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Pancreatic Morphology and Blood Glucose Level in Rats at Various Intervals after Duct Ligation*

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Pankreasmorphologie und Blutzuckerspiegel nach Gangligatur bei Ratten

Zusammenfassung. Bei 42 weiblichen Ratten wurde eine Unterbindung der Ausführungsgänge des Pankreas vorgenommen und die Entwicklung der Acinusatrophie über einen Beobachtungszeitraum von 1 Tag bis $8^1/_2$ Monaten nach der Operation untersucht. Außerdem wurden bei insgesamt 60 operierten Ratten das Körpergewicht und der Blutzuckerspiegel registriert.

Eine interstitielle Pankreatitis mit Vorkommen von aldehydfuchsinophilen Makrophagen war innerhalb der ersten 6 Versuchsmonate nachweisbar. Die interstitielle Entzündung war von einer Fibrose und Lipomatose begleitet. Die Acinuszellen waren innerhalb der ersten Versuchswoche vollständig geschwunden und zeigten keine Regeneration. Während der ersten Versuchswoche kam es zu einer Proliferation der Ductuli und zu einer Aussprossung von B-Zellen. Innerhalb der Inseln wurde eine leichte ödematöse Aufsplitterung beobachtet. Ovoide oder nierenförmige Inselkörperchen traten in Verbindung mit der Gangproliferation nach der ersten Woche auf und kamen innerhalb der ersten Versuchswochen sehr reichlich vor. Im weiteren Versuchsablauf kam es allmählich zu einer bindegewebigen Untergliederung dieser Inselkörperchen, so daß 5 Wochen nach der Unterbindung alle Inseln wieder einen normalen Aufbau aufwiesen.

Die Gesamtmenge des Inselgewebes in den atrophischen Bauchspeicheldrüsen nahm allmählich ab. Gleichzeitig kam es zu einem Anstieg des Blutzuckerspiegels, so daß bei den Tieren mit der längsten Versuchsdauer hyperglykämische Werte auftraten. Zucker im Urin konnte in keinem Fall festgestellt werden.

Aus den Ergebnissen wird der Schluß gezogen, daß die Unterbindung der Ausführungsgänge initial eine Schädigung der Inseln verursacht, auf die sekundär eine Regeneration von Inselgewebe aus proliferierenden Ductuli folgt. Die auf diese Weise entstehenden ovoiden und nierenförmigen Inselkörperchen erfahren im weiteren Versuchsablauf eine Umbildung zu normalem Inselgewebe. Die praktische Bedeutung dieser Beobachtungen für die Verwendung atrophischen Pankreasgewebes in chemischen und biologischen Untersuchungen von Inselgewebe wird besprochen.

Summary. The evolution of the atrophy of the acinar parenchyma obtained after ligation of the main pancreatic ducts was studied in 42 female rats at time intervals ranging from one day to $8^1/_2$ months after operation and the body weight and mean blood glucose level was followed in 60 duct-ligated female rats.

Marked interstitial inflammation persisted for almost 6 weeks with the occurrence of aldehydefuchsinophil macrophages. It was followed by fibrosis and ultimate fatty involution. The acinar cells completely disappeared within one week and never reappeared. The ductulus apparently proliferated during the first weeks and showed outbuddings of β-cells. Slight edematous splitting of the islets were noted. The previously described ovoid or kidney-shaped islet bodies appeared in connection with the proliferation of ductules one week after

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the ligation and then occurred rather abundantly for about 6 weeks. Then, they gradually became split by fibrosis into several islets of ordinary shape so that 5 months after the ligation all islets appeared normal.

The total amount of islet tissue in the atrophied pancreas progressively decreased and the mean blood glucose level gradually increased to attain hyperglycemic levels at the longest observation times. No cases with glucosuria occurred.

It was concluded that duct ligation caused some initial damage to the islets with subsequent regeneration from proliferating ductules via ovoid or kidney-shaped islet bodies to islets of ordinary shape as a final result.

The practical significance of these findings for the use of atrophied pancreatic parenchyma for chemical and biological studies of islet tissue is discussed.

Introduction

In a preceding investigation on rats (EDSTRÖM and FALKMER, 1967) it was observed in the atrophied pancreatic parenchyma obtained 5 weeks after ligation of the main ducts that ovoid or kidney-shaped islet bodies occurred, mainly containing islet cells and tubular structures. These islet bodies were tentatively supposed to represent signs of regeneration of the islet parenchyma, thus indirectly indicating that the duct ligation might have damaged also the islet tissue. In order to test whether our working hypothesis may have some validity, it was thought worth while to investigate more closely the evolution of the atrophic changes in pancreas after duct ligation over a wide range of time. Particular attention was paid to the occurrence of concomitant islet changes during the early phase of the atrophy of the acinar parenchyma as well as to the appearance and the fate of the ovoid or kidney-shaped islet bodies.

