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The Pancreas in Experimental Hypercholesterolemia in Normal and in Subdiabetic Rabbits* **

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Summary. Light and electron microscopic pancreatic changes were studied in 47 metabolically normal and in 59 subdiabetic rabbits that had received a diet containing 1% cholesterol for periods ranging from two weeks to 12 months. Subdiabetes had been induced by small doses of alloxan alone or by appropriate, sequentially administered injections of cortisone and alloxan. Nine animals served as normal controls. In keeping with the low avidity of rabbit pancreatic tissue for cholesterol as demonstrated biochemically by others (Ho and Taylor, 1968, 1971), the observed pancreatic lesions in normal and in subdiabetic, hypercholesterolemic animals were generally much less severe and less extensive than were alterations in other organs, such as the kidneys and parts of the cardiovascular system, of these same rabbits. Membrane-bound vacuolar inclusions harboring granular, membranous and amorphous material and giving the cytoplasm a foamy appearance were noted in the exocrine pancreatic acinar cells, and similar vacuoles as well as occasional cholesterol clefts were present in several endocrine A and B cells. Atheromatous plaques were much rarer in pancreatic blood vessels than in the arterio-arteriolar systems of the kidney and of the heart, and they were nearly always localized and nonocclusive. Comparatively more histologic lesions were noted in rabbits fed cholesterol for longer periods of time. There were no differences in the incidence of pancreatic alterations between metabolically intact and subdiabetic animals. It is concluded that all tissue components of the rabbit pancreas (exocrine and endocrine parenchyma, interstitium, and blood vessels) display a greater resistance towards the development of morphologically demonstrable lesions in hypercholesterolemia than do most other organs of this animal.

Zusammenfassung. Die Bauchspeicheldrüsen von 47 stoffwechselgesunden und 59 latent diabetischen Kaninchen, die 2 Wochen bis 12 Monate lang eine Diät mit 1%igem Cholesterinzusatz erhalten hatten, wurden licht- und elektronenmikroskopisch untersucht. Der latente Diabetes war durch kleine Alloxandosen oder mit Cortison-Alloxan-Gaben induziert worden. Neun unbehandelte Tiere dienten als Kontrolle. In Übereinstimmung mit der von Ho und Taylor (1968, 1971) biochemisch ermittelten niedrigen Affinität des Kaninchenpankreas für Cholesterin waren die morphologisch faßbaren Veränderungen in der Bauchspeicheldrüse bei den hypercholesterinämischen Kaninchen beider Gruppen erheblich weniger ausgeprägt und stärker umschrieben als etwa die in den Nieren und im kardiovaskulären System derselben Tiere. In den schaumig umgewandelten exokrinen Drüsenzellen fanden sich ultrastrukturell von Membranen umschlossene Vacuolen mit körnigen oder lamellären Strukturen oder mit amorpher Substanz. Einige A- und B-Zellen der Langerhansschen Inseln enthielten ähnliche vacuoläre Einschlüsse und gelegentlich auch Cholesterinnadeln. Atheromatöse Veränderungen waren in den Arterien und Arteriolen des Pankreas deutlich seltener als in der Niere oder im Herzen und zudem stärker umschrieben wie auch fast immer nicht-occlusiv. Mit der Fütterungsdauer nahm die Häufigkeit der Läsionen zu, doch zeigten sich keine quantitativ signifikanten Unterschiede zwischen stoffwechselgesunden und latent diabetischen Tieren. Morpho-

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logisch gesehen unterliegt also das Pankreas des Kaninchens in allen seinen Anteilen (exokrinen Drüsen, Langerhansschen Inseln, Zwischengewebe und Blutgefässen) dem schädlichen Einfluß einer langdauernden Hypercholesterinämie in erheblich geringerem Maße als das für die meisten anderen Organe dieses Tieres gilt.

