immune sera can be regarded as most suitable for the study of myosin. They have the great advantage over antisera against animal myosin usually used. The necessity for and value of thorough and extensive immunomorphological analysis of the specificity of the antisera which was undertaken must be particularly emphasized. The highly sensitive immunofluorescence method enabled ballast antibodies to be found in the immune sera despite their apparent purity as shown by precipitation in agarose. The crossed absorption of the antisera proved to be a sufficiently effective method of increasing their specificity. The impurities causing the formation of these ballast antibodies can be interpreted differently. In our view, preparations of skeletal muscle myosin contain C-protein. It may have been this which caused the formation of ballast antibodies and the crossed reaction with smooth muscles. The possibility likewise cannot be ruled out that myosin of vascular smooth muscles may have been responsible for this crossed reaction, for this is invariably present, even if in small quantities, in preparations of skeletal muscle myosin. As regards the antisera against smooth muscle myosin, C-protein or protein kinase were evidently the impurities against which ballast antibodies were formed. The problem of a true crossed reaction with myosin as a result of common antigenic determinants in striated and smooth muscle myosin likewise must be regarded as still unsolved.

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HEPATOCYTE ULTRASTRUCTURE DURING ACCUMULATION AND SECRETION OF BILE PRODUCTS

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KEY WORDS: hepatocyte ultrastructure; bile secretion; Golgi complex; clasmatosis.

The fine morphology of the hepatocyte during synthesis and secretion of bile products has not been adequately studied [1, 2, 13-15]. The object of this investigation was to study the dynamics of changes in the ultrastructure of hepatocyte organelles during the accumulation and secretion of bile products. The liver of the chick embryos is a convenient object for this purpose, for its mode of nutrition changes in the course of its development: until the 8th day of incubation an extraintestinal mode of nutrition with protein and fat of the yolk, and from the 9th through the 13th days of incubation, in addition to extraintestinal assimilation of yolk, an intestinal mode of nutrition connected with swallowing of amniotic fluid by the prefetus [4, 5]. It must be expected that the process of bile formation and bile secretion in the liver of the chick embryo begins early, and that the ultrastructure of hepatocytes can thus be studied during the period of formation of these functions.

EXPERIMENTAL METHOD

Hepatocyte ultrastructure was studied in chick embryos from the 6th through the 13th days of incubation. Pieces of liver were fixed daily in Palade's fixing solution at pH 7.2-7.4 and embedded in Araldite. Ultrathin sections were stained by Reynolds' method and studied in the EM-7A electron microscope.

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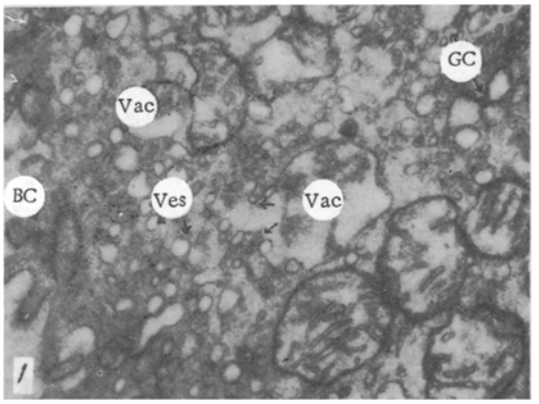