Moreover, a detailed quantitative analysis of the various pancreatic tissue components about $^1/_2$ year after the ligation was considered to be of interest both as a comparison with the preceding analysis at 5 weeks' observation time and as a contribution to the question of whether there is an increase (MANSFELD, 1924) or decrease (BEST, 1934; LARSSON, 1956) in the islet volume after duct ligation. Lastly, the blood glucose level was followed in these long-term experiments in order to get a rough idea about the functional capacity of the islet tissue of the atrophied parenchyma on various times after duct ligation. Another reason for studying the blood glucose level was that it is clinically well known that patients with cystic fibrosis (ANDERSEN, 1962; WALTERS, 1965) and other types of pancreatic cirrhosis (SEIFERT, 1966) may develop overt diabetes mellitus and it is reasonable to consider the atrophied pancreas obtained after duct ligation as an experimental model of various types of pancreatic duct occlusions in man.

However, the principal aim of the investigation was to study islet tissue regeneration and the biological properties of the islet parenchyma obtained after duct ligation as this apparently is becoming increasingly used in studies on islet tissue metabolism (KEEN et al., 1965).

Material and Methods

The basic experimental procedures were the same as in our preceding report (EDSTRÖM and FALKMER, 1967).

In all, 60 female Sprague-Dawley rats, being about 2 months old at start of the experiments and weighing about 200 g, were used for duct ligation. Of these, 18 were only used

for blood glucose assays or were merely included in a pilot study. One duct ligated rat died spontaneously late in the experimental period. As non-operated controls for following the blood glucose and body weight changes, 30 female rats of same size and age were used. The remaining 42 experimental animals were allotted into groups of 3 and sacrificed on the following time intervals after the duct ligation: 1, 3, and 5 days, 1, 2, 3, 4, and 6 weeks, as well as 3, 4, 5, 6, 7, and 8.5 months. Autopsy specimens were taken from pancreas, kidney, liver, and myocardium as shown in Table 1. The histological technique, including the quantitative morphological analysis, was essentially the same as previously. Serial sectioning of pancreas was made on one and three specimens from 5 and 6 months' observation times, respectively. The sections formed a complete series of planes about 400 μ apart.

From every rat to be sacrificed blood glucose assays were performed as duplicates from the tail blood before they were killed by an overdose of ether. In addition, blood glucose determinations on tail blood were also made on duct-ligated animals (selected at random) to be sacrificed later on, so that the total number of blood glucose values at each observation time varied between 7 and 30 (*cf.* Table 1). The non-operated control rats were used twice for blood glucose assays, *viz.* at the observation times 1 week and 8 months. Duplicate samples of tail blood were used on both occasions. The blood glucose assays were performed by the glucose oxidase procedure on animals fasted for 12—18 hours (*cf.* BOQUIST, 1967) and urinanalysis with Hemocombistix® paper strips on urine collected from hyperglycemic animals, randomly selected and kept in metabolic cages.

Table 1. *Survey of the animal material used for morphologic analysis and blood glucose assays*

Observation times	Number of animals					
	Microscopic examination				Blood glucose assay	
	Pancreas	Kidney	Liver	Myocardium	Ligated rats	Non-operated controls
Days						
1	2	1	1	1	—	—
3	3	1	1	1	—	—
5	3	1	1	1	—	—
Weeks						
1	3	1	1	1	13	7
2	3	1	1	1	30	—
3	3	1	1	1	23	—
4	3	1	1	1	16	—
6	3	1	1	1	—	—
Months						
2	—	—	—	—	9	—
3	3	1	1	1	19	—
4	3	1	1	1	8	—
5	3	1	1	1	12	—
6	3	1	1	1	9	—
7	3	1	1	1	7	—
8	—	—	—	—	7	6
8.5	3	1	1	1	—	—

Nomenclature

The terminology of the islet cells and the epithelium of the various parts of the efferent ducts follows that described in detail in another report from our laboratory (BOQUIST, 1968a). Thus, by "ducts" we mean those parts of the efferent duct system ("interlobular" and parts of the "intralobular" ducts) where the epithelium is light and high columnar and

sometimes ciliated. The term "ductules" is used for those parts where the epithelium is low columnar, cuboidal, or even flattened (parts of the "intralobular ducts", the "intercalated", and "centroacinar ductules"). By "tubular structures" we refer to islet-associated structures, light-microscopically indistinguishable from the "ductules" (and possibly related to them), that only seldom occur in normal pancreas in mammals.

Results

Weights of Animals and Pancreas

The variations in the body weight increase for the duct-ligated rats and the non-operated controls are shown in Fig. 1. Although the body weight increase

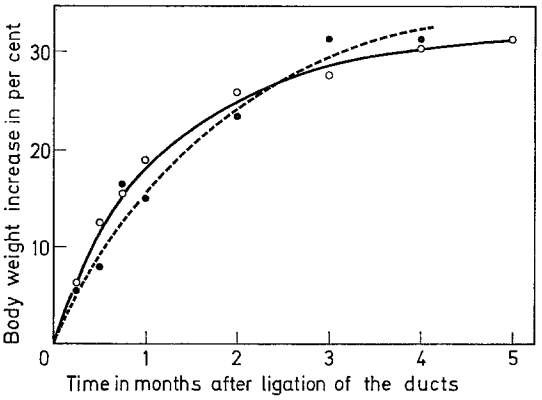

Fig. 1. Mean body weights of duct ligated rats (○- - - -○) and non-operated controls (●—●) on various time intervals after the operation. It seems as if the body weight increased somewhat more rapidly in the duct ligated animals than in the controls during the first weeks but there were apparently not any marked differences at any of the observation times

