PATHOGENESIS OF RETICULAR NECROSIS OF THE LIVER IN EXPERIMENTAL OBSTRUCTIVE JAUNDICE

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In the experimental-pathological studies on the occurrence of jaundice, Ohno and Hieda have observed the characteristic appearance of peculiar reticular necrosis in the liver of which the common bile-duct was ligated in rabbit or guinea pig. By means of serial sections, they have demonstrated that the necrotic lesion was due to a chemical activity of the bile leaking out of the duct which was broken down at the ampulla where the interlobular bile-duct within the sheath of Glisson connects to the bile capillaries. They maintained that the destruction of the ampulla has a great significance in the causation of jaundice due to reabsorption of leaked bile which is accepted as a basis for monistic theory of jaundice proposed by Ohno. Since then, a number of studies have been undertaken on the causes of this particular organ, some attributing it to bacterial infection, others ascerting that it was mechanical injury due to pressure within the bile-duct or that it was a result of an accompanying secondary disturbance in the blood vessels. Thus, there has so far been no conclusive theory established on this point.

Knowing the fact that the histological picture of the liver is peculiar, and this part of the structure is never accompanied with the formation of a demarcation line or the granulation tissue growth, which can be recovered within a short period of time without leaving a scar, and that, while it is frequently found in rabbits, but never seen in dogs. The present experiment was carried out to ascertain its pathogenesis. The result which has clarified certain points will be reported with an interest.

Chap. I HISTOROGICAL AND HISTOCHEMICAL OBSERVATION

EXPERIMENTAL METHODS

For experimental animals, 12 healthy rabbits roughly 2 Kg. in body weight were used.

First, the common bile-duct was blocked to cause the obstructive jaundice. At the intervals of 6, 12, 48 and 96 hours after operation, a group of three rabbits were killed by air embolism, and examination was conducted of liver lesion with the elapse of time. The surgery was conducted as far aseptic as possible with special attention paid to bleeding.

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HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION METHODS

For fixation, 10 percent neutral formalin solution, Carnoy's solution and Zencker-formol solution were principally used, and, especially for the fixation of the bile secretions in liver cells, Forsgren's barium-chloride-formalin fixation solution was used. After fixation, liver tissue sections, each according to their respective formula, were made into paraffin sections and freezing sections, which were respectively subjected for examination according to the following methods of staining:

1. Haematoxylin-Eosin double stain (H. E.)
2. Heidenhain's iron-alum Haematoxylin stain.
3. Van Gieson stain for collagen fiber.
4. Bielscheowsky's silver stain.
5. Sudan III's fat stain.
6. Periodic acid Schiff's reagent stains for polysaccharide (PAS reaction).
7. Forsgren's bile acids stain.
8. Unna-Pappenheim's pylonin-methylgreen stain.
9. Thionin stain for nucleic acid.
10. Feulgen reaction for high molecular, low molecular DNA.
11. Gomori's phosphatase reaction.

EXPERIMENTAL RESULTS

General State and Autopsy Findings: From the third day after operation, the rabbits showed inactivity of movement as well as decrease of appetite, excretion soft grey-colored feces and yellow-brownish urine. The icterus first appeared slightly on the ear lobes from about 24 hours afterward, then, became more pronounced around the eye lide conjunctiva, later involved all the organs. Necropsy revealed marked swelling of the common bile duct in all cases, while the gall-bladder also became strikingly swollen presented dark brownish green. Six hours after operation, the liver, both in size and hardness, showed no major change, the surface presented dark brown color, with white-yellowish millet-size patches both on the surface and cut surface. Twelve hours later, the liver capsul was slightly strained throughout with smooth surface and colored yellowish-green to yellowish-white or yellowish-green millet-sized or rice-sized disseminated, at times, patches partly speckled.

Histological and Histochemical Findings: With H. E. stain specimens, lesions of reticular necrosis which showed a characteristic histological picture was striking. At first, round-shaped necrotic process was noted throughout the lobules, followed by many semicircular lesions, not only in the periphery of the lobules but also in the central part of lobules. Many were seen to be V-shaped, mostly in the periphery of the lobules or midzonal regions. At times an extremely large necrosis extending to the central part of lobules was seen. These necrotic lesions were consisted mainly with destroyed liver cells with markedly reduced pigments, cytoplasm and nucleus

unaffected. The typical one had retained only the cell wall presented a peculiar reticular formation. Again, the focus was swollen more or less, and pressing the neighboring liver cells, showing definite demarcation. One or two cases showed the boundaries composed of normal liver cells in which their cytoplasm and nuclei stain ed deeply. They were pressed and shrunk showing shapes of either oblong or stellar or V-shaped. There were comparatively larger cells which contained two nuclei.

While the stellate cells are somewhat concentrated in the necrotic area, generally seen to be fairly well preserved. In an extensive necrotic area, liver cells were sometimes seen to be completely lost, often with a center, while, in the periphery, they are fused with those of disintegrated ones. The size of necrosis surrounded with capillary vessels is varied. At times a small amount of red blood corpuscles were observed in capillary vessels, though rarely seen. In the early stage, however, congestion was often noted in the neighboring capillary vessels, but their cellular activity could not be observed. Besides above typical findings of reticular necrosis, there were cellular swelling and vacuolization which appears to be transparent; the cytoplasm presented rough net like appearance, and a filmy or linear cytoplasm, with degenerated liver cells barely adhering to the cell wall or the surface of the shrunken nucleus. The degenerated cellular mass became an albuminous lump which is uniformly stained with eosin, but enveloped by the cell wall, some of which were still retained some cell activity in the periphery. In the non-necrotic area, vacuoles were observed in some parts, but changes were less striking. The cellular alignment of liver cell-cords appeared to be regular. In its early stage, the Glisson's cupsule was a slightly adematous in the interestices, and the fibers were coarse. Generally the small round cell infiltration was not noticeable. From the second day, proliferation of the interlobular bile duct was observed, but proliferation of the connective tissue was the least. On four days operation, the young connsctive tissue was definitely noted along with proliferation of the bile duct, but hard any around the large and medium bile ducts. In the extreme periphery of the hepatic lobules, however, the nuclei have arranged themselves in a ring like manner leaving a cavity in the center which at times gives an impressions of adenoma like growth. In a typical proliferated area, formed bile duct appears to be in a mass in the lobular periphery, and young connective tissue was seen to either infiltrating into the cellular spaces or encircling to form as isolated area. Interlobular' or central veins and intra lobular capillaries showed no special changes.

Silver Stain Findings: With silver stain method, the reticular fibers were in normal arrangement, and were seen to be preserved well even in necrotic area. In the larger necrotic center, silver was poorly deposited and the reticular fibers became indistinct. Along with proliferated bile ducts, there was observed the formation of collagen fibers which were tend to show marked affinity for silver stain.

Findings of Mitochondria: With the liver cells of the central and medium parts of lobules, mitochondrias were well stained and found relatively in large number.

Around 24 hours after operation, however, mitochondrias were found less in the neighborhood of the capillary bile duct, while, they seemed to collect near nuclei in the liver cells. They are largely of small and round, though some were appear to be rod-like. In the periphery of the lobules, outside of necrotic area, they are well stained and take either round or rod shaped. They were relatively concentrated around the nuclei but lesser in number toward the cell wall or adjacent to the capillary bile duct with reduced staining capacity. Those cells which have undergone complete necrosis have lost mitochondria and only the cell wall left and with their nuclei and karyoplasm no longer to be seen. Toward the periphery, the number of mitochondria becomes few, smaller in size, irregular and simple in alignment. There were a small number of cells in which nuclei have been degenerated and take dark indigo, but presented reticular appearance of cytoplasm. Mitochondrias, on the other hand, were either poorly stained or none at all and appear to be either round or in mass. At times, the cells having small spherical or rod-like mitochondria, with good staining property, were observed throughout necrotic lesion.

Findings of Sudan III Fat Stain: Fatty granule is generally few in reticular necrosis. At times microgranular fatty granules are deposited in the intercellular space and sometimes in the Disse's space. There were many granular-shaped fatty granules. Deposit of fat often observed in the periphery of the lobules, while, fatty granules were frequently noted in Kupffer's stellate cells, but, these granules were not generally observed in the central lobules. Marked proliferation was observed interlobular veins and the connective tissue around the bile duct.

Findings of PAS Stain: In most areas of reticular necrosis, the PAS positive substances were seen to have nearly completely disappeared, retaining only the reticular and light purple cell wall. While in the liver cells around reticular necrosis PAS positive substance was decreased generally; in some, it is completely disappeared, but others have retained only a trace or diffusely. In the early stage of necrosis, it is clearly demarcated from PAS positive area. PAS positive reaction was seen in the lobules where the bile duct was abundantly formed, but the reaction appeares to be entirely negative where only the cell wall remained which were stained with red-purple color. At times PAS positive substance was observed as either islet-like or linear fashion in the capillary bile, besides in the bile duct in the sheath of Glisson. In the non-necrotic area, PAS positive substance was found more commonly in the central lobules, twelve hours after operation, although it is less positive in the direction of the lobular periphery and became completely negative. With 24 hour post operative cases, PAS is least positive leaving granular positive substances in the central and medium lobules.

Findings of Nucleic Acid Staining: The distribution of the nucleic acid was examined with Unna-Pappenheim's Pylonin-methyl green stain, and Metachromasia reaction with thionin. DNA was observed in the residual stellate celluar nucleus, which has underwent complete reticular necrosis, otherwise, neither DNA nor RNA were detectable. Thus, the necrotic area could be clearly demarcated from the neighbouring liver

cells. In the isolated cells found in the periphery, DNA was found in the karyoplasma and nucleolus, while RNA, which appears to be minutely vesicular or microreticular, sould be seen in abundance over the entire area of cell body.