In previous work, the authors have reported on renal (Wellmann and Volk, 1970a, 1971) and on cardiovascular (Wellmann and Volk, 1970b; Volk and Wellmann, 1971) alterations in metabolically normal and in subdiabetic rabbits fed a cholesterol-enriched diet for periods ranging from two weeks to a full year. Since no systematic investigation of histologic changes in the pancreas has yet been recorded in experimental hypercholesterolemia, it was decided to utilize the pancreases of these same groups of animals for such a study. Only alterations related to, and presumably caused by, the hypercholesterolemic state will be discussed; pancreatic islet cell changes attributable to induced subdiabetes have already been described elsewhere (Lazarus and Volk, 1964; Volk, Lazarus, and Wellmann, 1965; Volk, Wellmann, and Lazarus, 1965; Volk, Wellmann, Lazarus, and Brancato, 1969; Wellmann, Brancato, Lazarus, and Volk, 1967; Wellmann, Volk, and Lazarus, 1967).

Material and Methods

A total of 115 adult white New Zealand rabbits (99 of them males) were utilized for this study. They were divided into four groups, according to the Table. Listed in the table are the number of rabbits in each group, their metabolic states, their diets, and their sacrifice schedules.

Subdiabetes was induced by pretreatment with appropriate doses of cortisone followed by a large dose of alloxan (Lazarus and Volk, 1964; Volk, Lazarus and Wellmann, 1965; Volk, Wellmann and Lazarus, 1965; Volk, Wellmann, Lazarus, and Brancato, 1969; Wellman, Brancato, Lazarus, and Volk, 1967) or with small doses of alloxan alone (Wellmann, Brancato, Lazarus, and Volk, 1967; Wellmann, Volk, and Lazarus, 1967). Procedural details and rationales of these methods may be found in our previous publications (Lazarus and Volk, 1964; Volk, Lazarus, and Wellmann, 1965; Volk, Wellmann and Lazarus, 1965; Volk, Wellmann, Lazarus, and Brancato, 1969; Wellmann, Brancato, Lazarus, and Volk, 1967; Wellmann, Volk, and Lazarus, 1967). The induced subdiabetic state is characterized by persistent normoglycemia, under ordinary conditions, which changes to hyperglycemia (of at least 190 mg%) following repeated, usually ineffective (nondiabetogenic) daily injections of small cortisone doses. All rabbits of Groups II and III were subdiabetic in the defined sense.

The diet for animals of Groups I, II and III consisted of Purina Rabbit chow containing 1% cholesterol. Since the addition of 2% corn oil in some of these rabbits failed to influence the results (Wellmann and Volk, 1970a, b), this practice was soon discontinued. Rabbits participating in the experiment for more than four months were fed cholesterol-containing food for periods of two months alternating with two-month periods in which no cholesterol was added to the diet. This interrupted feeding schedule has been shown to reduce mortality in long-term studies and permits the development of advanced vascular lesions (Constantinides, 1965). In addition to their solid diets, all rabbits received water with 5% glucose ad libitum.

The blood levels of glucose (Nelson, 1944), total cholesterol and cholesterol esters (Chiamory and Henri, 1954), phospholipids (Hawk, Oser and Summerson, 1954), nonesterified fatty acids (Duncombe, 1964), total lipids (Van Slyke and Plazin, 1965), creatinine (Owen, Iggo, Scandrett, and Stewart, 1954) and blood urea nitrogen (Hycel, 1964) were determined in all animals before, as well as at approximately bi-weekly intervals after, the initiation of cholesterol feeding. The daily as well as the total food intake of all rabbits was measured and tabulated.

Table. *Synopsis of experimental design indicating metabolic state, diet and sacrifice schedule of all rabbits*