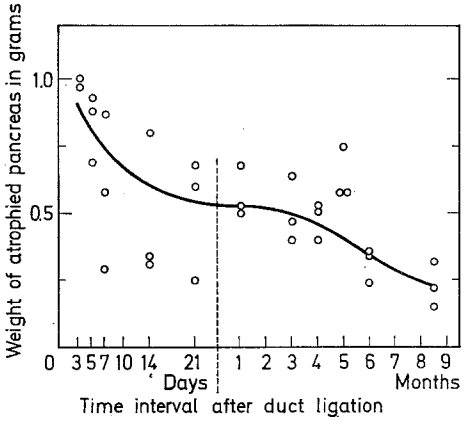

Fig. 2. Absolute weight of carefully dissected atrophied pancreatic parenchyma on various time intervals after duct ligation

possibly was somewhat more rapid in the duct-ligated rats during the first month (*cf.* EDSTRÖM and FALKMER, 1967), there did not seem to be any marked differences at any of the observation times.

The weight of the total pancreas as cut out by a pair of scissors at autopsy was close to that of normal or sham-operated rats (EDSTRÖM and FALKMER, 1967)

at all observation times. When, however, the adherent and grossly visible interstitial fat, nerves, vessels, and adjacent connective tissue was cut away by careful naked-eye (or micro)dissection (*cf.* EDSTRÖM and FALKMER, 1967), the remaining more or less atrophic parenchyma showed a gradual decrease in weight with increasing observation times (Fig. 2). This indicates a successive replacement of the parenchyma by fat, *i.e.* a fatty involution. This was also quite apparent at gross inspection at autopsy.

Gross Appearance of Pancreas

In addition to the progressive fatty involution mentioned in the preceding section and the expected occurrence of fat necroses, edema, and bleedings during the first weeks after duct ligation, the gross appearance of pancreas distally to the ligatures essentially conformed at all remaining observation times to that described previously 5 weeks after duct ligation (EDSTRÖM and FALKMER, 1967). The ligations did not fail in any of the 60 cases.

Microscopic Appearance of Pancreas

The changes evoked in various parts of pancreas by duct ligation at different time intervals have been summarized in Table 2 and illustrated in Figs. 3, 4, and 7—9.

It is obvious from Table 2 and Fig. 3 that both the exocrine and endocrine parenchyma suffered from the ligation and that a neoformation occurred of islet tissue but not of acinar cells. It is also evident that a "proliferation" of ductules preceded the neoformation of islet tissue, that β-cells and small islets budded out from the ductules, that ovoid or kidney-shaped islets appeared at the height of this islet neogenesis (Fig. 9), and that these atypical islet bodies ultimately were transformed into islets of ordinary shape (Fig. 4). The stationary end result for the parenchyma was arrived at about $^1/_2$ year after the ligation (Fig. 9). After this, only progressive fatty involution occurred. The fact that marked signs of inflammation with aldehydefuchsinophil macrophages (β-cell *débris* ?) persisted as long as 3 months after the operation indicates that the degenerative processes were rather protracted.

Quantitative Studies of the Atrophied Parenchyma about $^1/_2$ Year after Duct Ligation

When the relation between the atrophied parenchyma, including the islets, and the mesenchymal tissues were assessed planimetrically, large variations were noted between individual animals (Table 3). However, it is obvious from Table 3 that on an average the mesenchymal tissues predominated and that the islet tissue did not comprise a markedly higher percentage of the atrophied parenchyma than normally. As there is a progressive decrease of the atrophied parenchyma (Fig. 2), this implies that duct ligation gives an absolute decrease in the amount of islet parenchyma with increasing time intervals after the duct ligation.

A total number of 674 islets from the serial sections were grouped according to their mean diameters into 9 classes with an interval of 26 μ (Table 4). The percental frequency distribution is given in Fig. 5. In order to allow direct comparisons, the corresponding frequency distribution of the islets and the ovoid

Table 2. *Survey of the main microscopical changes noted on various time intervals after duct ligation*

Time interval after duct ligation	Interstitial soft tissue	Acinar parenchyma	Ducts	Ductules	Islet parenchyma
1 day	Some fat necroses and hemorrhages	Early necrosis of isolated lobules	Normal	Normal	Normal
3 days	Marked edema, fat necroses and inflammation	Marked shrinking of acinar cells with loss of zymogranules. Some lobules intact	Normal	Appear wide and increased in number (effect of acinar atrophy ?)	Normal
5 days	Marked edema and inflammation	Almost complete loss of acinar cells with zymogen granules. (Transformation to ductules ?)	Wide	Wide and seemingly markedly increased in number. (Transformation of acinar cells ?)	Edema and splitting into trabecules
7 days	Moderate edema but still marked inflammation. Many macrophages	Complete loss of normal acinar parenchyma	Fairly marked dilatation	The same. Outbudding of β-cells from tubular structures in patches	The same. Ovoid or kidney-shaped islet bodies appear
2 weeks	Slight edema and moderate inflammation. Slight fibrosis. Many macrophages	The same	The same	The same but now marked β-cell proliferation from tubular structures	The same. Ovoid or kidney-shaped bodies occur abundantly
3 weeks	The same	The same	Wide. Signs of proliferation in patches	The same but no further increase in number	The same
4 weeks	No edema. Slight inflammation. Moderate fibrosis. Several macrophages	The same	Somewhat wide	The same but the β-cell proliferation only sparse	The same
6 weeks	Slight inflammation. Several macrophages. Marked fibrosis	The same	Normal	The close connection to islets split by fibrosis	Islets of ordinary shape normal. Ovoid or kidney-shaped islet bodies split by fibrosis

