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Dietary effects on pancreatic lesions induced by excess arginine in rats

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1. The effect of nutrition on the incidence of pancreatic damage was studied. Injection of excess arginine was found to cause more massive necrosis of the acinar cells after 24 h in malnourished rats (those given 50 g casein/kg diet) than in well-nourished rats (those given 200 g casein/kg diet).

2. Ultrastructural examination showed that whorl formation of the endoplasmic reticulum, decreases in the number of zymogen granules and formation of vacuoles in the cytoplasm were more marked in rats given 50 g casein/kg diet. Degradation of zymogen granules within vacuoles in the damaged cells was frequently observed in rats given 200 g casein/kg diet.

3. Necrosis of adipose tissue was associated with pancreatic damage more frequently in rats given 200 g casein/kg diet; rats with large amounts of zymogen granules in the acinar cells showed particularly severe necrosis of adipose tissue. Rats given 50 g casein/kg diet did not show necrosis of adipose tissue.

4. These results indicate that in the malnourished state there were more marked arginine lesions of the pancreas in which to study cellular and histologic changes than in the well-nourished state and that the occurrence of necrosis of adipose tissue may be related to a high content of zymogen granules in the acinar cells before pancreatic damage.

The incidence and clinical pattern of human pancreatitis are thought to be influenced by dietary habits. In a study of the geographical distribution of pancreatitis, Sarles (1973) obtained strong evidence for a link between alcoholism and pancreatitis, particularly in countries where high-protein and high-fat diets were consumed. Moreover, the incidence of pancreatitis at an early age is high in countries where there is protein malnutrition. Various explanations have been proposed for the role of nutrition in the pathogenesis of human pancreatitis. However, few investigators have studied the effect of nutrition on experimental pancreatic damage.

In previous studies (Kishino & Kawamura, 1984; Mizunuma *et al.* 1984) we observed extensive degenerative changes of the pancreatic acinar cells of rats after intraperitoneal injection of excess arginine. The present paper, using this method, describes the effects of nutritional state on morphological changes of the pancreas and the occurrence of necrosis of adipose tissue as a complication.

MATERIALS AND METHODS

Male Wistar rats obtained from Tokushima Jikken-Dobutsu Kenkyusho, Tokushima, Japan were used. The average weight of the rats was 70 g on the 1st day of the experiment. Groups of twelve rats were offered 200 and 50 g casein/kg diets *ad lib.* The composition of these diets is given in Table 1. Animals were housed individually in wire-bottomed cages in a room at a temperature of approximately 22°. Water was provided *ad lib.* Food intake and body-weight were carefully recorded daily. After 4 weeks on these experimental diets, six animals from each group were injected intraperitoneally with L-arginine monohydrochloride (5 g/kg body-weight; Otsuka Pharmaceutical Co., Tokushima) in saline (9 g sodium chloride/l) at 10.00 hours and then starved for 15 h before death. Control rats were injected with the same volume of saline alone. The rats were killed by decapitation 24 h later. At autopsy, the appearances of the pancreas and abdominal adipose tissue were

Table 1. *Composition of experimental diets (g/kg dry matter)*

Ingredient	200 g casein/kg	50 g casein/kg
Casein	200	50
Maize starch	570	720
Sucrose	100	100
Soya-bean oil	80	80
Salt mixture*†	40	40
Vitamin mixture*‡	10	10
Total energy (kJ/kg)	17600	17600
Total energy (kcal/kg)	4200	4200

* Prepared by Oriental Yeast Co., Ltd, Japan.

† Contained (g/kg mix): $CaHPO_4 . 2H_2O$ 145·6, KH_2PO_4 257·2, $NaH_2PO_4 . H_2O$ 93·5, NaCl 46·6, calcium lactate 350·9, ferric citrate 31·8, $MgSO_4$ 71·7, $ZnCO_3$ 1·1, $MnSO_4$ 1·2, $CuSO_4 . 5H_2O$ 0·3, KI 0·1.

‡ Contained (g/kg mix): retinyl acetate 1, cholecalciferol 0·0025, thiamin hydrochloride 1·2, riboflavin 4, pyridoxine hydrochloride 0·8, vitamin B_{12} 0·0005, ascorbic acid 30, DL-α-tocopherol acetate 5, menadione 5·2, biotin 0·02, folic acid 0·2, calcium pantothenate 5, *p*-aminobenzoic acid 5, niacin 6, inositol 6, choline chloride 200, cellulose powder to 1 kg.

observed in detail. The pancreas was removed and weighed, and portions were placed in the following fixatives. For light microscopy, tissues were fixed in formalin (100 ml/l) and embedded in paraffin. Tissue sections of 7 μm thickness were stained with haematoxylin and eosin. For electron microscopy, tissues were fixed in 25 ml glutarladehyde/litre 0·1 M-cacodylate buffer, pH 7·2, for 2 h. Then they were postfixed in a 10 g osmium tetroxide/litre cacodylate buffer, dehydrated by passage through a graded acetone series and embedded in Epon 812. Ultra-thin sections were cut with a glass knife on an LKB ultrotome (LKB Instruments, Rockville, MD, USA) and stained with uranyl acetate and lead citrate. The stained sections were examined in an HU-12 electron microscope (Hitachi Corp., Tokyo, Japan) at 75 kV.