In the early stage, smaller nuclei were congregated in the periphery, some stained well with methyl-green, while, other tend to be stained well with pylonin, in which well stained DNA with an appearance of reduced polymerization could be identified. The cellular body was stained pale with pylonin and thionin, and only the trace of RNA could be seen. The nucleic acid to be observed in the non-necrotic area showed the same picture as the controls, possessed DNA in the karyoplasma and around the nucleolus, and an extermely small amount of RNA could been seen in the nucleolus. In the cell body, RNA could be detected in heavy patches throughout the cell presenting normal pattern. In the periphery of the lobules, RNA mass in the cell body was seen to disintegrated and spread over the entire plotoplasm, leaving granular edge suggesting the picture of reticular formation. With the cases 12 hours after operation, on the other hand, RNA in the cell body similar to the periphery of the lobules, were found to be diffused over the entire area of the cell body, and finally became reticular or vesicular appearance. The liver cells of 2–4 days after operation, these showed their nucleoli took pylonin but stain little.

Findings of Feulgen Reaction for High-Polymerization and Law-Polymerization DNA: Through Feulgen's test for high-polymerized DNA, it was found that the karyoplasm of liver cells, Kupffer's stellate cells, bile duct cells, and connective tissues was found to have Feulgen positive DNA. The nuclei of liver cells were of big-size, while DNA generally had low density, mainly found in the nucleic membrane and nucleus. while the nucleolus apparently looked vacuolar.

Kupffer's stellate cells were of small size, with DNA possessed of a comparatively high density. The nuclei of the bile duct cells were of medium size, with high stainability ane with clearly visible nuclear membrane. The density was much higher than the nuclei of liver cells. With cells of reticular necrosis, Feulgen's test was practically negative, except for some Kupffer's stellate cells or the congregated nuclei of liver cells in the marginal necrotic area. The findings of Feulgen's test on low-polpmerization DNA were roughly similar to those of the high-polymerized DNA, though of the lesser degree, other than the area of necrotic lesion. At times, a vague and slightly pink-color portion was observed in the cell body in the non-necrotic area.

Finding of the Bile Secretory Granules: These granules take purple-colored stain which are to be seen in the lobular cell protoplasm. The nucleus generally was stained blue, and partially yellow. These secretory granules tend to fill the cells around the bile duct, both intermediate and periphery parts and gradually to the central part; and by 24 hours, cases after operation stasis of bile could be seen and filled the entire lobules. In the area of reticular necrosis, bile secreting granules were no longer to be seen. Leaving only the cell membrane which took blue stain but with disintegrated cellular body which appeared to be reticular and with total loss of nuclei.

Sections of the capillary bile duct around these degenerated liver cells were seen to be filled with an orange-stained substance, surrounding the necrous liver cells. In some parts of necrotic areas and part of the neighboring liver cells, these granules were lost and formed of vacuoles. Many of such cells had a transparent nuclei, at times, became vacuolar having the nucleic content expelled. A small numbers of bile granules could be seen in the nuclei of cells filled with bile. With Kupffer's stellate cells, when normal, few bile granules could be found, but, from about 24 hours after obstruction of the bile duct, the stellate cells mainly in the central and medium regions were seen to become swollen into long trianglar or long oval shape, with a considerable number of bile granules being observed in the cell body. At first few granules were noted in the stellate cells in the periphery, but, from about the tenth day after, they came to be observed in the entire area of the lobules.

Even after the liver cells became necrotic, the stellate cells were able to preserve their original shape for a comparatively long period of time, with a large amount of bile granules being found in the cell body even in the area necrotic. The bile usually fills the capillary bile duct, from the periphery of the lobules, which creates enlargement and dilatation. The part of the interlobular bile duct was also filled with bile depositing granules.

Findings of Phosphatase Reaction:

1. Alkalinity Phosphatase Reaction (pH 9.24).

The alkalinity phosphatase test for reticular necrosis was strikingly weak. The nncleus and karyoplasm of the degenerated liver cells in the periphery of the lobules reacted vaguely light-brownish, while the central part was nearly negative. Outside the necrotic area, the nucleus reacted light-brownish or brownish in the central, medium part and periphery of the lobules, with the nuclear membrane and 2–3 granules in the nucleus being stained clearly. The reaction of the cell body was weaker than that of the nucleus, having stained with similar light-brownish granules or in lump. Likewise, Kupffer's stellate cells, had their nuclei and cell bodies stained brownish and light-brownish, respectively. The capillary bile duct reacted not strongly in the periphery of the lobules, taking dark-brownish streaks, while those of the central and intermediate regions showed weaker reaction.

With the sheath of Glisson, the test was generally weak, the nuceus of the connective tissue mercly reacted light-brownich. The interlobular bile duct and pseudo bile cannaliculus were strongly stained dark brownish with that nuclei and karyoplasm often indistinguishable.

When compared with the control cases, the test revealed marked reactivity of the capillary bile duct in the locular periphery as well as the interlobular bile duct, but less with the Kupffer's stellate cells. There was no increased sign with alkaline phosphatase as far as hepatic cell goes.

2. Acid Phosphatase Reaction (pH 5.6).

The acid phosphatase reaction is practically negative with reticular necrotic tissue ; it is weaker in the central and intermediate lobular regions. Their nuclei

and cell bodies stained light brownish. The liver cells in the periphery, took deeper staining than the cells of the central or intermediate regions, and the cell body as well as cellular membrane took light brown color. The capillary bile duct tissue revealed distinct black-brownish stain from the intermediate region to the periphery. Kupffer's stellate cells and interlobular bile duct had their nuclei and cell bodies staind light brownish. Ths surface of the interlobular bile duct was stained with markedly black-brownish color.

SUMMARY

It has been found that the reticular necrotic changes occured most commonly in the lobular periphery following the ligation of common bile duct. In most instances, typical reticular formation was to be seen, though various pictures of necrotic process such as with congestion, bleeding or with mild cellular influence were present. The lattice-shaped fibers seemed to retain their form relatively well despite necrotic invasion, however, with eventual disappearance. Mitochondrias mostly were small spherical type, and became less in number in the tissue of capillary bile duct, and seemed to be congregated near nucleus. In the presence of necrotic process, mitochondria completely disappeared and presented net-like structure with only the remaining cell wall. From the early stage, PAS stain was positive indicating a distinct polysaccharidic localized lesion. The test was characterized by either decreased or disappearance of PAS positive substances in the direction of the central region from the lobular periphery. In certain cases, however, moderately positive PAS substances, have been observed in the necrotic liver cells and the neighboring capillary bile ducts, and so with the interlobular bile duct. This is concidered to be due to the reaction of certain ingredients filling or infiltrating into the capillary bile duct, necrotic liver cells and interlobular bile duct, or, of certain necrotic substances. In most necrosis, fat was negative, with fatty drops being often noted in the necrotic liver cells of the periphery. As bile acid stain was carried out, a large quantity of red-purple-colored and micro-granular bile acids were observed in the liver cells and stellate cells in the periphery of necrosis, but not in reticular necrosis. In the periphery of the capillary vessels or in what was though to be capllary bile duct a lump-shaped, pink-purple colored substance was found. It is not known whether this is a constituent of the bile or escaped necrotic cells. As the distribution of the nucleic acid was examined by means of thionin metachromasic reaction, both RNA and DNA were found to have disappeared in reticular necrosis, though minute quantity, was still remaining in certain liver cells and in necrotic stellate cells. Even by means of pylonin-methyl green, high-molecular, low-molecular Feulgen's reaction, DNA was similarly found to be provided with a peculiar property of turning low-molecular. Phosphatase reactions, both alkaline and acid, were negative in necrosis, with a striking stimulation of reaction in the capillary bile duct in the periphery of the lobules. This was noted to be especially strong with alkaline phos-

phatase reaction.

In the light of the afore-mentioned findings of various stains, no specific findings were obtained with reticular necrosis unlike other forms of necrosis, pertaining either to peculiar low-molecularization of DNA in nucleus, or, to the reduction of the staining property transiently.

Chap. II ELECTRON MICROSCOPIC OBSERVATION

From the histological and histochemical observations made in the preceding report, the evidence has already been brought out that the so-called reticular necrosis is a condition of colliquative necrotic process as a result of direct action of bile ran out of the ampulla rather than transient reduction of stain ability of the hepatic cells. It is assumed that the net-like formation of the cells in the result of cell membrane which will be retained considerably a longer period. Assuming that the morbid hepatic cells would be simply a transient reduced staining, it would be seen possible that there should be no advanced disintegration of ultra minute structure of the liver cells as far as electromicroscopic observation goes and without using staining technique; and a remnant of basic structure should have been noted. With this in view, further study was made on this particular aspect, and the present experiment was undertaken for the purpose of ascertaining changes occurring in the microstructure of the liver cells at the time of obstructive jaundice,

EXPERIMENTAL METHOD

The experimental animals and the method of operations were roughly similar to those mentioned in the preceding report.

The healthy, adult rabbits (of which, three were controls) were used. Forty-eight hours after the duct ligation, they were sacrificed causing air embolism through the ear vein. The specimens of liver tissue were removed, subjected to 3-hour fixation, at 5 °C, in 1 percent 0_S 0_4 solution prepared at pH 7.2–7.3 with veronal acetate buffer following Palade. The tissues were washed in water, dehydrated with alcohol, and polymerized for about 10–20 hours at 40 °C–48 °C with synthetic resin mixed with eight parts of N-Butyl methacrylate; two parts of methyl methacrylate and 2 percent of Benzoyl peroxide, as a polymerizing catalizer. The ultra thin section was prepared by Shimazu's ultra microtome, with the use of glass knife. Hitachi's HU-10-tope electron microscope was used for observation without removing monomar.

ELECTRONE MICROSCOPIC FINDINGS OF CONTROL RABBITS' LIVER CELLS

Nucleus: The nucleus was generally round or oblong in shape, bordered with a distinct nuclear membrane of which the interior was filled with micro-granular

nuclear substance of uniform size, some of which present lighter appearance, while some areas formed granular mass of relatively higher density. There was no nuclear network to be observed.

Nucleolus: The nucleolus was filled with micro-granular substances of high density, but no visible limiting membrane could be seen.

Nuclear Membrane: The nuclear membrane was of distinct double structure. Its inner membrane generally was smooth, but the outer was thin with uneven boundary, and what appeares to be an endoplastic structure could be seen in the cytoplasm.