Table 2 (Continued)

Time interval after duct ligation	Interstitial soft tissue	Acinar parenchyma	Ducts	Ductules	Islet parenchyma
3 months	No inflammation but still some macrophages. Marked fibrosis	The same	Normal	The ductules seem collapsed. No close connection to islet cells	The same but now marked fibrotic subdivision of large islet bodies
4 months	No macrophages. Marked fibrosis	The same	Normal	The same	Almost all ovoid or kidney-shaped islet bodies transformed to islets of ordinary shape
5 months	Fibrosis and signs of fatty involution	The same	Normal	Small groups of ductules occur embedded in fat or connective tissue	All islets of ordinary shape
6 months	Marked signs of fatty involution	The same	Normal	The same	Normal
7 months	Marked signs of fatty involution	The same	Normal	The same	Normal
$8^1/_2$ months	Marked signs of fatty involution	The same	Normal	The same	Normal

Table 3. *Volume of various tissue components in mm³, calculated from planimetrical determinations of the areas in serially sectioned atrophied pancreatic tissue obtained 5 and 6 months after duct ligation*

Rat No.	Volume (in mm³)				
	Total amount of tissue used for serial sectioning	Atrophied pancreatic tissue (ducts, ductules, and tubular structures) including islet parenchyma	Islet parenchyma (without tubular structures)	Remaining fat	Other remaining soft tissues
05/65	72.1	43.3	1.1	22.1	6.8
07/65	16.2	8.3	0.4	6.7	1.2
09/65	32.8	5.7	0.2	27.0	—
10/65	28.1	10.6	0.3	16.0	1.6
Mean	37.3	17.0	0.5	18.0	2.4
Per cent	100	45	1	48	6