RESULTS

As early as 1 week after the beginning of the experiment, the average body-weight and food intake of the 50 g casein/kg group began to diverge from those of the 200 g casein/kg group (Fig. 1). The rats in the 50 g casein/kg group did not grow, and their final mean body-weight was about 30% of that of the 200 g casein/kg group. Rats in the 50 g casein/kg group ate about 70% as much as those in the 200 g casein/kg group per d. These results indicate that rats given 50 g casein/kg diet were deficient in both protein and energy. Changes in pancreatic weight showed the same pattern as those in total body-weight. However, as a percentage of the total body-weight, the relative weight of the pancreas tended to be higher in the 50 g casein/kg group than in the 200 g casein/kg group, but the difference was not significant.

After injection of excess arginine, the relative weight of the pancreas was significantly increased in the 200 g casein/kg group (Fig. 2). In four of six rats in the 200 g casein/kg group, the pancreas became swollen. Chalky white spots appeared on the surface of adipose tissue in the abdominal cavity, such as epididymal, mesenterial and peripancreatic adipose tissues. In the 50 g casein/kg group, injection of excess arginine had no significant effect on the relative weight of the pancreas and had no obvious effect on the appearance of abdominal adipose tissues.

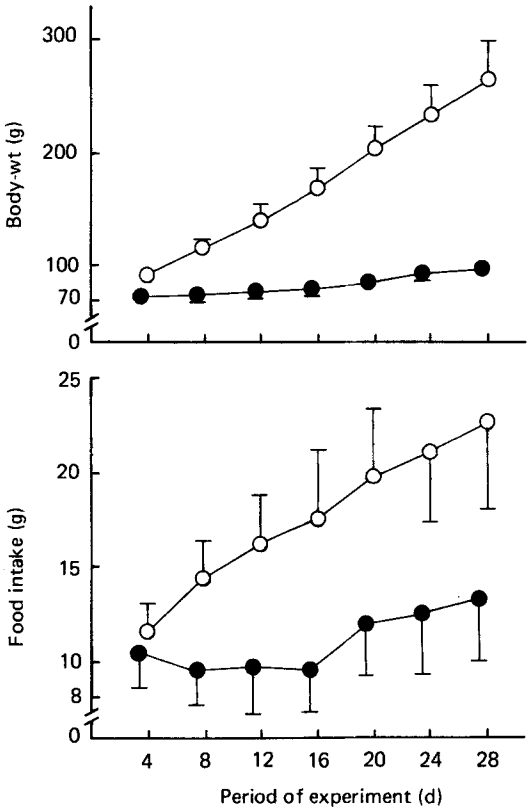

Fig. 1. Daily values for body-weight and food intake of rats. Male rats were fed on diets containing 200 g (○) or 50 g (●) casein/kg as described in Table 1. Values are means of daily food intake and body-weight at 4 d intervals, with their standard errors represented by vertical bars.

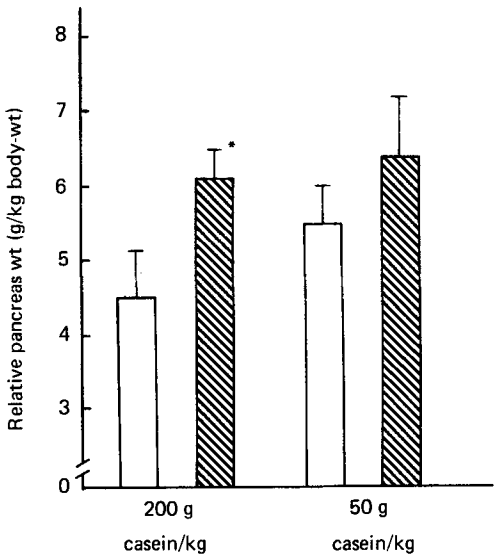

Fig. 2. Pancreas weight per kg body-weight in rats. (□), Relative weights of the pancreas in rats given 200 g and 50 g casein/kg diets for 4 weeks after saline (9 g sodium chloride/l) injection. (▨), Relative weights of the pancreas in each group after arginine injection. Values are means with their standard errors represented by vertical bars for six rats. *Mean value after arginine injection was significantly different from the value after saline injection ($P < 0{\cdot}025$).