Cytoplasm: The ground cytoplasm generally has a low electrotic density. In some parts, presenting micro-granular substances, were seen while, in other parts showed uniform light shade.

Some of endoplasmic reticulums pressnted either a flat lamella, or vesicular appearance, or minute vacuolar appearance. Many of them contain flat filaments which appeared to be oblong, hollow-shaped structure, made up with an indefinitely shaped and light substances having a wall made of a membrane with less than 10 μ thickness, and filled with small dark granules of 130–200 Å in diameter and cyst like structure irregularly distributed. Reticulum was tend to form chains.

Mitochondria: The mitochondrias are mostly spherical, oblong or rootshaped, but round or oblong types were predominating. These mitochondrias have double membrane, in which cristae mitochondriales of axile shape having double-membrane structure could be seen. Their density was appeared to be higher than cytoplasm, and was dark hued, and many of which cristae mitochondriales were hardly visible.

Golgi Complex: This consists of three elements, namely, golgi membrane, golgi vacuole and golgi granule. The golgi membrane was equipped with a double-membrane structure, and, with a curve, presents a lamella, adjacent to which golgi vacuole or big vacuoles and golgi granule or round, small granules were said to be seen. They were comparatively difficult to identify in liver cells, and, in a few cases, they were only observed near the bile capillaries.

Cell Membrane: The liver cell has a distinct cell membrane, and runs parallel to adjacent cell membranes at a definite interval. Some points fuse each other. The cell membrane facing the liver cells is usuary of smooth structure, but cell membrane on the side facing sinusoid has many small irregular projections, visible interstices between sinusoides.

Bile Capillaries: The bile capillaries were observed as small slit-like cavities between liver cells, with their cell membranes projected with tiny villi in the direction of the inner cavities.

Glycogen, fat and bile component were to be detectable in these cell according to Fawcett, glycogen stored in abundance, however, it was not identifiable actually.

Fat could be fixed with osmic acid, and to be seen as a round or a confetto-shapsd lump.

Small spherical granules of high density, presumably of bile constituent noted

near the bile capillaries, at times. The liver cells were either light or dark, the latter having cytoplasm of high-density filled abundantly with endoplasmic reticulum and mitochondrias.

ELECTRON MICROSCOPIC FINDINGS OF LIVER CELLS WITH LIGATION OF THE COMMON BILE DUCT

In the early period following ligation of the common bile duct, the nucleus and nuclear membrane looked irregular to some extent and with clearly visible double membrane, though the charges were of lesser degree. The filament structurs of the karyosome was definitery seen. Most of the mitochondrias were semi-circular but no particular change to be noted other than filled with rich substance of high density and without cristae. Vesicles with flat lamina seemed to disappear gradually from the aspect of bile capillaries and transform into vacuole like appearance within the cytoplasmic ground presenting clear uniform shade.

While, the other side showed plentiful filamentous vesicles aligned with lamella where ground cytoplasm seemed to be denser than the former, and highly granular. In these instances, the mitochondria was often compressed to one side. Eventually, endoplasmic reticulums containing flat and lamella structure were no longer to to be seen but became vesicular in which micro-granular substances of high dentity were seen to be wandering. Occasionally several dozen semi-circular minute bodies of high density were observed.

In some cases, appearance of porous, felt-like mass was visible in portions of the cytoplasms, while in some cases, minute bodies which appeared to oval shape with high density containing heavy stripes with size of mitochondria or several times larger would be noted in the cellular bodies, or, again, these appear edvacuoles of various size taking semi-circular shape containing low-density granules or unidentified body structure which threw same clearness as cytoplasmic ground. The bile capillaries contained low-density, granular substances in their cavities which were dilated.

The nuclei of the stellate cells of Kupffer could be seen several small substances containing one or two micro-vacuoles, mixed with mitochondria. In the necrotic liver cells, the nuclei were finally broken down and lost, at times, they may remain as irregular granular mass. Vesicular bodies also underwent dissolution finally. The endoplasmic reticulum with its lamella structure would be lost but mould into vesicles which continueed sign or disintegration and became irregular and indistinct nebular condition. The process of dissolution further involved mitochondria and disappeared. Those liver cells which were completely necrotic could only leave broken down debris and adhered to the cellular membrane. The so-called dark cell seemed to possess ability to resist injury though cellular reticulum became indistinguishable with swollen mitochondria and with uneven cell membrane, and least affected with dissolution. The cellular membrane and the stellare cells of Kupffer were able to preserve their structure relatively well.

SUMMARY

It has been found when electron microscopic observation was made on the liver cells of rabbit which had been subjected of obstructive jauudice by ligating their common bile duct, the first change noted was the formation of vesicles from the aspect of the bile capillaries, which gradually extened to the opposite side, with endoplasmic reticulum presenting lamella to be seen on the capillary side. With further progress, endoplasmic reticulum would be filled with formed vesicles throughout the cell, containing granular substances of low density as well as high density. The bile casillaries as a whole showed dilatation of cavities. In the stellate cells of Kupffer, however, granular substances, were relatively larger than mitochondria but with considerable high density. While, the necrotic liver cells underwent swelling and dissolution of endoplasmic reticulum involving the nucleus and mitochondria and lost finally. Only the cellular membrane would be retained for much longer period leaving cellular debris, the product of disintegrated mass adherent to the cell membrane.

These findings seemed to suggest that the so-called reticular necrosis is not the result of transient loss of staining properties, but due to the complete necrotic manifestation with unaffected cellular membrane.

From the endoplasmic variation observed seemed to indicate that the metabolic direction of bile acid is assuming one definite channel through Disse's cavity running from bile duct to the capillary. It is very likely that the vili lined Disse's cavity as well as inner cavity of the bile capillaries take some role of positive activity not merely dependant on osmotic pressure.

The bile, in itself, will not be fixed as a physical substance, with osmium acid; but, it is presumably subjected to certain change within endoplasmic reticulum, and those parts which had undergone concentration will be detectable by electron microscopically as either granular substance or physical object. Glycogen in the liver cells in all probability did not seem to present as a distinct picture.

Chap. III ON THE EFFECT OF BILE AND BILE ACID ON HEPATIC CELLS

Although pronounced liver damage characterized by reticular necrosis was observed in rabbit or guinea pig following ligation of common bile duct but with dog, this is hardly possible; the effect is no more than a clear non-necrotic lesion. Several reasons have been advanced, perhaps one reason may be due to the difference in the property of bile between dog and rabbit.

In order to clarify these points, the present writers undertook to examine the character of bile of two different animals. Moreover, in view of the ascertion made by certain investigators that bleeding is closely related to the occurrence, an approach was made by administering direct injection of the combined sera of animals, into the liver, and examined the extent of involvement with possible appearance of

reticular necrosis. Furthermore, a comparative study was made of desoxycholic acid, considered to be a principal constituents of rabbit's bile, and of cholic acid which is the chief chemical substance found in bile of dog.

EXPERIMENTAL METHODS

The experimental animals used included dogs, rabbits and mice.

Three dogs and three rabbits have had their common bile duct ligated by following the same procedure as described before to make experimental jaundice. Twenty days after in dogs, 48 hours after in rabbits, the animals were operated for laparotomies, and bile specimen were taken aseptically to be used for the experimental material. At the same time, blood specimens were taken by means of cardio puncture, and sera were also used. Cholic acid and desoxycholic acid in physiological salt solutions were made 1 percent and 0.25 percent, respectively.

These materials were injected directly to the liver tissue in a divided dosage more than ten different place and the wounds were closed.

Twenty-four hours after the laparotomy, they were killed by introduced air through the ear vein to cause air embolism. The liver tissue were removed and fixed in 10 psrcent formalin, with which paraffin sections were prepared and observed after staining.

A group consisted of two dogs and rabbits were used, while about 0.5 cc. each of cholic acid and desoxycholic acid was injected into the peritoneal cavity of the mice (ten for each group) with about 20 gm. weight. Twenty four hours afterwards, they were killed, and following post-mortem examination, the liver tissue sections were fixed in 10 percent formalin, observation made after stained with haematoxylin eosin and Sudan III fat stain.

EXPERIMENTAL RESULTS

Dog's Bile injected to Dog's liver Tissue: When a small amount of dog's bile was injected, coagurative necrosis of the liver cells accompanied with cellular reaction would result but with a larger dose not only did coagulative necrosis of the liver cells develope but the tissue frequently show sign of haemorrhage and swelling, cellular disintegration, reduction of stain properties and loss of the nuclei presenting a picture of colliquative necrosis with marked stasis of bile and infiltration.

Dog's Bile and Serum injected to Dog's Liver: When dog's bile was combined with serum, hemorrhage in the necrotic lesion became most marked and degenerated cellular membrane of fibrous substance formed an irregular network; the liver cells broke down in the periphery and showed coagulative appearance. In the affected areas were infiltrated with wandering phagocytes which contained much hemosiderin. The necrotic areas which were, free from bleeding, were appear to be coagulated.

Dog's Bile injected to Rabbit's Livsr: Slight atrophy at first seen in the liver cell cord was less affected. The liver cells in the peripheries of necrotic legion were atrophied but cells took eosin stain well, and nuclei were pyknotic. The liver cells were more or less swollen disintegrated and forming granules in the necrotic center and reduced staining quality, while, the nuclei stained poorly with hematoxylin containing large vacuoles. In some areas, the nuclei have undergone marked disintegration and lost and formed net-like formation with broken down vacuolized hepatic cells with dilated sinusoids where neutrophic mobilization was more noticeable.

Dog's Bile and serum injected to Rabbit's Liver: The liver arease where injections were made undergo degenertion, the cell cords were atrophied and no longer retain normal alignment. The cell body took eosin better, and with vacuolized appearance and minute structure was lost. Vacuoles were tend to be formed on the capillary side. The nuclei were either pyknotic or poorly stained, or completely gone. The stellate cells were least affected and preserved original form fairly well though their nuclei became pyknotic. The sinusoids were dilated but erythrocytes were not seen in most cases. In some parts were infiltrated with cells.

