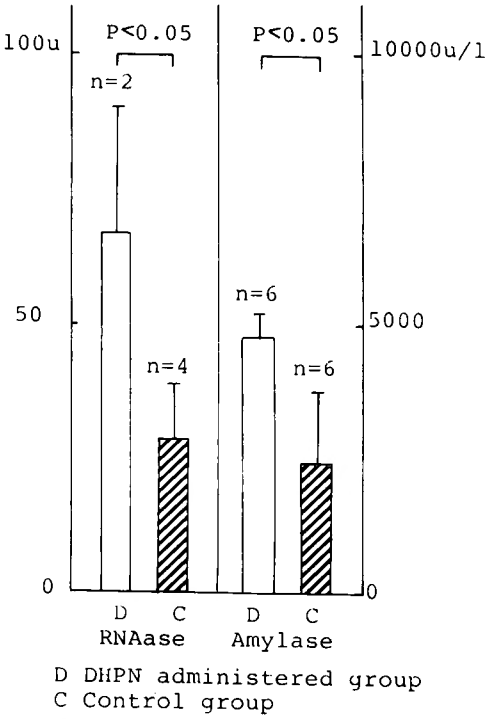

Fig. 14. Serum RNAase and amylase of DHPN administered group and control group. Mean±S. E.

carcinomas are listed on Table 2. In control group, ^{3}H-thymidine uptake was seen at all tissue components. Its uptake of the acinar cells, intralobular ductules, interlobular ductules and main pancreatic ducts were larger than it of the islets. By DHPN administration, ^{3}H-thymidine uptake of the acinar cells, intralobular ductules, interlobular ductules and main pancreatic ducts increased significantly but it of the islets did not. It of such lesions as flat epithelial multiplication, hypertrophic epithelial multiplication and multilayer hyperplasia of the interlobular ductules were large. Above all, it of hypertrophic epithelial multiplication were significantly larger than it of flat epithelial multiplication. In adenocarcinomas, it of the epithelium of adenocarcinomas was by far larger than other lesions and normal tissue components.

(*Serum RNAase and amylase*)

The difference of serum RNAase and amylase activity between DHPN administered group and control group is listed on Fig. 14.

Discussion

To know the histogenesis of pancreatic cancer is one of the most important matter among investigations against it.

HAYASHI mentioned[4] that in one case, the view of atypical hyperplasia and atypical hyperplasia with mitosis of the interlobular ductules and main pancreatic ducts coexisting with adenocarcinomas indicated that adenocarcinomas originated from them and in the other case, the view of coexistance of ductal adenocarcinoma, islet cell carcinoma and acinar cell carcinoma indicated that adenocarcinomas originated from intercalated ductules. SOMMERS et al. mentioned[14] that papillary hyperplasia and atypical hyperplasia coexist with most of adenocarcinomas but do not coexist with normal pancreases. This observation supports the theory that adenocarcinomas originated from the interlobular ductules and main pancreatic ducts.

On the other hand, the histogenesis of hamster pancreatic cancer induced by DHPN[1,5] or N-nitroso-bis(2-oxopropyl)amine[10,11,12,15,16] was examined for clarifying the histogenesis of human pancreatic cancer. Among the reports, POUR et al. reported it most definitely[12] In this experiment, the view of coexistance of hyperplasia of ductules and ducts, hypertrophic epithelial multiplication of ductules, intraductal carcinoma of a main pancreatic duct and small adenocarcinomas at 5 weeks later and appearance of adenocarcinomas after 22 weeks indicated that the majority of adenocarcinomas originated from hypertrophic epithelial multiplication via small adenocarcinomas and the minority of adenocarcinomas originated from the spread of intraductal carcinomas of ducts. Macroscopical tumor nodules appeared after 22 weeks.

Many cancers were examined histochemically[7]. For example, alkaline phosphatase activity was strongly proved at the epithelium of human stomach adenocarcinomas but did not proved at the epithelium of human colon adenocarcinomas. This experiment clarified the distribution and intensity of ALPase, SDH, LDH, G-6-PD and NADH-TR at normal pancreases and pancreatic adenocarcinomas induced by DHPN. Strong activity of SDH and LDH at epithelium of adenocarcinomas indicates the rise of both TCA cycle and glycolysis. Strong activity at

epithelium of pancreatic adenocarcinomas of such enzymes located at normal ductules and ducts suggested that adenocarcinomas originated from ductules and ducts.

Labeling indexes of human cancers are investigated broadly[9]. LEVITT et al. reported[5] the labeling indexes of ^{3}H-thymidine uptake of ductules, ducts, acinar cells and islet cells in hamster pancreatic carcinogenesis induced by DHPN. The worth of this experiment is to clarify labeling index of every lesion appeared in the carcinogenesis and to prove very large increase of it at epithelium of pancreatic adenocarcinomas.

Like a case of human pancreatic cancer, serum RNAase and amylase[2] of DHPN administered group were proved to be increased significantly against control group.

These data must contribute to elucidation of human pancreatic carcinogenesis.

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和文抄録

N-nitroso-bis (2-hydroxypropyl) amine 誘発ハムスター膵発癌の組織化学的解明

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N-nitroso-bis (2-hydroxypropyl) amine (DHPN) 誘発ハムスター膵発癌を，肉眼的，組織学的，組織化学的に観察した．DHPN 500 mg/kg 体重皮下注射，5週後，小葉内導管，小葉間導管，主膵管の hyperplasia，ラ氏島内腺構造形成と，小腺癌が現われた．21週後，hypertrophic な上皮の multiplication が増加した．22週以降，腺癌が現われた．この時期には，肉眼的腫瘍結節も見られた．これ等の組織は，酵素組織化学的にも検索された．Alkaline phosphatase, succinate dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, NADH tetrazolium reductase の，正常ハムスター組織における分布が明らかにされた．腺癌上皮には，これら酵素は全て，その活性が認められた．更に，オートラジオグラフィーにより，正常膵の各組織構成々分と，膵癌の組織発生の諸過程における各病変と，腺癌の Labeling index を算出し，各病変におけるその増加と，腺癌における非常な増加を証明した．これ等 data は，ヒト膵発癌の解明に貢献すると考えられる．