Fig. 1

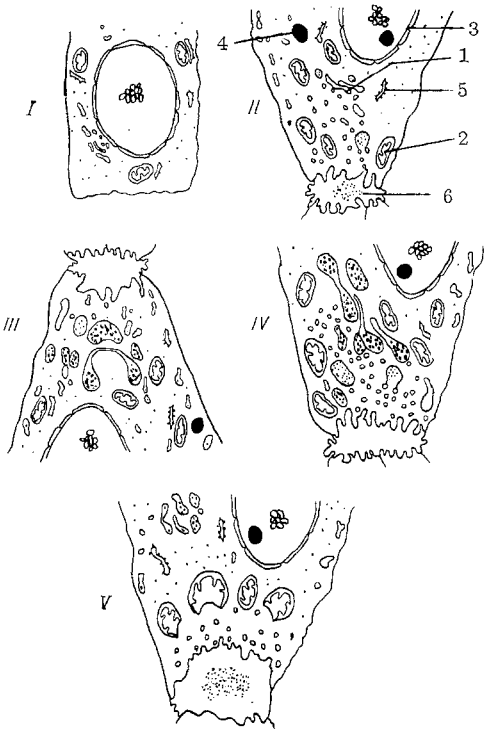


Fig. 2

Fig. 1. Hepatocyte of chick embryo on 7th day of incubation. Golgi complex (GC) lies at a distance from bile capillary (BC), around which are concentrated many small vesicles (VES) and vacuoles (VAC), from which vesicles also are being pinched off (arrow). 31,500×.

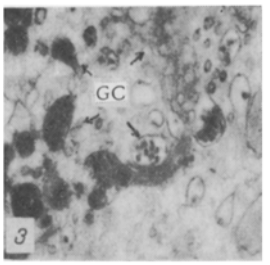
Fig. 2. Dynamics of changes in ultrastructure of biliary pole of chick embryonic hepatocytes at 6th-10th days of incubation. I-V) 6th, 7th, 8th, 9th, and 10th days of incubation respectively. 1) Golgi complex, 2) mitochondria, 3) nucleus, 4) fat, 5) RER, 6) bile capillary.

EXPERIMENTAL RESULTS

On the 6th day of incubation bile capillaries begin to be formed in the liver of chick embryos and on the 7th day the tubular structure of the liver is already formed. At all times of the investigation no significant changes could be found in the ultrastructure of the nuclei, in which droplets of fat could be nearly always seen. The looped nucleoli were large until the 11th day of incubation, after which they decreased in size appreciably, but on the 12th day they grew larger again. In the cytoplasm of the hepatocytes free ribosomes were numerous from the 6th until the 12th days of incubation, whereas the number of small cisterns of the rough endoplasmic reticulum (RER), with ribosomes unevenly distributed on them, was small until the 12th day of incubation. Lipid drops could be seen in the cytoplasm at all times of investigation. Glycogen was present in the cytoplasm of the hepatocytes after the 6th day of incubation, it disappeared completely on the 11th day, and reappeared on the 13th day. Vesicular profiles of the smooth endoplasmic reticulum (SER) with particles resembling lipids of very low electron density (VLDL) [12] were very numerous at all times of the investigation between the sinusoidal and biliary poles. This abundance of vesicular profiles of SER in the early embryogenesis of the chick and later can be explained as follows. By the 7th day all the blood which flows from the yolk sac, saturated with nutrients [7], is known to pass through the liver [5]. As a result, in early embryogenesis the materials are available for synthesis of bile products. The components of bile and, in particular, its lipid complex, are formed in the hepatocytes from products brought in with the blood [6]. An active part is played in this process by the SER profiles, through which products entering from the blood are transported around the cell, and in which cholesterol and bile acids also are synthesized [8-11].

On the 6th day of incubation the Golgi complex lies in the immediate vicinity of the nucleus, so that its genesis from the outer nuclear membrane can be postulated. Later during the investigation the main changes are found in the arrangement and ultrastructure of the Golgi complex and mitochondria.

On the 7th day of incubation the Golgi complex was appreciably larger than at the previous stage because of the accumulation of finely granular and floccular material in the terminal expansions of its cisterns and in



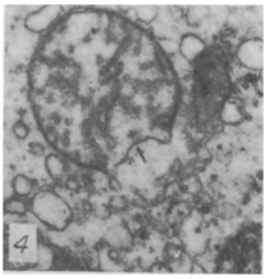


Fig. 3 Fig. 4

Fig. 3. Increase in size of Golgi complex (GC) in hepatocytes of chick embryo on 8th day of incubation. 33,000×.