Rabbit's Bile injected to Rabbit's Liver: Injected part as a focus, the liver tissue involved semi-circularly; these areas have shown degeneration presenting picture of either vesicular or having nuclei swollen or granular appearance, or showing pyk nosis though some vanished. The cell wall was clearly visible, in parts, what appeared to be reticular like lesion was seen. Cellular infiltration could not be identified.

Rabbit's Serium injected to Rabbit's Liver: Following injection, the micro-structure of the cytoplasma was seen to collapse; both protoplasm and nucleus were not too well stained. In the periphery of necrotic lesion, nuclei became pyknotic, while, in the central regions, they were either swollen or lost. Vacuolization was common but some areas were swollen and became reticular.

The sinusoids were markedly dilated and congested. The central region was swollen and presented a picture of hemolysis, losing much of staining ability. Cellular infiltration was seen.

Rabbit's Bile and Serum injected to Rabbit's Liver: With the injected area as a pivot a semi-circular involvement or along the direction of needle, a V-shaped like scattered necrosis of typical reticular type was observed. Cellular infiltration was extremely weak, in some parts was, totally absent.

Rabbit's Bile injected Dog's Liver: Mild reticular necrotic process was noted around the injected site, having lost cellular structure except for cell wall. In some cases, nuclei were pyknotic, deformed and vesicular. There were many transitory forms. Practically no cellular reaction was seen.

Rabbit's Bili and Serum injected to Dog's Liver: The liver cells in necrostic periphery showed either extensive or limited involvement, but most area became coagulative, but disintegration was most noticable in the center; the nuclei were either

pyknotic or lost, though the cells appeared to be vesicular which looked more like reticular. The cellular infiltration was least effected.

Rabbit's Serum injected Dog's Liver: In a small area where the injection was made, the liver cells were degenerated and became necrotic while coagulation was relatively slight.

A Group with Cholic Acid injected to Liver: When injected, the areas underwend degeneration, the cell transformed into granular, lightly stained with eosin, and generally became transparent. The nuclei were generally, pyknotic and partly become less stainable with hematoxylin, and was often observed as a small mass thinly stained with eosin.

In the degenerated liver cells, the nuclei were pyknotic, and the micro-structure of protoplasm became indistinct, being comparatively thickly stained with eosin. Thus, it sometimes loooed like a mild coagulations necrosis, while, at times, the nuclei disappeared with vacuolization. Neutrophile leucocytes infiltration was mild.

A Group with Desoxycholic Acid injected to Liver: Of the degenerated liver cells with necrosis in the injected region, those in the peripheries of necrosis showed coagulative necrosis, while in the central regions, protoplasm was seen to collapse and from micro-granules and liquefy. The cell transformed into a fine network and with formed vacuoles, but nuclei have vanished, and developed into reticular picture. Neutrophilic leucocyte were mildly infiltrated.

A Group with Cholic Acid and Desoxycholic Acid injected in Abdominal Cavity:

In either case, alignment of the liver call cord remained normal, and, cellular dissociation to be seen. Vacuolization was unusually strong. But no hemorrhage or necrotic process could be seen. The cell groups found in the liver margin or larger vessels were somewhat edematous and transparent, while the nuclei were slightly pyknotic and practically transparent as above. Proliferation of the interstitial connective tissue as well as cellular infiltration could not be identified.

SUMMARY

Experimental results described above revealed that the higher the density of either rabbit's or dog's bile becomes, the more strongly the disturbances manifest in the liver cells, thus, necrosis of the liver parenchyma is invariably results. In general, however, dog's bile was more liable to cause coagulative necrotic degeneration, while rabbit's bile tended to cause reticular necrosis.

This tendency is common, weather it is the liver cell of rabbit or liver cell of dog which speaks for the fact that reticular type of necrotic process is limited to rabbit.

Again, when serum only added to either rabbit's or dog's bile, retcular necrosis was most likely to be developed. Most cases, coagulated necrotic degeneration would be the case when serum alone was subjected. It was also found that transformation of necrotic process from reticular type the coagulative had been substained.

While the cellular defense mechanism was not uniform but it was less reactive in the area where reticular degenerative change was evident, whereas it was definitely active where coagulative necrosis was in progress.

With the case when cholic acid or desoxycholic acid was directly injected into the liver, the peripheral change would be congulative necrosis, while the central regions showed liquefaction closely simulating reticular necrosis. This effect was stronger with desoxycholic acid. If cholic acid and desoxycholic acid were injected in the abdominal cavity, the liver cells would be under the influence of vacuolization, though cells of pelivascular part may become transparent.

DISCUSSION

Despite numerous attempts made to elucidate the mechanism of causation of jaundice for years, little progress has been made on the whole pathological interpretation. In recent years, with the progress of studies made by biopsy method, reports on Gilbert's disease or Dubin-Johnson's spndrome, additional information have been advanced but views became more complicated. Even with the problem of obstructive jaundice which is supposedly less complicated has created much discussions since the reports of Eppinger and Ohno.

Eppinger was the first scholar who made successful attempt on histological method of the bile copillary. He proposed a theory based on the rupture of biliary space and explained that, as bile accumlated in biliary ducts, the bile capillary in the hepatic lobules will expand, which, eventually rupture, will force bile out of the ducts at two points, lead it into the adjacent lympatic space, where it is absorbed into blood stream which developed icterus.

Hieda on the other hand, carried out a detail investigation on obstructive jaundice in rabbits, and, in the light of a specific change of hepatic tissue and basing on histological and dynamic approach of the so-called reticular necrosis, expressed his doubt on the theory of ruptured biliary space proposed by Eppinger; and in turn asserted that the finding of biliary space was simply a secondary finding reexamination but proposed his own theory of rupture of intra-lobular biliary ducts (ampulla). This subsequently became one of the most important basis for Ohno's monistic theory of jaundice.

The so-called reticular necrosis, which is a most appropriable evidence to support histological aspect of this theory is that a characteristic hepatic degenerative change would result when the biliary ducts of rabbits or guinea pigs were ligated. It was already recorded as taches jaunese by Charcot et Gombault (1876, guinea pigs) and as Netzbezirk by Tischner (1904, rabbits). These were substantiated later by a number of students. Ohno and Hieda, howevsr, were the first scholars to clarify its nature and its significance. Some of the theories advanced for elucidating the nature of the reticular necrosis can be summarized here.

Jagic theory of infection states that an infection is the direct cause at the

time of surgery, which induces cellular dissolution and degeneration. Theory of Mechanical Disturbance interpreted by Foa et Salvioli, Belleussow, Canalis Sumida, and Momoe, states that bile accumulates in biliary ducts, and, as a result of the rise of internal pressure, the hepatic cells are directly affected by the pressure and degenerate into necrosis. But, theory of disturbed blood circulation proposed by Janowski, Tischner, Chambard, Löffler, Rous & Larimore, and Iijima, is that necrosis is caused by disturbance of blood circulation due to expansion of bilary ducts. Later, theory of intoxication that is direct chemical activity of bile causing necrosis received good support.

Despite various theories have been reported and supported by others, there has been no agreement of views reached on the issue of bile, some attributing it to the rupture of bile capillary (Charcot et Gombault, Eppinger, Abramow u. Samoilowicz, Bürker), while, others favored the activity of bils ran out from the ruptured (Ohno, Hieda, Nagaoka, Kikuchi and Ito) but, still others maintained that it is the result of the stagnation of bile in hepatic cells (Kajimoto, Fukuda).

Ohno and Hieda, however, on the basis of detailed experimental facts were critical of Eppinger's and also the others theories. They held that reticular necrosis is caused by forced chemical activity of escaped bile due to the ruptured ampulla. The fact that there is total loss of hepatic tissue in a short time, unaccompanied with cellular reaction is least understood in the light of what we know about morbid change of degeneration and necrosis. From this, the condition might not be true death of tissue cells but that might be the condition of temporary imperfect staining of hepatic cells resulting from the chemical activity of bile, presenting the picture of necrosis microscopically and as soon as bile activity ceases, the hepatic cells will recover their staining ability, reestablished normal function without undergoing regeneration or hyperplasm, nor infiltration with wandering cells or granulative formation or leaving scar tissue. Thus, they interpreted that the reticular necrosis does not really represent a true process of necrosis but a type of pseudonecrosis resulting from failure of the staining ability of the hepatic cells, which were affected directly by bile. Subsequently, Watanabe and others have contended that through venous or intraperitoneal injection of sodium salt of bile reticular necrosis or transparency of cells could be induced in the liver of some rabbit's and assumed that the heightened activity of polynucleotidase is the responsible factor in the causation of degeneration and by additional information from smear preparation taken from peripheral blood of rabbit. They explained that bile acid is taking an important role in connection with metabolism of nucleoprotein or nucleic acid from the fact that the reticular type of necrosis fully recovers leaving no evidence of retrogressive change.

Therefore, it can be stated that reticular necrosis is caused directly by the bile action, especially, bile acid which constitutes the content. But, admitting that morbid change is due to loss of staining ability in association with nuclear metabolism, it should follow that there must be certain histochemical findings indicating

the process of the nuclear metabolism unlike that of true type of necrosis or evidences of remnant microscopic structure of the hepatic cells as they should have been observed independent of the stain method.

If, again, such a pecular necrotic degeneration does really exist, it will provide no important evidences for concerning further information on cytological, cytochemicrl or super-microscopic structure of cells. With this in view, chapter I and Chapter II were devoted for the above. As stated in the summary of the preceding Chapter, the experimental results obtained by the present writer had failed to include findings that reticular necrosis was different from common necrotic degeneration.

Thus, it is assumed that the reticular necrosis is no way associated with loss of staining ability of the hepatic cells but to colliquative necrosis and hydropic degeneration of heaptic cells caused by bile, and, therefore, that cellular membrane is least affected and leaving characteristic appearance of reticular formation.