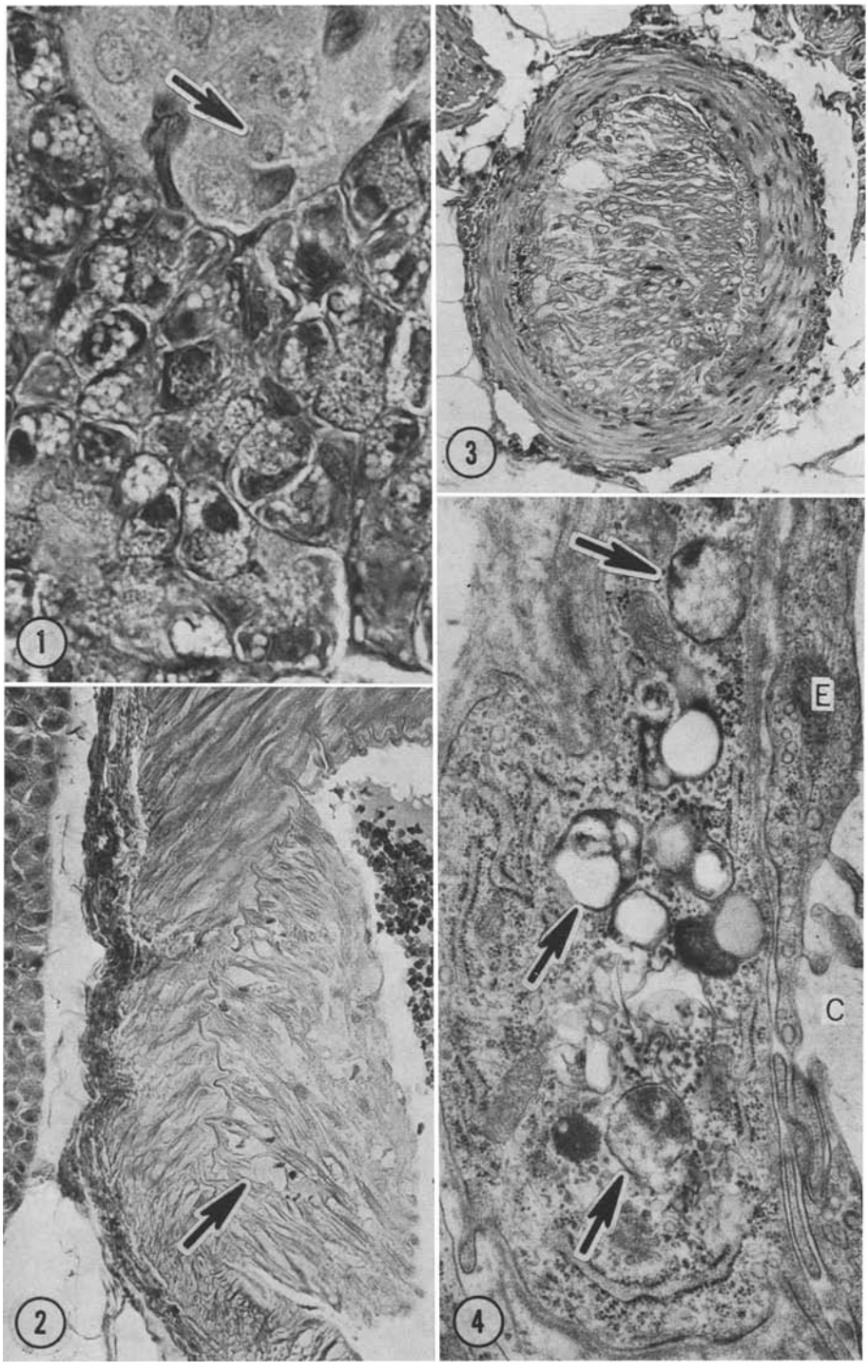
Group	Number of rabbits	Metabolic state	Diet	Number of rabbits killed											
				Weeks			Months								
				2	4	6	2	3	4	6	7	8	9	10	12
I	47	Normal	Cholesterol	4	4	4	4	4	4	5	4	4	4	4	2
II	32	Subdiabetic (alloxan alone)	Cholesterol	4	4	4	4	2	4	1	1	2	2	2	2
III	27	Subdiabetic (cortisone and alloxan)	Cholesterol	4	4	4	4	—	4	1	2	1	1	1	1
IV	9	Normal	Normal						9						