Light microscopy

Pancreas. In rats given the 200 g casein/kg diet, the pancreatic structure was well preserved. The acinar cells were swollen relative of those of the 50 g casein/kg group and filled with prominent zymogen granules, and showed distinct perinuclear basophilia (Plate 1 (*a*)). In animals treated with arginine, degenerative changes of acinar cells were seen (Plate 1 (*b*)), which tended to spread along interlobular septa. Undamaged acinar cells contained large amounts of zymogen granules, particularly at the margin of degenerative or necrotic lesions. Ductal and Langerhans' islet cells appeared to be unaffected. There was also oedema of the stroma.

In all rats of the 50 g casein/kg group, the acinar cells were small and had small amounts of zymogen granules. There was also considerable reduction of perinuclear basophilia, but no nuclear change (Plate 2 (*a*)). After injection of excess arginine, the acinar structure was destroyed extensively. Numerous cytoplasmic vacuoles appeared but these did not stain for fat. These cellular changes were often associated with nuclear alterations such as pyknosis and karyorrhexis (Plate 2 (*b*)).

Adipose tissue. In rats of the 200 g casein/kg group, chalky white areas in gross appearance showed necrosis of adipose tissue. Necrotic adipose tissue was filled with opaque material and had shadowy outlines of cellular membranes which were demarcated by a wall of leucocytes (Plate 3). No necrosis of the pancreas or adipose tissue was seen in two rats in the 200 g casein/kg group. On the other hand, in rats given 50 g casein/kg diet, no necrosis of adipose tissue was observed despite the severe and extensive destruction of the acinar cells (Plate 4).

Electron microscopy

In the 50 g casein/kg group, the number and density of zymogen granules were less than in the 200 g casein/kg group. The structure of the nuclei appeared normal. The endoplasmic reticulum was reduced in amount and it occasionally had a wavy and whorled appearance. After injection of arginine (Plate 5), the nuclei often contained coarse granules with increased osmiophilia, and they occasionally showed clumping of chromatin and vacuolation at their periphery. The membranes of the endoplasmic reticulum were more widely separated than in the 200 g casein/kg group. Ribosomes were scattered freely in the cytoplasmic matrix. Many vacuoles in the cytoplasm contained fine, granular particulate matter but few zymogen granules. Large vacuoles were enclosed by an agranular membrane. Most mitochondria were swollen and contained short cristae attached to the inner margins of the outer limiting double membrane. The degradation of various organelles indicates that the cells were either necrotic or undergoing necrosis. After injection of arginine in the 200 g casein/kg group (Plate 6), the slight dilatation of the endoplasmic reticulum and vacuoles of various sizes were seen. The membranes of these vacuoles were continuous with the membranes of the endoplasmic reticulum. Vacuolar areas were seen containing destroyed zymogen granules in various stages. Remaining zymogen granules had a normal appearance. Most mitochondria and nuclei appeared normal in size and appearance.

DISCUSSION

In the 50 g casein/kg group, the microscopic changes in the pancreatic tissue were essentially atrophy of the acinar cells with reduction of zymogen granules. In contrast, in the 200 g casein/kg group, the acinar cells were rich in zymogen granules within the cytoplasm. After injection of excess arginine, the changes in the acinar cells consisted of a decrease in basophilia and vacuolar formation in the cytoplasm with concomitant decrease in zymogen

granules, particularly in the 200 g casein/kg group. Furthermore this damage was greater in the 50 g casein/kg group which showed whorl formation, focal distension of the endoplasmic reticulum and free scattering of ribosomes. It seems, therefore, that one target of excess arginine in the pancreas is the endoplasmic reticulum, which is involved in the processes of protein synthesis. Nuclei of the acinar cells in the 50 g casein/kg group showed coarse granularity and clumping of chromatin. These findings were almost identical with those found in the degenerating pancreas of rats following ethionine poisoning. The lesions induced by ethionine possibly reflected interference with protein and nucleic acid metabolism (Herman & Fitzgerald, 1962). A protein-deficient diet might induce changes of the structure and stability of cellular membrances (van Deenen, 1966; O'Brien, 1967) and in protein metabolism in the endoplasmic reticulum, and could easily potentiate the toxic action of excess arginine, resulting in massive necrosis.

The present results are not consistent with the report of Maki *et al.* (1967), where 270 g casein/kg diet resulted in the highest incidence of severe pancreatitis on ligation of the common bile duct. They suggested that the acinar cells, which contain large amounts of digestive enzymes, were susceptible to the injury leading to pancreatitis. This difference in findings can be explained by the difference in experimental methods used to induce pancreatic damage. Ligation of the bile duct might be expected to cause mechanical stress of the pancreatic acinar cells, and the extent of damage produced by a pressure sufficient to injure acinar cells would depend on the concentration of enzymes in the cells. However, in the pancreatic damage induced by a chemical agent such as ethionine or excess arginine, the severity of damage would depend inversely on the ability of the cells to resist the challenge.