In rabbit, reticular necrosis does develop when the common duct is ligated whereas in dog, only the transparent lesion is possible. But it is our interest to know that when the condition is let alone for a prolong period of time, rabbits develop biliary cirrhosis, but not in dogs, according to a report, similar to experimental imposibiaity of gall-stone in dogs.

Possible reasons for the difference between rabbits and dogs are summarized as follows:

1. Difference in resistance of hepatic cells against bile obstruction.
2. Difference in the composition of bile.
3. Difference in the amount of bile produced in unit time, as well as difference in the variation of the internal pressure of bile ducts.
4. Structural difference of the ampulla of bile ducts. Ohno and Hieda have reported on series of studies on those points. The present writers have placed the utmost emphasis on the difference in the composition of bile between rabbits and dogs. The Chapter III dealt on this problem. It was found that rabbit's bile has a tendency to produce reticular necrosis, while dog's bile tends to produce the ordinary necrotic lesion. This tendency is true with the hepatic cells of both animals. It seems possible that it is not intrinsic difference of hepatic cells which is inducible to reticular type of necrosis, but it should be rather regarded as functional difference of bile peculiar to the animal.

Generally, bile contains bile acid, biliary pigment, mucus, cholesterol, lipoid, inorganic salt (Cl^-, HPO_4^-, $H_2PO_4^-CO_3^-$, HCO_3^-, Na, K, Ca, Mg,) CO_2, NH_3, urea, and purine; of these components, bile acid differ greatly between two animals. While rabbit's bile consists mainly of desoxycholic acid, but in dog, cholic acid comprise the chief constituent. Thus, the difference in the chemical constituent of the two animals is the nature of bile acid which varies.

The following chemical difference between desoxycholic acid and cholic acid were summarized:

1. Fatal dose: When it is administered in rabbit's veins, the fatal dose of desoxychoric acid is 0.015 gm/kg, while that of cholic acid is 0.05 gm/kg.

2. Hemolytic activity: Maximum limit dilution of bile acid in hemolysis against goats erythrocytes.

Cholic Acid	320 times
Desoxycholic Acid	2500 times

3. Leucocytolysis:

Cholic Acid	800 times dilution
Desocycholic Acid	3000 times dilntion

4. Neufeld's phenomenon: Desoxycholic acid (2500–3200 times dilution) has a stronger bacteriolytic activity as compared with cholic acid. It also has a stronger toxic property.

Thus, it is shown that desoxycholic acid is prossessed of stronger chemical action than cholic acid.

It has been accepted that the nature of hemolytic activity of bile acid is its chemical adherance to cellular surface (Gibbs' Law) affecting lowering of surface tension, which in turn, causes hemolysis. Nuefeld's phenomenon is considered to be due to action of bile acid which will dissolve the bacterial membrane freeing enzymic content resulting autolysis. This is also applicable to leucocytes which undeago leucocytolysis. These activities shown by bile acid are due largely to the physiological function of bile acid, particularly its reducing action of surface tension and lypolytic activity.

The fact that rabbit's bile, which contains the chief contituent desoxycholic acid, has an influence which will produce reticular necrosis implies powerful intoxicating character by combining with fatty acid and convert into cholein acid which is possessed with marked reduction of surface tension.

This property of desoxycholic acid was recently utilized by Palade and others for breaking endoplasmic reticulin and isolating microsome, and, also, by Tashiro, for clarifying the structure of endoplasmic reticulum as anionic surface active agents.

The occurrence of reticular necrosis, therefore, can be explained as follows based on all these informations. When bile ran out the ampulla of bile ducts, it first affects the cells, and, due to its specific property, increases its permeability, when entering into the cells, they became adherent to the protein membrane of endoplasmic reticulum, breaking down secondary and third structures and loosening the membrane, and invades further and dissolves lipoid micelle. This process will introduce a large amount of water into microsome causing marked swelling and dissolves endoplasmic reticulum.

This cellular change extends to all other cells, forces dissolution and loss of protoplasm and with remained in the cells for a long period would eventually develop into necrotic reticular formation.

From the first experiment, it was found that the action of bile acid which had been stagnent in the hepatic cells was less injurious, but, once it was secreted in

the capillaries, it became markedly topic. This means that development of reticular necrosis is not by bile stagnent in hepatic cells, which destroys the latter, but by bile secreted into extra-cellulary which acts from without.

Kajimoto has suggested that dissolution of hepatic cells starts from the capillary vessels side his finding did present some bearing on this issue. It has been assumed and stated in Chapter III that the bile acid is largely responsible for reticular necrosis that desoxychol acid is possessed of a stronger action than cholic acid.

As to cholic acid, it will be considered that in dogs, a greater amount of bile escaping from the ampulla of bile ducts containing higher concentration of cholic acid, speaking for possiblity of producing not only a transparent lesion but also advanced reticular necrosis.

In rabbits, on the contrary, if the concentration of desoxycholic acid is higher, occurrence of transparent lesion may be possible. Tanaka recently reported that, a transparent lesion could be developed in rabbit's liver with Indian ink injected into bile ducts simultaneously with the ligation of the common bile ducts, and, also, reported on the finding of reticular necrosis in the liver of a young dog in whom cholecystectomy was performed simultaneously with the ligation of bile ducts. These findings are good evidence to substantiate the observations.

While Iijima and others held that bleeding is the cause of the formation of reticular necrosis but no histological findings coinciding with his view was found in the author's experiments. It has been frequently observed that typical reticular type of necrotic process with congestion was found in the cases of early post operative period. Considering Chapter III and from the report made by Kadomae that a small quantity of desoxynucleodepolymerase was found in blood serum, it is possible to assume that, while the blood serum itself has no activity to produce reticular necrosis, but there might be some factor which would accelerate reticular lesion.

Again, this focus is characterized by the fact that it will disappear in 7 – 10 days and will not be reformed even when the common bile ducts are ligated again. This feature has been discussed by many students. Based on present results obtained and from the previous reports by others, the process of disappearance of this lesion may be elucidated as follows.

That highly concentrated bile escaping from the ampulla of bile ducts is diluted and weakened by the tissue fluid, but the condition can be fully remedied by powerful regenerative capacity of hepatic cells in due course; when this is diluted, bile no longer affects cellular integrity and there is least possible means of developing necrosis.

When, however, bile acid content reaches abnormally high, the hepatic cells are to cease secreting bile. Therefore, when icterus is caused by ligating the bile ducts, the concentration of bile acid in the bile will be fixed after an elapse of time and it will be unaffected. Thus, again, serves as another reason for the fact

that, after a certain time, no progress of necrotic process is observed.

The reason for less cellular reaction can be explained by the fact that this is essentially morbid changes by dissolution or hydropic degeneration of liver cells, and therefore, that when reticular necrosis is fully developed, the inflamatory products which will mobilize cell commonly seen in typical necrotic lesion is no longer possible because of dilution of proinflammatory products reactive to cells.

CONCLUSION

Based on foregoing experimental findings and by incorporating with some of the essential findings previously reported by investigators, the following conclusion has been reached.

1. When rabbit's common bile ducts were ligated to cause jaundice in least possible time, bile becomes highly concentrated to escape from the ruptured ampulla, the adjacent liver cells cause reticular necrosis.

2. It is a condition of advanced hepatic necrosis which is unrelated with transient reduction of stainability.

3. The basic reason for occurrence of reticular necrosis limited only rabbit and not in dog is attributable to the difference in constituent of bile ; that is dissimilarity of physiological action and toxicity of desoxycholic acid and cholic acid which are chief constituents of respective animal's bile, which explained the fact that an experimental biliary cirrhosis can be easily induced in rabbits but not in dogs.

4. Taking into consideration of cholic acid and desoxycholic acid and evaluating the results, the patholoy of reticular necrosis of liver cells in rabbits is no more different than transparent change of hepatic cells in dogs as far as essential nature of degenerative process goes and it should be construed as the matter of degree in its involvement. Thus it is possible to produce a transparent necrosis in rabbits and a reticular necrosis in dogs by experimental means.

5. It is the action of bile acid directly responssible for development of reticular necrosis. The essential pathogenesis may be explained as follows:

Due to the physiological activity of bile, especially the activation of lipolytic enzyme and toxicity of the free bile acid, the cell-wall will be first injured causing transparent appearance, while bile, together with body fluid will migrate intracellularly acting on endoplasmic reticulum and injure cellular components including nucleoprotein, mitochondria and lipoid, and finally liquefy due to cellular expansion due to water-logging.

Thus, the entire cell became hydrapic and except the cell wall undergoes complete dissolution resulting reticular type of necrosis.

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Exposition of Figure

Fig. 1. H. E. Stain, reticular necrosis at an extremely early stage.
Fig. 2. H. E. Stain, reticular necrosis showing a typical construction.
Fig. 3. H. E. Stain, hydropic degeneration is strong, and, in the central zone, protoplasm is seen absolved and swollen, in some zones even isolation of the cell membrane is seen.
Fig. 4. H. E. Stain, one type of reticular necrosis. Compared with the typical case where complete vacuolization has occurred retaining only cell membrane, this is seen still retaining zones where cytoplasm affected with pyknotic nucleus and degenerated necrosis remains.
Fig. 5. H. E. Stain, the shifting part consisting of the part showing a typical reticular structure which showing approximately normal structure, with various degree of degenerated hepatic cells, including those with concentrated nucleus and those with lost nucleus or with the remanant cellular debris.
Fig. 6. Birschowsky's silver stain. The reticula fiber at the zone of necrosis still retains its original form.
Fig. 7. PAS stain. In the zone of reticular necrosis, PAS positive polysaccharide is not seen.
Fig. 8. PAS stain. Lump-shaped PAS positive substance is seen in the perilobular canals and necrosis.
Fig. 9. Thionin stain. In the necrotic area, both the RNA and DNA of hepatic cells show either striking decrease or disappearance.
Fig. 10. Phosphatase reaction (pH 9. 54). Absence of reticular necrosis. Marked reaction is seen in the hepatic tissue around necrotic area, especially in the bile capillaries in the peripheries of the lobules.
Fig. 11. H. E. stain. Example of injection in rabbit bile into the rabbit liver. Colliquative necrosis of liver cells, and, parcially, reticular necrosis are seen.
Fig. 12. H. E. stain. Example of injection in rabbit bile and rabbit serum into the rabbit liver. In part of the liver cells, necrosis showing a striking hydropic degeneration is seen. Around necrosis, infiltration of polynuclear leucocytes is seen.
Fig. 13. H. E. stain. Example of injection in dog bile into the dog liver. Coagulationary necrosis of liver cells in the injected zone is seen.
Fig. 14. H. E. stain. Example of injection in rabbit bile into the dog liver. Dropsical necrosis of liver cell is seen. In the peripheries, congestion, slight bleeding and cell infiltration are seen.
Fig. 15. H. E. stain. Example of injection of desoxycholic acid in the rabbit liver. Necrosis of liver cell and vacuolar change are seen.
Fig. 16. H. E. stain. Example of injection of desoxycholic acid in abdominal cavity. Vacuolization of liver cell is strikingly seen.
Fig. 17. Normal liver cell, semi-circular nucleus, spherical or bacilli-form mitochondria, and, parallel to these, many small layer-shaped endoplasmic reticulums are seen. In part small bodies presumed to be high-density irregular semi-circular fatty granules are seen.