All animals were killed by the intravenous injection of an overdose of Nembutal. Diced blocks, measuring less than 1 mm in greatest diameter, of various organs, including the pancreases, were fixed for two hours in icecold 1% osmic acid solution containing 20.83% acetate-Veronal buffer, as well as 4.5% sucrose (Caulfield, 1957), adjusted to a pH of 7.4, and embedded with Epon 812 (Luft, 1961). Ultrathin sections were then cut with glass knives on a Porter-Blum microtome, stained with lead citrate and uranyl acetate (Reynolds, 1963), and viewed and photographed with an RCA EMU-3G electron microscope. The remaining pancreatic tissue was fixed in Zenker-formol solution. Paraffin-embedded sections were cut and stained with hematoxylin and eosin, PAS-trichrome, and a modified aldehyde-fuchsin trichrome stain as previously described (Lazarus and Volk, 1962).

Results

Biochemistry. The blood glucose, urea nitrogen and creatinine levels of all rabbits generally stayed within their normal ranges during the entire period of the experiment. All blood lipid fractions rose rapidly after the initiation of cholesterol feeding and remained abnormally elevated for as long as the cholesterol-enriched diet was supplied. Both subdiabetic groups (Groups II and III) showed considerably higher average levels of cholesterol, phospholipids and total lipids, even before cholesterol was added to the diet, and for the first few months of cholesterol feeding their average figures stayed in a higher range than those of the metabolically intact animals. During the later stages of cholesterol administration, these initially distinct differences in the blood lipid levels between the metabolically normal rabbits of Group I and the subdiabetic animals of Groups II and III tended to disappear. A more detailed account of the blood lipid changes in these rabbits has been presented elsewhere (Wellmann and Volk, 1970a, 1971).

Light Microscopy. The islets of Langerhans did not show detectable light microscopic alterations in any of the 106 cholesterol-fed rabbits apart from changes attributable to subdiabetes, such as B cell degranulation (Fig. 1), which have been described elsewhere (Lazarus and Volk, 1964; Volk, Lazarus, and Wellmann, 1965; Volk, Wellmann, and Lazarus, 1965; Volk, Wellmann, Lazarus, and Brancato, 1969; Wellmann, Brancato, Lazarus, and Volk, 1967; Wellmann, Volk and Lazarus, 1967). Not infrequently, small or large groups of exocrine pancreatic



Figs. 1–4

acinar cells were characterized by a finely foamy quality of their cytoplasm. This change was barely noticeable in many cells, but in others it was quite unequivocal (Fig. 1). In 15 rabbits, including six of 66 (9%) fed cholesterol for up to four months and nine of 40 (23%) on the cholesterol diet for more than six months, cytoplasmic vacuolization was extensive and distinct. Among these 15 animals there were nine subdiabetic ones. One the other hand, interstitial foam cells, as readily found in heart and kidneys (Volk and Wellmann, 1971; Wellmann and Volk, 1970a, b, 1971) were not identified by light microscopy in any of these pancreases. The pancreatic ducts were unremarkable.

In the majority of cases, no arteriosclerotic lesions were noted. Atheromatous plaques in large and medium-sized arteries (Fig. 2) were found in only seven of 66 (11%) rabbits before the end of the fourth month and in 11 of 40 (28%) animals after at least six months of cholesterol feeding. Of these 18 rabbits with vascular involvement, 10 were subdiabetic. Almost invariably, the observed arteriosclerotic plaques were flat and circumscribed. Occlusive lesions or participation of very small arteries and of arterioles in the atheromatous process were encountered only very occasionally (Fig. 3).

Electron Microscopy. In only two of the 106 cholesterol-fed rabbits could interstitial foam cells be identified by electron microscopy. In each of them, a single such cell was noted in the exocrine pancreas in the immediate vicinity of a capillary (Fig. 4).

Many exocrine pancreatic acinar cells were seen to contain occasional membrane-enclosed cytoplasmic lesions. In several cases—most of them the same that had revealed foamy exocrine cells by light microscopy—the cytoplasm was riddled with such vacuolar inclusions which harbored granular, lamellar or amorphous material of varying electron density (Figs. 5 and 6). No ultrastructural alterations were noted in the pancreatic duct cells.