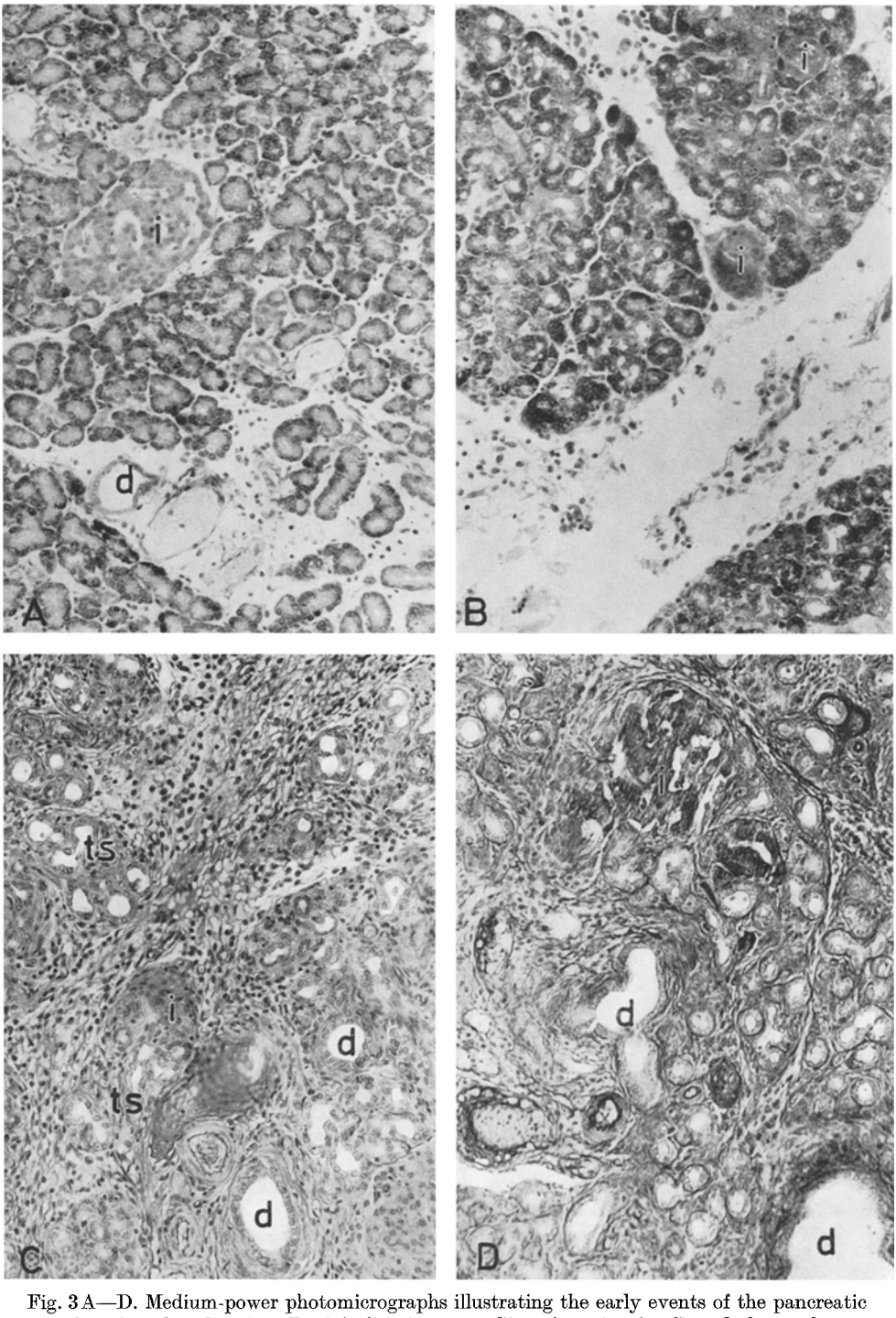

Fig. 3 A—D. Medium-power photomicrographs illustrating the early events of the pancreatic atrophy after duct ligation. Bouin's fixative; van Gieson's stain (A—C) and chrome-hematoxylin (D). ×120. A *First day:* There is only a slight interstitial edema with teleangiectases and a few inflammatory cells. The acinar parenchyma, the ducts (*d*), and the islet tissue (*i*) are all normal. B *Third day:* Marked interstitial edema and shrinking of acinar cells with loss of zymogen granules. The centroacinar ductules appear wide (effect of acinar atrophy?).

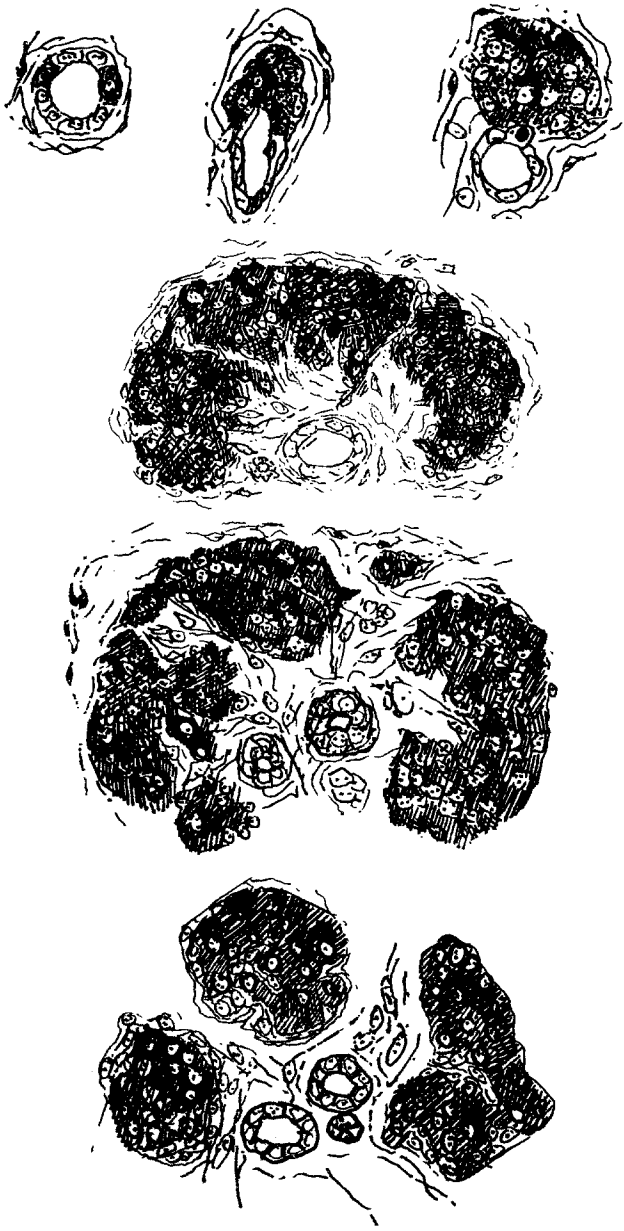

Fig. 4. Schematic drawings of our present concept of the evolution and ultimate fate of the ovoid or kidney-shaped islet bodies. The three top pictures show the occurrence and outbudding of β-cells from ductules (tubular structures) and the two following pictures (in the middle) illustrate the appearance of a kidney-shaped islet body at 4 weeks and 3 months, respectively, after the duct ligation. The bottom figure shows the splitting of the islet body seen 5 months after the duct ligation. Approx. magnifications: $\times 185$

The islets (*i*) are normal. C *Fifth day:* There is now almost complete loss of acinar cells with zymogen granules (transformation to ductules?) with groups of tubular structures (*t.s.*), often intimately associated with small nests of islet parenchyma (*i*). The ducts (*d*) are wide. There is marked interstitial inflammation. D *Seventh day:* Essentially the same picture as at the fifth day, but the close association between the tubular structures (ductuli) and the islet parenchyma (*i*) is more apparent here where the β-cells stand out dark (same signs and symbols as in A—C)

Table 4. *Distribution of 674 islets occurring in the atrophied pancreatic parenchyma obtained 5 and 6 months after duct ligation. The structures were grouped according to their mean diameter into 9 classes with a class interval of 25 μ*

Length of mean diameter (in μ)	Number of islets	Number of islets in per cent of total	Length of mean diameter (in μ)	Number of islets	Number of islets in per cent of total
45.5—70.5	301	44.7	175—200	15	2.2
71—96.5	179	26.6	201—226	5	0.7
97—122	83	12.3	227—252	4	0.6
123—148	55	8.2	253—278	1	0.2
149—174	31	4.6			

or kidney-shaped islet bodies of the atrophied parenchyma obtained 5 weeks after duct ligation (Fig. 4 of our preceding report: EDSTRÖM and FALKMER, 1967) has been included in this histogram. It is obvious from Fig. 5 that the islet distribution curve about $^1/_2$ year after duct ligation essentially coincides with that of the islets of ordinary shape seen 5 weeks after the ligation.