Fig. 18. Liver cell with its common bile duct ligated. In case soon after operation, no striking change is seen in the nucleus and mitochondria. E. R. is deprived of its layer-like structure in the vicinity of capillary duct, and, by degreas, those of vesicular structure are increasingly seen.

Fig. 19. Liver cell with its common bile duct ligated. E. R. of the whole protoplasm is deprived of its layer-like structure, with those of vesicular shape increasing.

Fig. 20. Liver cell with its common bile duct ligated. Shape of nucleus and mitochondria become somewhat irregular. E. R., too, is mostly of vesicular shape, and, in the interior, a small number of high-density small granulos are seen.

Fig. 21. Liver cell with its commen bile duct ligated. The picture of E. R. become indistinct, and, in cytoplasm, small granules, big and small, presumed to be bile granules, are diffusedly seen. Also swelling of capillary duct and cellular space.

Fig. 22. Liver cell with common bile duct ligated. In the swollen E. R. of coarse vesicular shape, high-density micro-circular small bodies are seen.

Fig. 23. Liver cell with common bile duct ligated. In the cell body, a congregation consisting of small granular bodies, presumed to the bile components, is seen.

Fig. 24. Liver cell with common bile duct ligated. From the side of the capillary duct, collapse of E. R. gradually occurs, while, mitochondria is pressed on the opposite side.

Fig. 25. Liver cell with common bile duct ligated. In the cell bodies, formation of vacuoles, big and small, are seen. A small number of fatty drops, also, is seen.

Fig. 26. Liver cell with common bile duct ligated. Vacuoles mutually conglutinate, while, some of them break. Dissolution of cell bodies begins.

Fig. 27. Liver cell with common bile duct ligated. Collapse of the micro-structure of protoplasm is seen in progress.

Fig. 28. Liver cell with common bile duct ligated. The micro-structure of protoplasm completely collapsed and disappeared retaining cell membrane only.

Fig. 1.

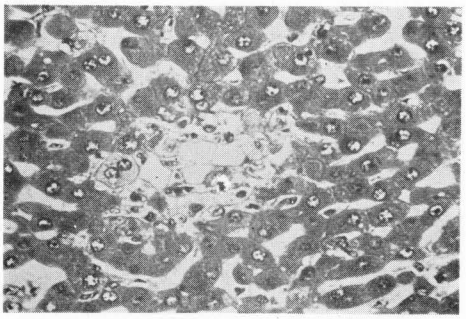


Fig. 2.

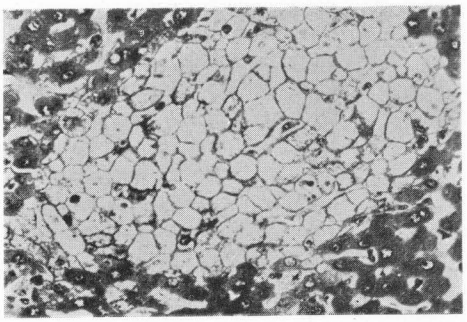


Fig. 3.

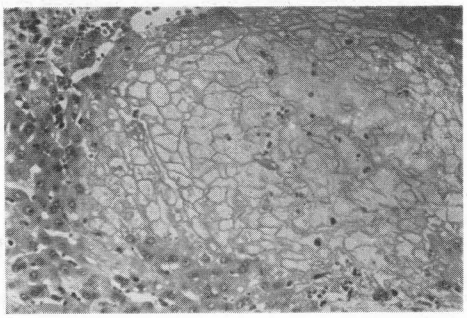


Fig. 4.

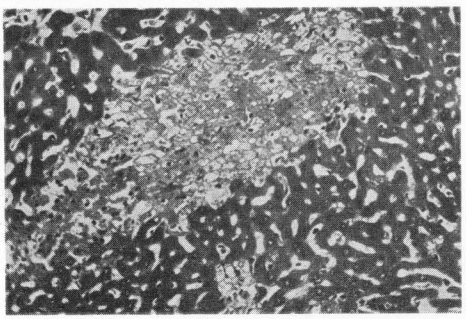


Fig. 5.

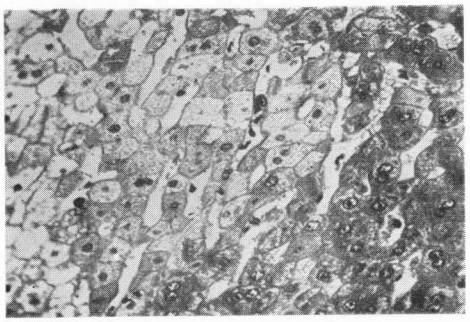


Fig. 6.

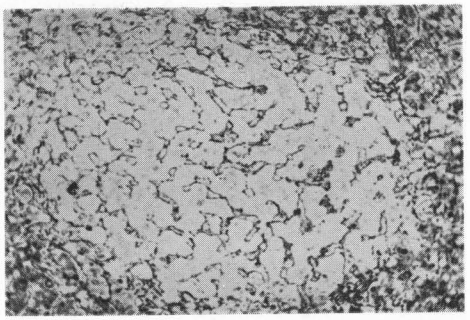


Fig. 7.

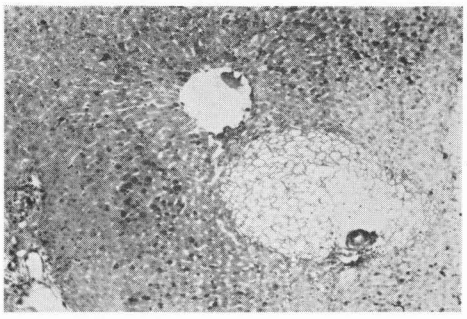


Fig. 8.

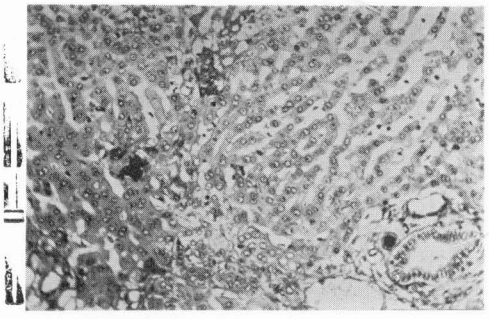
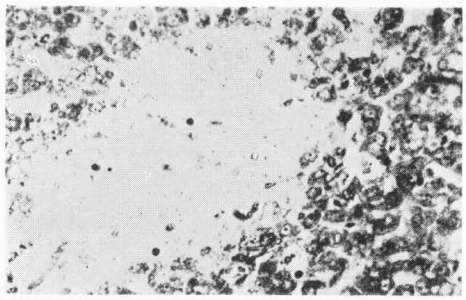


Fig. 9.



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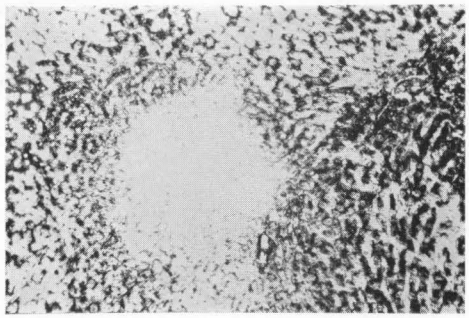

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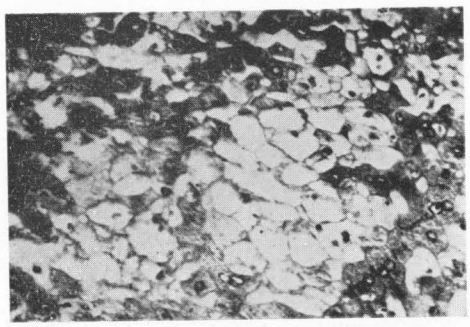

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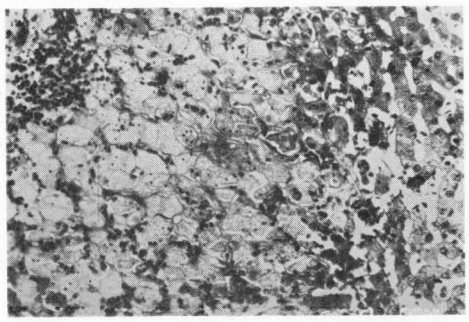

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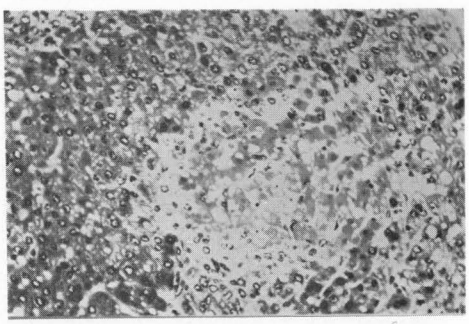