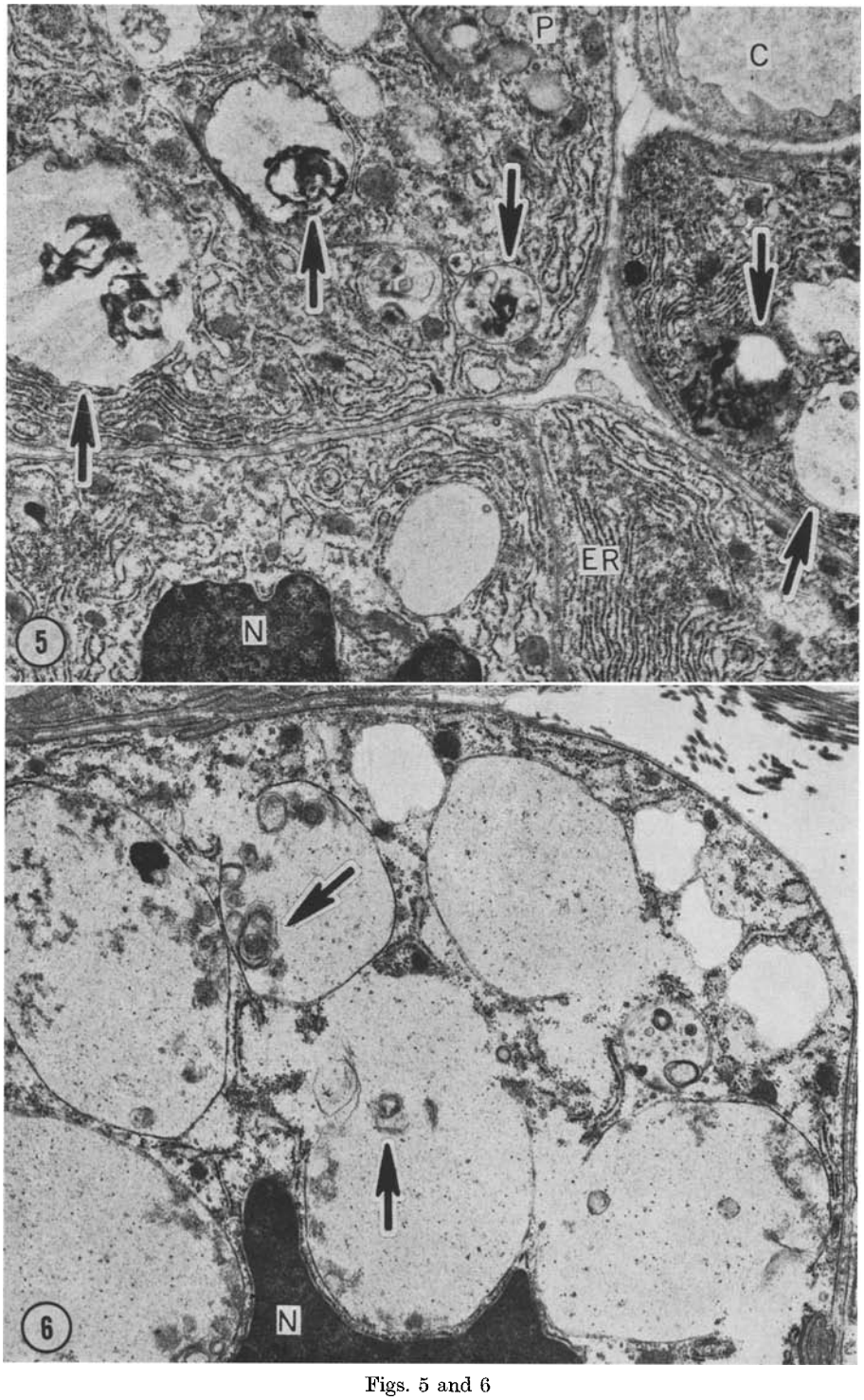
The islets of Langerhans revealed few electron microscopic changes other than those attributable to subdiabetes which have been discussed elsewhere (Lazarus and Volk, 1964; Volk, Lazarus and Wellmann, 1965; Volk, Wellmann and Lazarus, 1965; Volk, Wellmann, Lazarus, and Brancato, 1969; Wellmann, Brancato, Lazarus, and Volk, 1967; Wellmann, Volk, and Lazarus, 1967). Four cases with interstitial (Fig. 7) or intracytoplasmic (Fig. 8) cholesterol clefts were encountered.