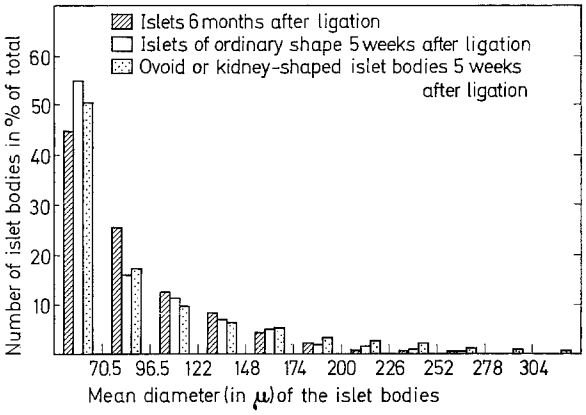

Fig. 5. Percental frequency distribution histogram of the mean diameters of 674 islets in rat pancreas 6 months after ligation compared with those obtained 5 weeks after ligation (395 ovoid or kidney-shaped islet bodies and 385 islets of usual appearance; EDSTRÖM and FALKMER, 1967). The distribution of islets of ordinary shape is obviously essentially the same at both observation times

Lastly, planimetrical determinations of α- and β-cell areas were performed on 92 islets of the serial sections. The results are given in Table 5. Here also, large variations occurred between individual animals. On an average, however, the β/α-ratio was of the same order of magnitude as found in our preceding report, *i.e.* a more marked predominance of β-cells than in normal rat islets.

Microscopic Examination of Other Tissues

Apart from slight fatty degeneration of the liver cells in some rats, no pathologic changes were noted in the kidneys, the liver, and the myocardium at any of the observation times.

Blood Glucose Assays and Urinanalysis

The results of the blood glucose determinations are given in Fig. 6. There were no differences between the values obtained at the two assays of the control animals and the pooled values from both form the basis for what has been considered the normal range in this investigation. Fig. 6 shows that after an initial decrease during the first week the blood glucose level increased progressively to attain moderately hyperglycemic values at the longest time intervals after the duct ligation.

Table 5. *Planimetrically determined relationship between the areas of β- and α-cells in the parenchyma of 92 islets in pancreas 5 and 6 months after ligation of the ducts*

Class No.	No. of islets	β/α ratio
46	12	2.2 ± 0.4
58	12	4.7 ± 0.7
70	17	4.0 ± 0.9
83	13	2.7 ± 0.4
96	12	3.9 ± 0.7
122	15	3.8 ± 0.6
148	6	8.2 ± 0.8
200[a]	5	5.3[b]
		Mean: 4.4 ± 1.8

[a] Pooled classes No. 174 to 226;
[b] Range 2.2—22.3

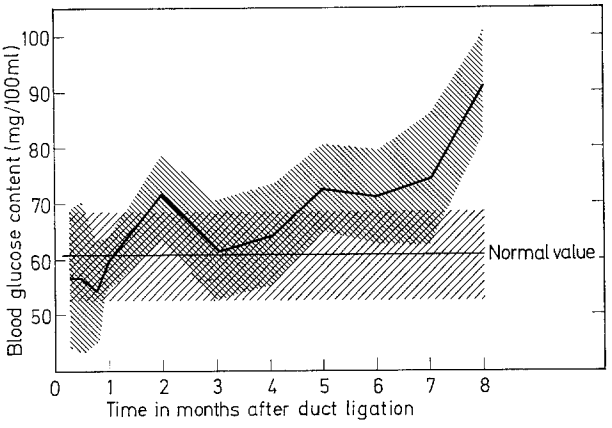

Fig. 6. Mean blood glucose level with its standard deviation (shaded area) of duct ligated rats (upper curve) on various time intervals after operation. The horizontal line with its surrounding shaded area gives the mean value of non-operated control rats and the range of ± the standard deviation, respectively

The results of the urinanalysis were normal at all observation times.

Discussion

It seems from the results obtained in this long-term study as if there would be some truth in our working hypothesis that the ovoid or kidney-shaped islet bodies with their tubular structures really represent morphological signs of islet tissue regeneration (Edström and Falkmer, 1967). Our present results agree with the hypothesis (Benseley, 1911; Vranic, 1965; Bunnag, 1966; Hajdu and Rona, 1967; Zweens and Bouman, 1967) that the islet cells develop from the ductules. Obviously, there was a marked increase in the number of ductules at the end of the first week after the ligation. It was impossible to decide with certainty light-microscopically whether these ductules represented atrophic acinar cells or proliferated ductules. This problem is also met with in human pathology where, *e.g.*, it is much controversy whether or not there is any bile duct proliferation in cirrhosis of the liver. It is well known that there is much support for the hypothesis that there is a real neoformation (*i.e.* a hyperplasia) of ductules in both cases (*cf.* Tasso, 1967; Vranic, 1965).