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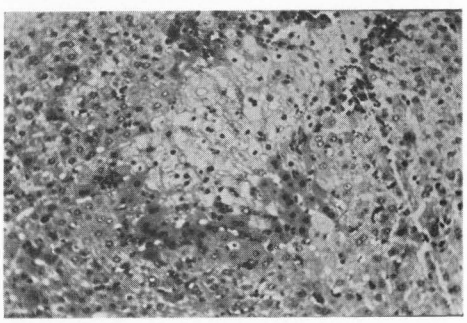

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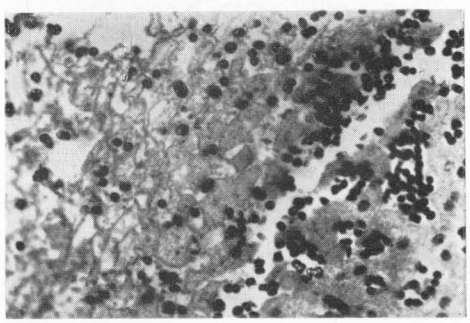

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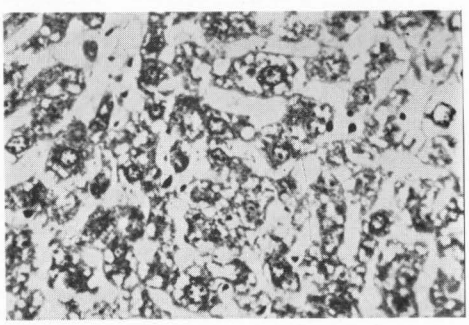

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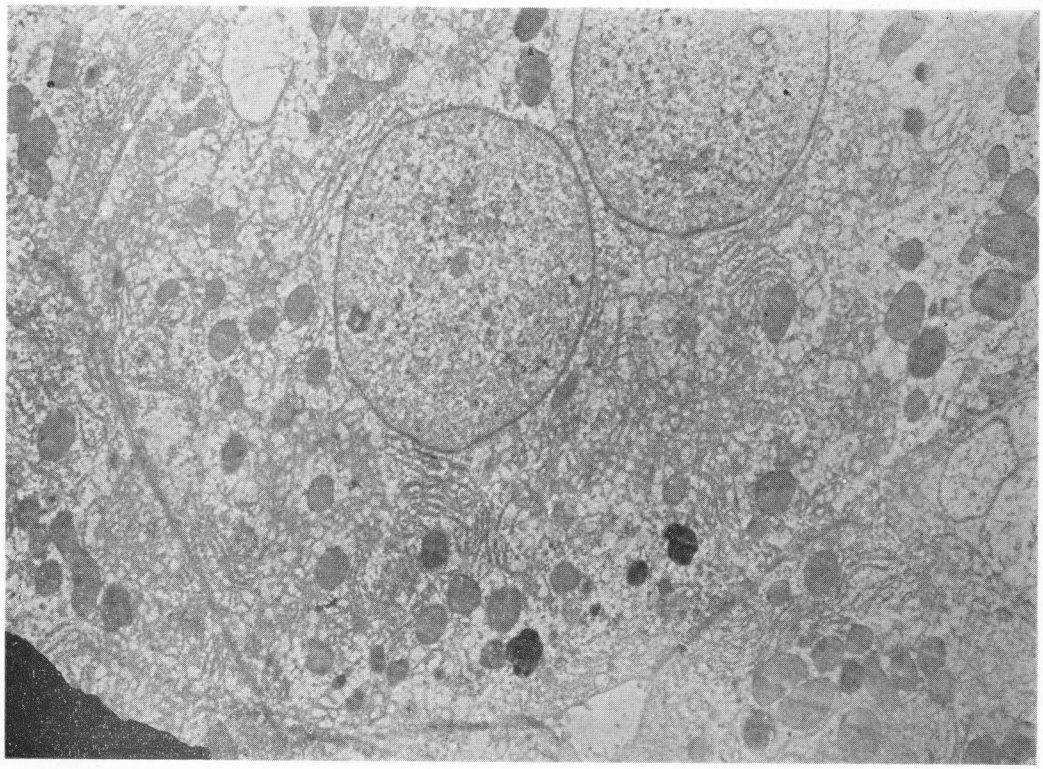

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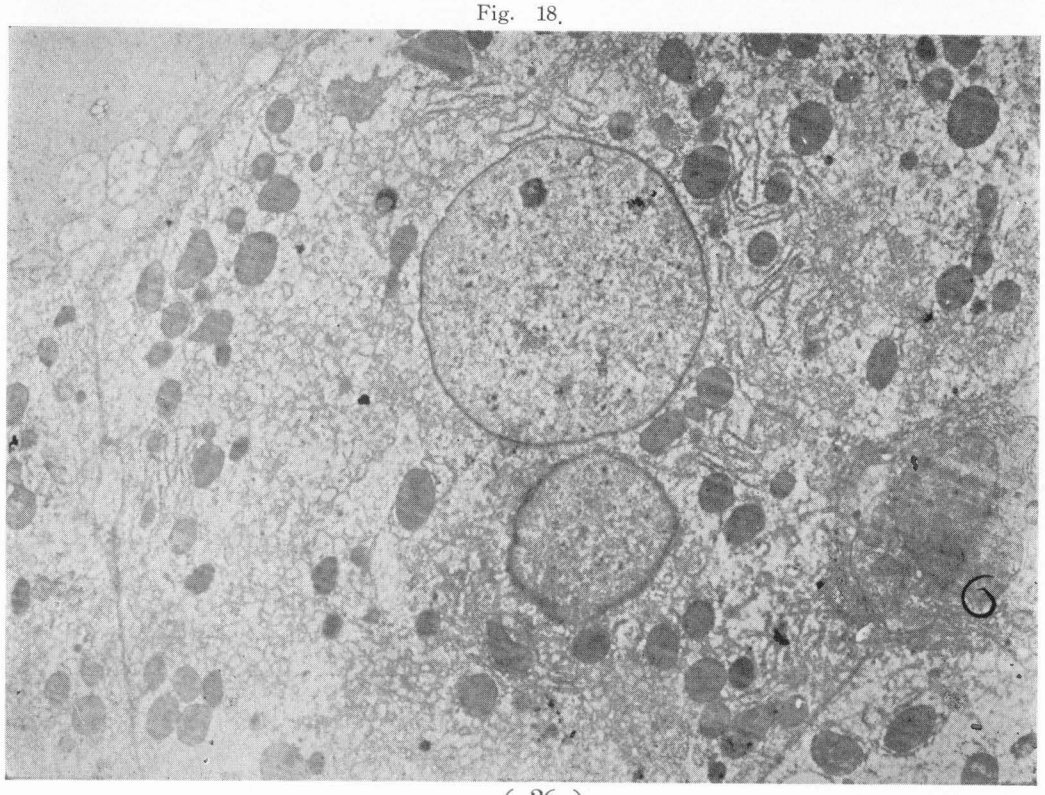

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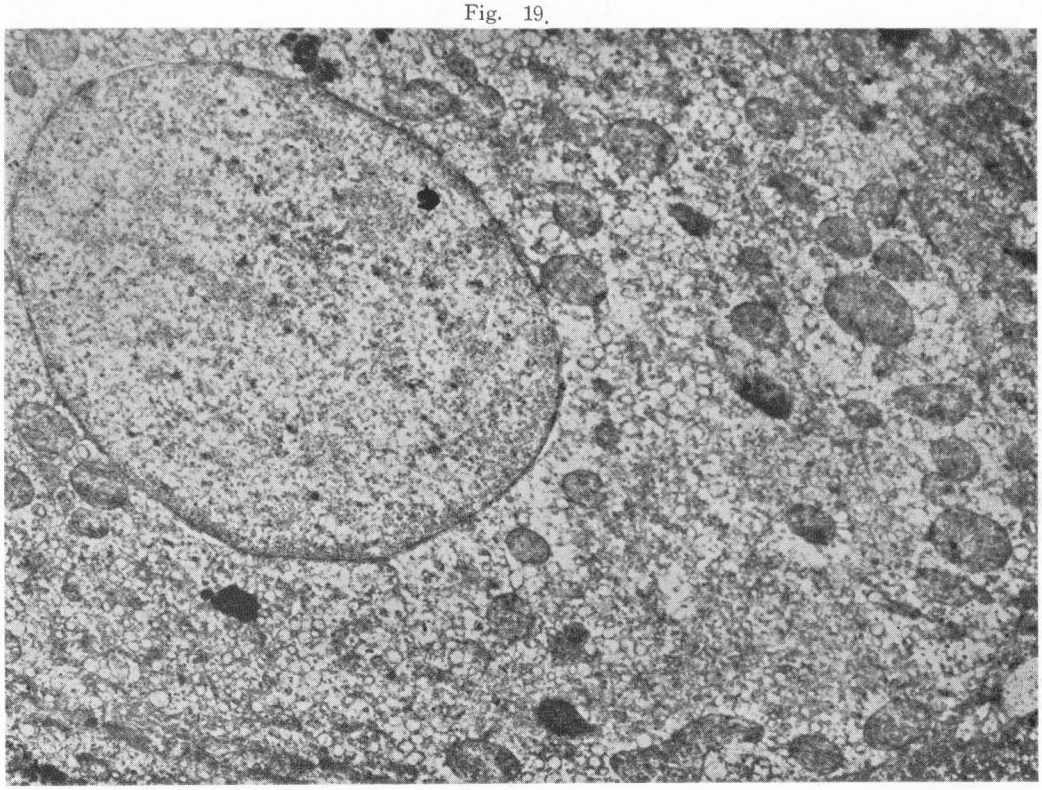