Fig. 1. Pancreas of subdiabetic rabbit of Group II fed cholesterol for 3 months. The acinar epithelial cells contain numerous small vacuoles in their cytoplasm. The B cells (arrow) of the islet of Langerhans display degranulation attributable to subdiabetes. PAS-trichrome stain. ×960

Fig. 2. Portion of large pancreatic artery branch of normal rabbit of Group I fed cholesterol for 8 months. A fibrotic intimal plaque containing a few foam cells (arrow) is present. Hematoxylin and eosin. ×280

Fig. 3. Small pancreatic artery with occlusive intimal plaque. Subdiabetic rabbit of Group III fed cholesterol for 10 months. Hematoxylin and eosin. ×190

Fig. 4. Electron micrograph of a pericapillary cell from a metabolically normal rabbit of Group I fed cholesterol for 4 weeks. The cytoplasm contains membrane-enclosed vacuolus which appear either empty or harbor granular or amorphous material (arrows). *C* capillary; *E* endothelial cell. ×18700



Figs. 5 and 6

In several A and especially B cells of three rabbits, membrane-bound cytoplasmic vacuoles were noted. Most of these appeared empty, but some contained electron-dense material and small crystalloid structures (Fig. 9).

Discussion

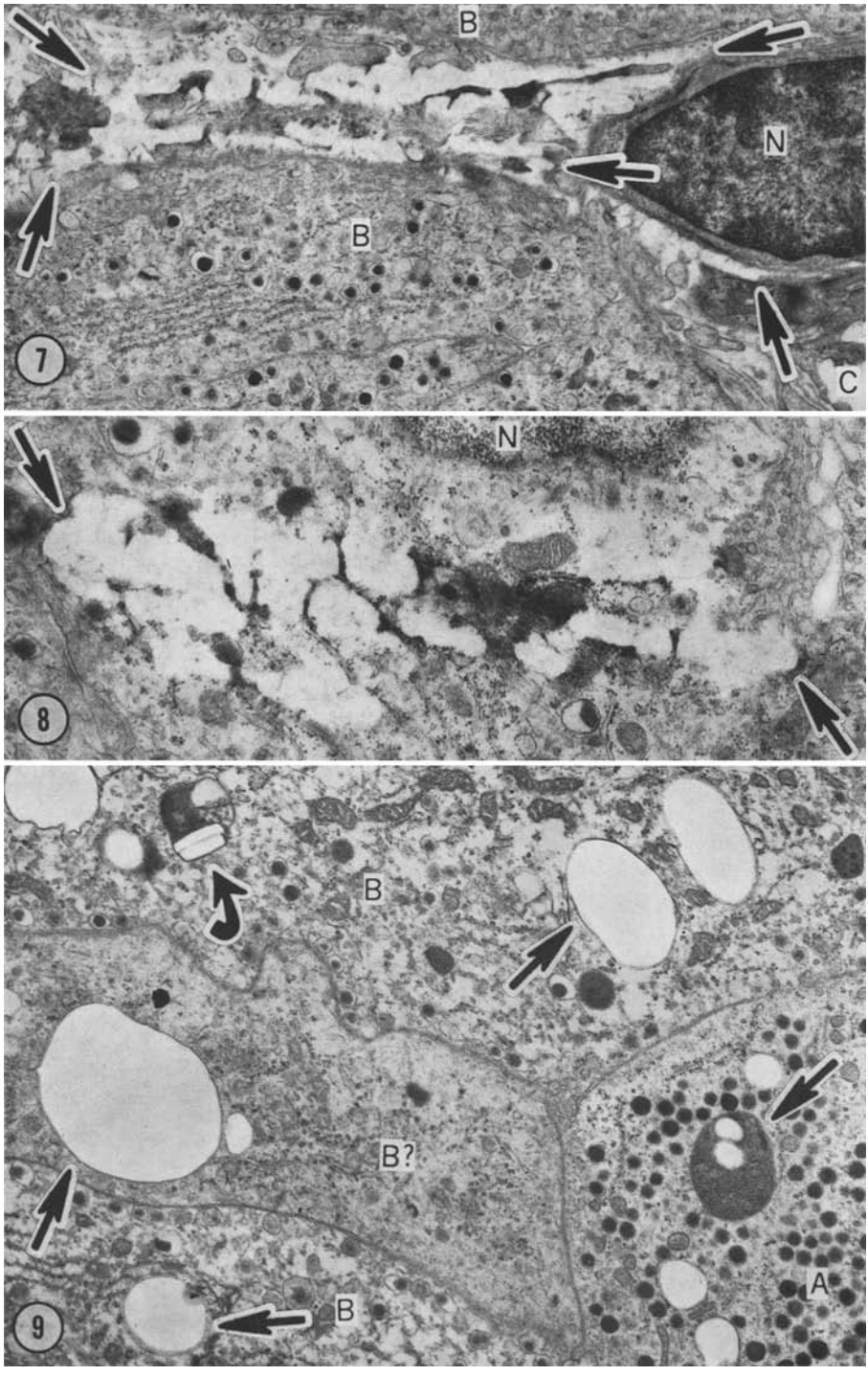
Rabbits apparently lack such homeostatic mechanisms as limitation of absorption, suppression of synthesis, and acceleration of excretion of sterols (Cook, Kliman, and Fisser, 1954; Ho and Taylor, 1968, 1971), and their only means of coping with a great extraneous influx of cholesterol is to reversibly deposit the excess material in their tissues. However, not all tissues can store cholesterol to the same degree. In comparative studies on tissue cholesterol content, Ho and Taylor (1968, 1971) exposed the relatively low avidity of the rabbit pancreas for cholesterol even when this substance is offered in large quantities and over a prolonged period of time. These authors found that of all the major organs and tissues of that species only the brain and the pancreas failed to increase