Fig. 4. Disturbance of integrity of outer membrane of mitochondrion (arrow) as a result of clasmatosis of fragment of mitochondrion in cytoplasm of hepatocyte on 10th day of incubation.

the vacuoles, whereas the number of flat cisterns was small. In some hepatocytes on the 7th day of incubation the changes observed in the Golgi complex and mitochondria was such that it could be deduced that secretion of bile products into the bile capillary begins at this time. The Golgi complex was at a distance from the bile capillary. The region around the cisterns of the Golgi complex and the whole space between it and the bile capillary were occupied by many tiny vesicles and large vacuoles with floccular contents, from which similar small vesicles also were being pinched off (Figs. 1 and 2). They formed tracks in the direction of the bile capillary membrane, and some merged with it, as a result of which the outline of the bile capillary membrane had deep invaginations. Among the numerous vesicles between the cisterns of the Golgi complex and the bile capillary mitochondria with a translucent matrix could be seen.

Subsequent changes in the ultrastructure of the Golgi complex consisted of a sharp increase in its size on the 8th day of incubation (Fig. 3), probably as a result of intensive accumulation of synthesized products. The number and size of the dictyosomes and also of the terminal expansions of their cisterns and of the large vacuoles containing many particles of VLDL type, also were increased. Furthermore, large vacuoles with floccular contents were distributed in the zone of the Golgi complex. The Golgi complex was fairly close to the bile capillary. Compared with the previous time the matrix of the mitochondria was denser. The bile capillaries were dilated and floccular material could often be seen in them.

On the 9th day of incubation intensive secretion of bile products into the bile capillary began and continued also on the 10th day. The dimensions of the cisterns of the Golgi complex were reduced on the 9th day of incubation, and large vacuoles with particles of VLDL type, vacuoles with finely granular and floccular contents, and numerous vesicles with electron-optically pale contents were found around the dilated bile capillaries, between them and the Golgi complex, and often they could be seen to fuse with the membrane of the bile capillary. Floccular material and many fragments of microvilli, evidently resulting from clasmatosis during secretion of the products into the bile capillary, could be seen in the dilated bile capillaries. The mitochondria were polymorphic, numerous, with a comparatively dense matrix and many cristae, far more than mitochondria on the 8th day of incubation.

On the 10th day the Golgi complex was smaller than on the 9th day. Characteristically the Golgi complex was located at a considerable distance from the bile capillary, and in its immediate vicinity there were numerous vesicles, separate vacuoles with floccular contents and, beyond, mitochondria. The distinguishing feature of this period was swelling of the mitochondria, considerable translucency of the matrix, and disorientation of the cristae; in many mitochondria the integrity of the outer membrane was disturbed for some distance and clasmatosis of individual fragments of mitochondria was taking place (Fig. 4) [3]. The bile capillaries were sharply dilated and their lumen contained floccular material, electron-dense small granules, and myelin-like figures. Microvilli were almost absent in these bile capillaries and fragments of them could be seen in their lumen, probably as a result of clasmatosis of part of the hepatocyte during intensive secretion of bile products.

On the 11th-13th day of incubation the size of the Golgi complex and its location relative to the bile capillary differed in different hepatocytes, evidence of absence of synchronization of the subsequent function of the hepatocytes. The normal density of the matrix was restored in the mitochondria and the number of cristae was increased.

The investigation thus showed that synthesis and secretion of bile products in individual cells begin on the 7th day of incubation and on the 9th-10th days most cells participate in this process. An important role in the synthesis, accumulation, and secretion of bile products is played by the numerous SER profiles, the Golgi complex, and the mitochondria. The considerable increase in size of the Golgi complex, its location near the bile capillary, and the concentration of material of varied density in its cisterns and vacuoles are evidence that bile products synthesized by the hepatocyte accumulate gradually in the Golgi complex and are probably transformed. The "dissemination" of the cisterns and vacuoles of the Golgi complex in numerous vesicles approaching the membranes of the bile capillaries and merging with them is evidence of the mode of secretion of the bile products into the bile capillary. The close topographical contacts between mitochondria and secretory vesicles, translucency of the matrix of the mitochondria, and clasmatosis of their fragments indicate an active role of the mitochondria in this process as sources of energy. The fact that the RER is poorly developed from the 6th through the 10th days of incubation is evidence, in the writer's view, that protein synthesis for export in the hepatocytes was negligible during this period, possibly because of the excess supply of ready-made protein products from the yolk sac.

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