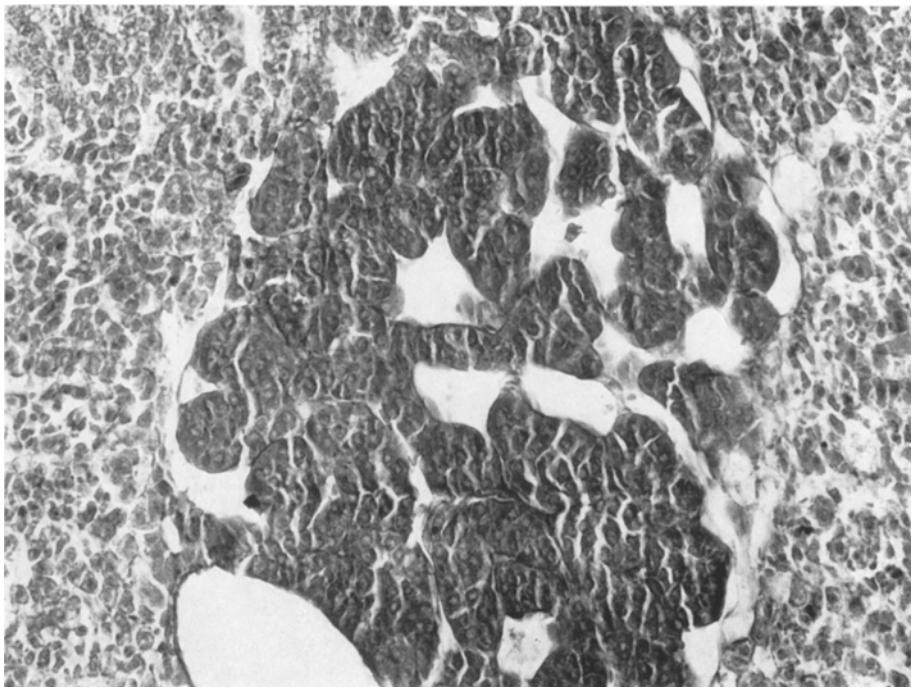

Fig. 7. Large islet surrounded by a hemorrhagic necrosis found in a specimen obtained 2 week after ligation. There is practically no cells left with tinctorial features of β-cells. Bouin's fixative. Chrome-hematoxylin stain. $\times 450$

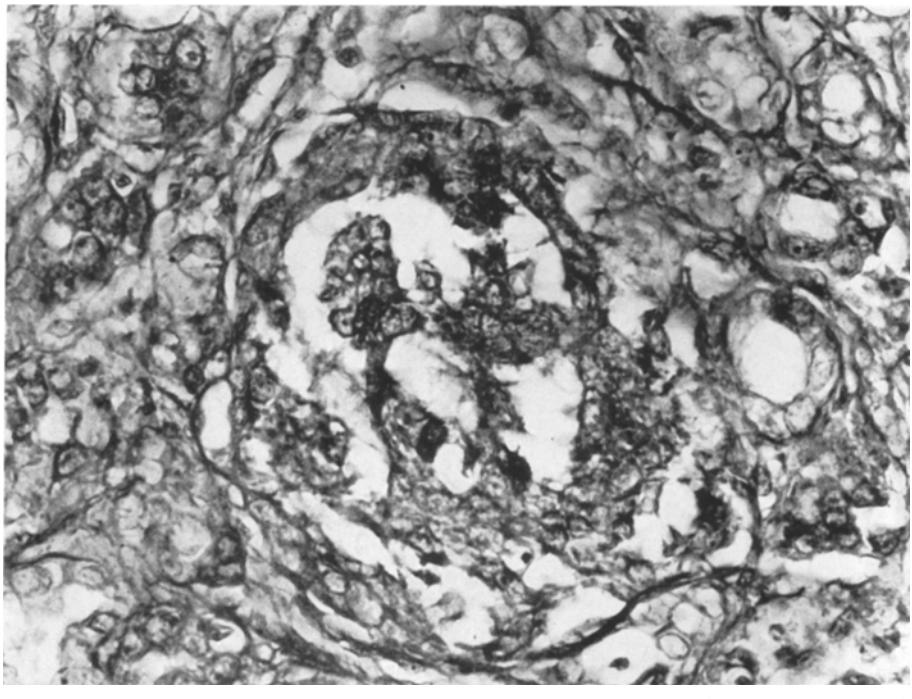

Fig. 8. Edematous splitting of islets in a specimen taken 5 days after duct ligation. Bouin's fixative. Chrome-hematoxylin stain. $\times 450$

Anticipating that a real hyperplasia of the ductules occurs, it is apparent from our results that this supposed hyperplasia precedes the appearance of the ovoid or kidney-shaped islet bodies. The sparsely occurring outbuddings of β-cells from these ductules or tubular structures described previously 5 weeks after duct ligation (EDSTRÖM and FALKMER, 1967) were obvious and quite frequent at 1—3 weeks' observation time (Fig. 4). During the evolution of the ovoid or kidney-shaped islet bodies the supposed ductule hyperplasia successively declined. Later, also the ovoid or kidney-shaped islet bodies disappeared and the final result became the well-known picture of long-standing pancreatic cirrhosis in man, *i.e.* essentially normally shaped islets in fat and connective tissue with some small groups of ductules and remnants of ducts.