their cholesterol content to a significant degree after three months of exposure to a diet containing 2% cholesterol (Ho and Taylor, 1968). However, continuous feeding, for nine months, of the cholesterol-enriched diet resulted in a threefold elevation of the pancreatic cholesterol content; nevertheless, even this degree of augmentation amounted to only a fraction of the total increase observed in most other tissues of this animal. Conversely, cholesterol loss from the pancreas after the cessation of cholesterol feeding was found to proceed at a somewhat faster rate than was true for many other organs.

The present study confirms, on morphologic grounds, the biochemical data as presented by Ho and Taylor (1968, 1971) for the rabbit pancreas. In contrast to the conditions in other organs of which we have investigated the kidneys (Wellmann and Volk, 1970a, 1971) and parts of the cardiovascular system (Volk and Wellmann, 1971; Wellmann and Volk, 1970b), the pancreatic alterations attributable to induced hypercholesterolemia were characterized both by their relative paucity and by their limited extent. Whereas the great majority of the kidneys, the hearts, the coronary artery branches and the aortas of these same rabbits contained lesions demonstrable by light and electron microscopy, especially after prolonged cholesterol feeding, only 18 of 106 hypercholesterolemic animals displayed unequivocal pancreatic arterial atheromatous plaques most of which were focal and nonocclusive at that.

By comparing the histologic lesions from different sites in these rabbits it has also become obvious that the mode of cholesterol storage differs markedly from

Fig. 5. Portions of several exocrine pancreatic epithelial cells from a subdiabetic animal of Group II fed cholesterol for 2 months. There are many membrane-bound cytoplasmic inclusions containing lamellar, vesicular or granular material of varying electron density (arrows). *C* capillary; *ER* endoplasmic reticulum; *N* nucleus; *P* prozymogen granules. ×11200