After treatment with excess arginine, extensive necrosis of abdominal adipose tissues occurred in the 200 g casein/kg with large numbers of zymogen granules in the pancreatic acinar cells. On the other hand, there was no necrosis of adipose tissues in the 50 g casein/kg group, although necrotic change of the acinar cells was more severe and extensive in this group. The precise relation of enzyme content to necrosis of adipose tissue is not clear, but it seems to be secondary to pancreatic necrosis and can be explained by leakage of active pancreatic enzymes into the abdominal cavity. This is because loss of zymogen granules from the damaged acinar cells was greater and destruction of these granules was often observed within the vacuoles of the damaged acinar cells in the 200 g casein/kg group. Greenbaum *et al.* (1959) reported that the destruction of zymogen granules could lead to activation of zymogen by lysosomal enzymes in the foci of cytoplasmic degradation. Grossman *et al.* (1942) and Magee & White (1958) showed that rats given a high-protein diet had elevated pancreatic levels of proteolytic enzymes and lipase (*EC* 3.1.1.3) respectively. On the other hand, a low level of dietary protein can cause decreases in all pancreatic enzymes (Magee & White, 1958). Neal & Ellis (1930), Panabokké (1958) and Storck (1971) thought that the combined actions of lipase and trypsin (*EC* 3.4.4.4) were responsible for the development of necrosis of adipose tissue. Colipase, as a cofactor of lipase, may attack the membranes before triglyceride is exposed to lipase (Lee *et al.* 1979; Ouaqued *et al.* 1980). Ibrahim *et al.* (1964) also found that phospholipase A (*EC* 3.1.1.4) facilitated the transport of substances and that pancreatic lipase split the triglyceride of adipocytes in necrosis of adipose tissue. A sequence of events such as this would account for the contrast between the paucity of necrosis of adipose tissue in the 50 g casein/kg group and severity in the 200 g casein/kg group. However, the precise mechanism by which various factors attack the adipose tissue and cause necrosis of adipose tissue is not known and requires further study.

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EXPLANATION OF PLATES

Plate 1. Light micrographs of the pancreas of rats given 200 g casein/kg diet for 4 weeks and then starved for 15 h. (*a*) Acinar cells of a control rat are swollen due to large amounts of zymogen granules. (*b*) Acinar cells from a rat 24 h after injection of excess arginine. Note the partial destruction of acinar structure and loss of zymogen granules (→). Some of the acinar cells with vacuolated cytoplasm and pyknotic nuclei (⇉) are present. The remaining acinar cells contain zymogen granules. Haematoxylin and eosin stain.

Plate 2. Light micrographs of the pancreas of rats given 50 g casein/kg diet for 4 weeks and then starved for 15 h. (*a*) Acinar cells of a control rat are atrophic and contain very few zymogen granules. (*b*) 24 h after injection of arginine. Note the extensive destruction of acinar structure and the presence of scattered necrotic cells and pyknotic debris in the cells (→). Some nuclei are fragmented (⇉). The cytoplasm is often vacuolated (►). Zymogen granules are not seen in the cells. Haematoxylin and eosin stain.

Plate 3. Light micrograph of peripancreatic adipose tissue of a rat in the 200 g casein/kg group 24 h after injection of arginine. Adjacent to the damaged pancreas tissue there is necrotic adipose tissue (→), which has shadowy outlines of cellular membranes and contains pink, fibrillar or opaque material. Polymorphonuclear leucocytes infiltrate at the periphery of the necrotic areas. P, pancreas tissue. Haematoxylin and eosin stain.

Plate 4. Light micrograph of peripancreatic adipose tissue of a rat in the 50 g casein/kg group after injection of arginine. No necrosis of adipose tissue is observed. Pancreatic acinar cells are destroyed extensively. P, pancreas tissue; A, arteriole. Haematoxylin and eosin stain.

Plate 5. Electron micrograph of a pancreatic acinar cell of a rat in the 50 g casein/kg group 24 h after arginine injection. The cell is reduced in size. Spaces between endoplasmic reticula are increased (→). Whorl formation of the endoplasmic reticulum (⇉) is shown. Ribosomes are scattered freely in the cytoplasmic matrix. Many vacuoles contain fine granules but very few zymogen granules. Most mitochondria are swollen (►). Nuclei show increased osmiophilia, clumping of chromatin and vacuolation at their periphery (>). L, acinar lumen.

Plate 6. Electron micrograph of an acinar cell of a rat of the 200 g casein/kg group 24 h after arginine injection. Widening of the endoplasmic reticulum is partially seen. Vacuoles contain destroyed zymogen granules in various phases (→). Remaining zymogen granules are seen in the upper left. The nucleus and mitochondria appear to be intact.

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