It is biologically interesting to note that these ductules did not show any light-microscopical signs of neoformation of acinar cells. The reason for this is unknown. It may, however, be due to the fact that we used "subtotal ligation" in the sense of HULTQUIST and JÖNSSON (1965) which implies that some acinar parenchyma was left between the ligature and the gut, thus still supplying the body with some pancreatic juice. In speculation, it may then be said that the impetus to the ductules to form acinar cells peripheral to an occlusion of the efferent ducts reasonably should be lower than that to form islet cells.

It was light-microscopically difficult to detect any marked and wide-spread damage to the islets although isolated islets were found to be almost completely surrounded by hemorrhages (Fig. 7) and marked edematous splitting of the islet parenchyma frequently was observed (Fig. 8). A supplementary ultrastructural investigation is presently in progress (BOQUIST and EDSTRÖM) in order to obtain more relevant information about the occurrence of degenerative and regenerative changes in the islet cells. It may perhaps also elucidate why the β-cells predominate in the islets of the atrophied parenchyma (JÖNSSON, 1965). So far, we can offer no explanation for this observation although it has recently been shown that the regeneration of α-cells in several aspects differ from that of β-cells (BOQUIST, 1968a and b; HULTQUIST, 1968).

As stated in the results (Table 3; Fig. 2), it is obvious that both the total amount of atrophied parenchyma and the absolute amount of islet tissue undergo a progressive decrease with increasing time intervals after the duct ligation. This may be a morphologic explanation for the observation (Fig. 6) that the fasting blood glucose level gradually increased to attain hyperglycemic values at the longest observation times. Our results thus agree with those of BEST (1934) and LARSSON (1956) and also give some experimental aspects on the underlying mechanisms for the clinical observations of the occurrence of diabetes in pancreatic cirrhosis (*cf.* Introduction). However, it remains to be settled by glucose loadings that the hyperglycemia really is associated with a pre-diabetic state (*cf.* KLIMAS, 1968). It can be speculated that the initial hypoglycemias may be due to release of insulin from damaged β-cells, like the well-known early hypoglycemia that occurs after alloxan administration. The plenty occurrence of aldehydefuchsinophil macrophages also indicates the possibility of some kind of β-cell damage in the early phase of the changes evoked by the duct ligation. An increased release of insulin during the first few weeks may also be an

explanation to the seemingly more rapid body weight increase of duct ligated rats in this period.

The recent report by HAJDU and RONA (1967) gives some aspects both on the hyperglycemia at long observation times and the evolution and fibrosis of the ovoid or kidney-shaped islet bodies. The spontaneous pancreatic islet fibrosis and enlargement as well as the functional disturbance observed in aging rats (HAJDU and RONA, 1967) showed, however, a marked predominance for males. As the present report is based exclusively on female rats, and as our non-operated blood-glucose controls did not show any hyperglycemia, we think it is unlikely

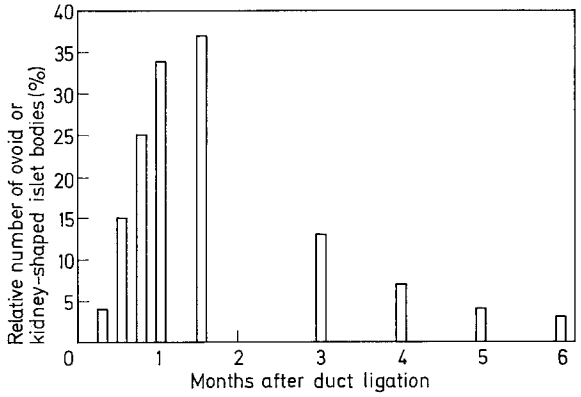

Fig. 9. Number of ovoid or kidney-shaped islet bodies in per cent of total number of islet structures in the atrophied parenchyma on various time intervals after duct ligation

that the rise in the blood glucose level noted by us should be a spontaneous aging phenomenon (KLIMAS, 1968). Whether the ultimate fibrotic splitting of the ovoid or kidney-shaped islet bodies may be related to increase in age of the experimental animals, is impossible to state at the present.

The practical implications of these investigations are that it may be so that the "fetal nature" of the islets of duct ligated pancreas (*cf.* LARSSON, 1956) does not disappear until 4 or 5 months after the operation (Fig. 9). As stated in our preceding report, this implies that the use of islet tissue from duct ligated atrophied pancreas in *in vitro* and *in vivo* studies on the biological properties of the islet parenchyma (KEEN et al., 1965) may be apt to rather give those of a regenerating tissue than of a fully developed one, unless the specimens are taken at least 3 or 4 months after the ligation. Even then, however, the relative amount of islet tissue does not become markedly higher than 1 per cent (Table 3) despite the lack of any acinar regeneration and the progressive decrease of the ductules. Thus, the statement by HULTQUIST (1967) that duct-ligated atrophied pancreatic tissue of rats does not lend itself particularly well to chemical studies of the islet tissue can be confirmed and widened to hold true for all reasonable time intervals after the operation.

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