Fig. 6. Portion of another exocrine pancreatic epithelial cell, from a subdiabetic animal of Group III fed cholesterol for 4 months. Much of the cytoplasm is occupied by large membrane-bound vesicles filled with granular material of low electron density and a few lamellar structures (arrows). *N* nucleus. ×11200



Figs. 7–9

one organ to the next, a fact that cannot be deduced from tissue cholesterol studies alone. Thus, in the kidneys (Wellmann and Volk, 1970a, 1971) huge quantities of cholesterol are sequestered in grossly visible interstitial foam cell plaques located below the cortico-medullary junction. Renal parenchymal and arterio-arteriolar involvement are present but are not quite as impressive. In the heart (Volk and and Wellmann, 1971; Wellmann and Volk, 1970b), vascular plaques—often large or occlusive and sometimes associated with ischemic myocardial lesions—predominate by far; parenchymal lipid vacuoles are fairly frequent but interstitial foam cell nests are always small and rather rare in this organ. In the pancreas, on the other hand, interstitial foam cell accumulation is virtually nonexistent, and vascular involvement is minimal in most cases. The greater part of whatever cholesterol does get stored in the pancreas must therefore be lodged in the parenchymal cells. This appears, indeed, to be the case; yet in only a minority of animals in our series was lipid storage extensive enough to induce many parenchymal vacuolar lesions detectable by light and electron microscopy.

The morphologic features of the observed pancreatic acinar cell inclusions differed from those seen in renal (Wellmann and Volk, 1970a, 1971) and cardiovascular (Volk and Wellmann, 1971; Wellmann and Volk, 1970b) alterations of these same rabbits mainly by containing fewer electron-dense lamellar and other large particulate components. Undoubtedly, these structural differences reflect hydrolysis and recombination of the stored lipids in a manner peculiar to the individual organ or tissue. That breakdown, resynthesis and modifications of lipids derived from the circulating blood do occur in various tissues of the rabbit (and of other mammals, including man) has been amply documented (Baes, van Gent and Pries, 1968; Büttner, 1966; Day, Fidge, Gouldhurst and Wilkinson, 1965; Day, Gould-Hurst and Wilkinson, 1964; Jepson, Billimoria, and MacLagen, 1965; Newman and Zilversmit, 1959; Stüttgen and Vogelberg, 1965; Wilson, 1963). There is thus no reason to doubt that similar processes also take place in the exocrine pancreas which is, furthermore, richly endowed with enzymatic tools.

In addition to membrane-bound lesions, occasional elongate acicular structures, so-called "cholesterol clefts", were encountered in the cytoplasm of some of the pancreatic islet cells, as mentioned. The fact that none of these were surrounded by a limiting membrane can only mean that their phagocytosis preceded crystallization inasmuch as a crystalloid body phagocytized as such should be invested with a membrane. The possibility that crystallization occurred only during the fixation process cannot be excluded.

Fig. 7. Cholesterol clefts (arrows) in the pericapillary interstitium between two pancreatic B cells (*B*) of a metabolically normal rabbit of Group I fed cholesterol for 2 weeks. *C* capillary; *N* nucleus. ×8500

Fig. 8. Cholesterol clefts (arrows) in cytoplasm of pancreatic B cell of same animal as in Fig. 7. *N* nucleus. ×14000

Fig. 9. Several B cells (*B*) and one A cell (*A*) of metabolically normal rabbit of Group I fed cholesterol for 7 months. All cells contain membrane-bound vacuoles (straight arrows) some of which harbor ill-defined electron-dense material. Two rectangular crystalloid structures are seen in one of the vacuoles (curved arrow). ×10500

Comparatively more histologic lesions were seen in rabbits fed cholesterol for six months and longer than in those on the cholesterol-enriched diet for four months and less. This is in keeping with the biochemical data of Ho and Taylor (1968, 1971) and parallels our observations in other organs of these same rabbits (Volk and Wellmann, 1971; Wellmann and Volk, 1970a, b, 1971). In contrast to the conditions found in these other organs, however, there were no apparent differences in the incidence or severity of pancreatic alterations—exocrine, endocrine, and vascular—between the metabolically intact rabbits and the animals with induced subdiabetes.

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