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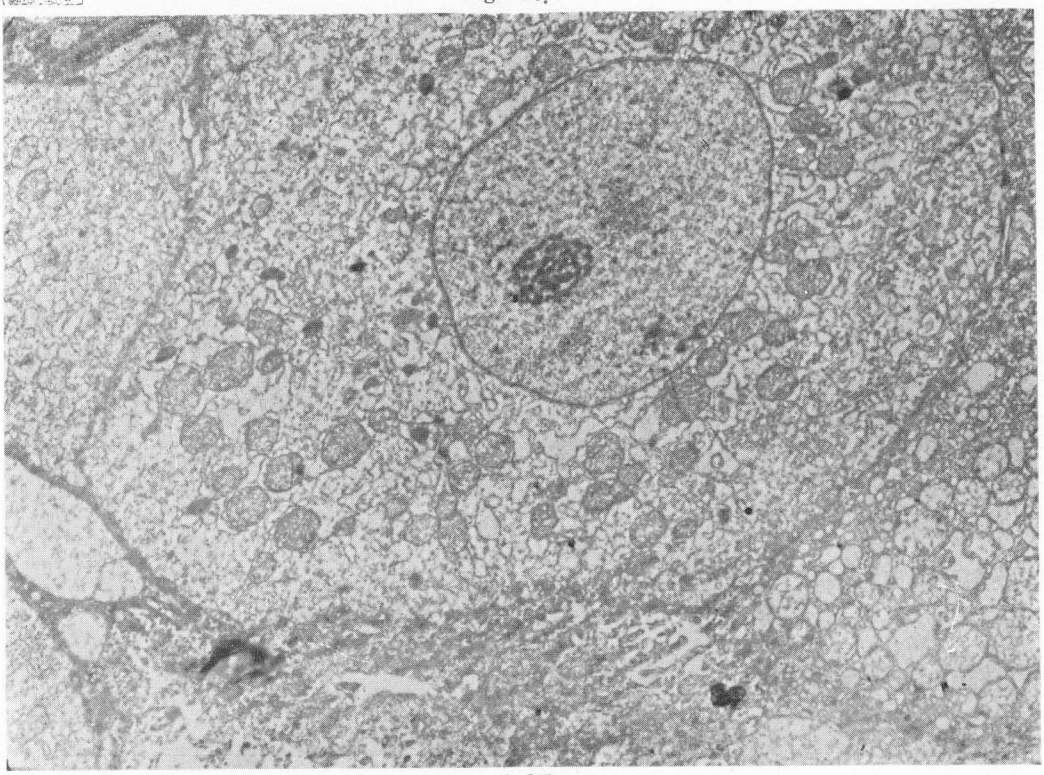

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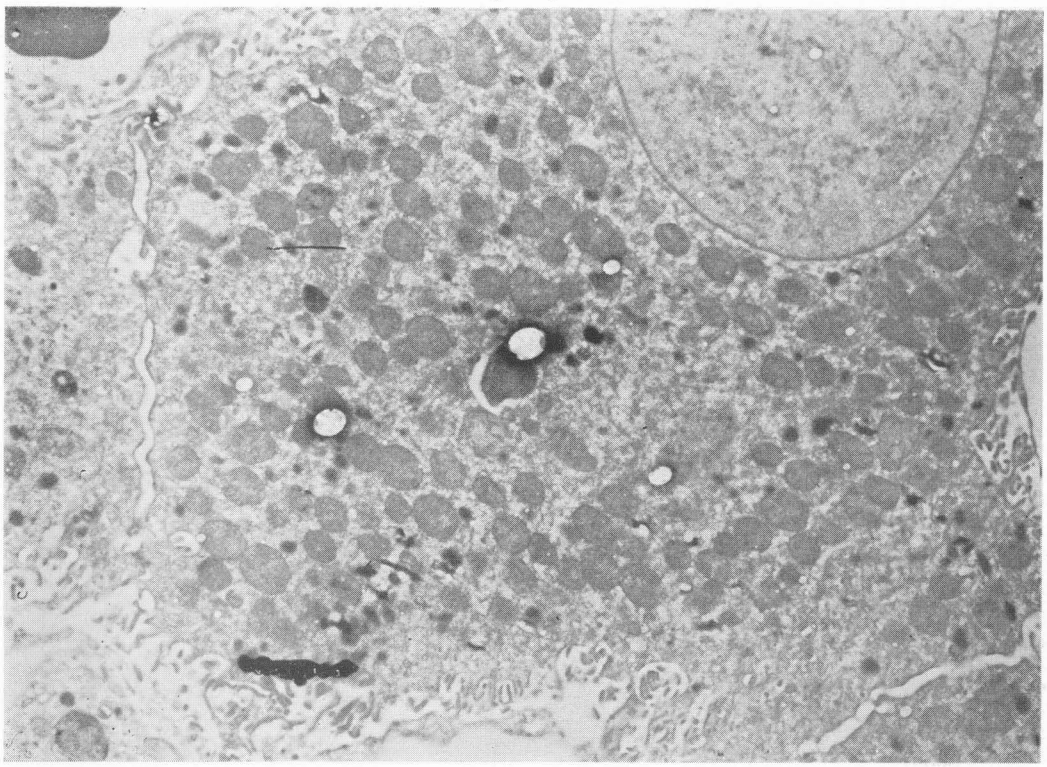

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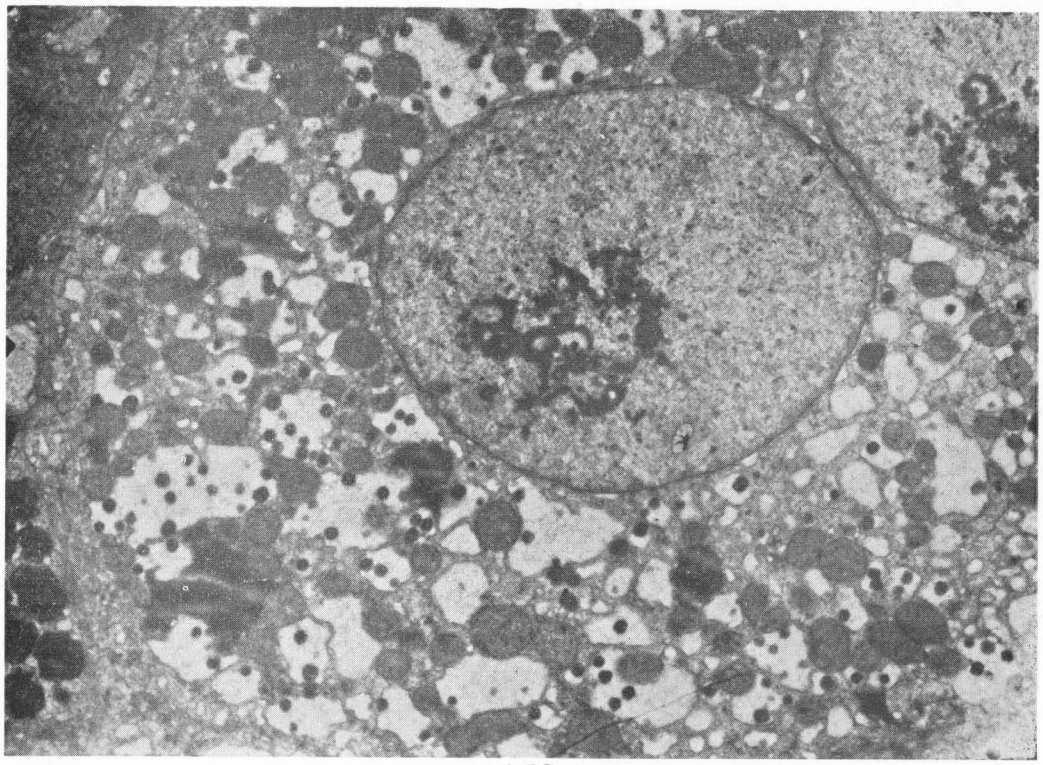

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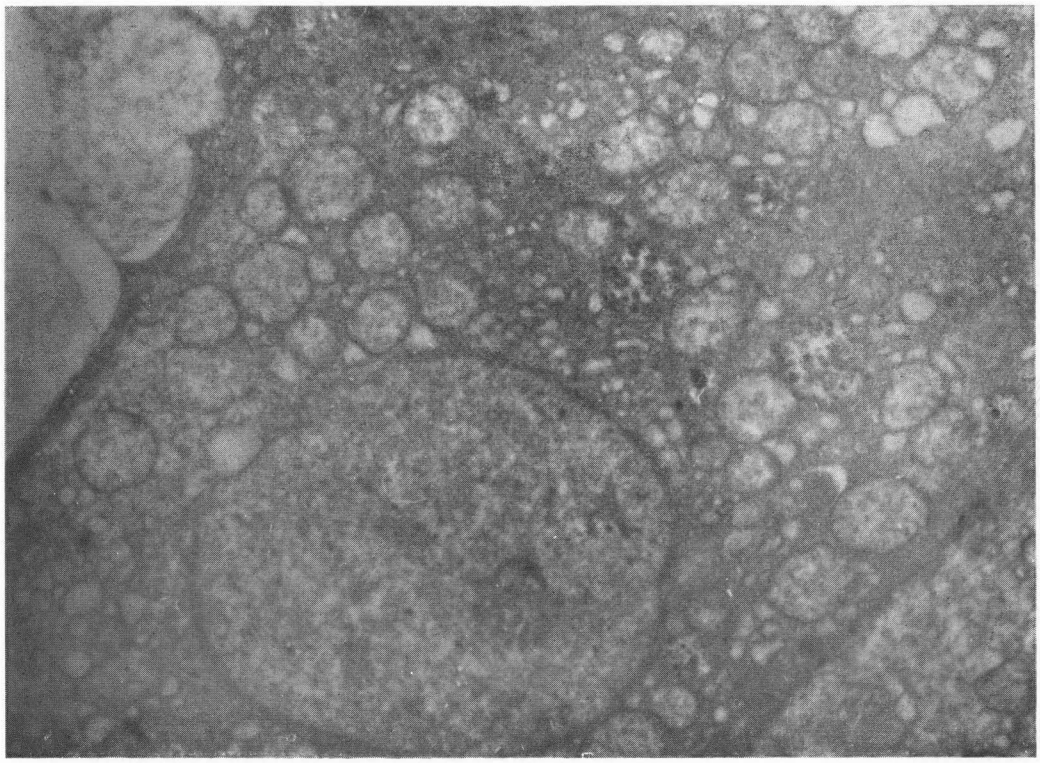

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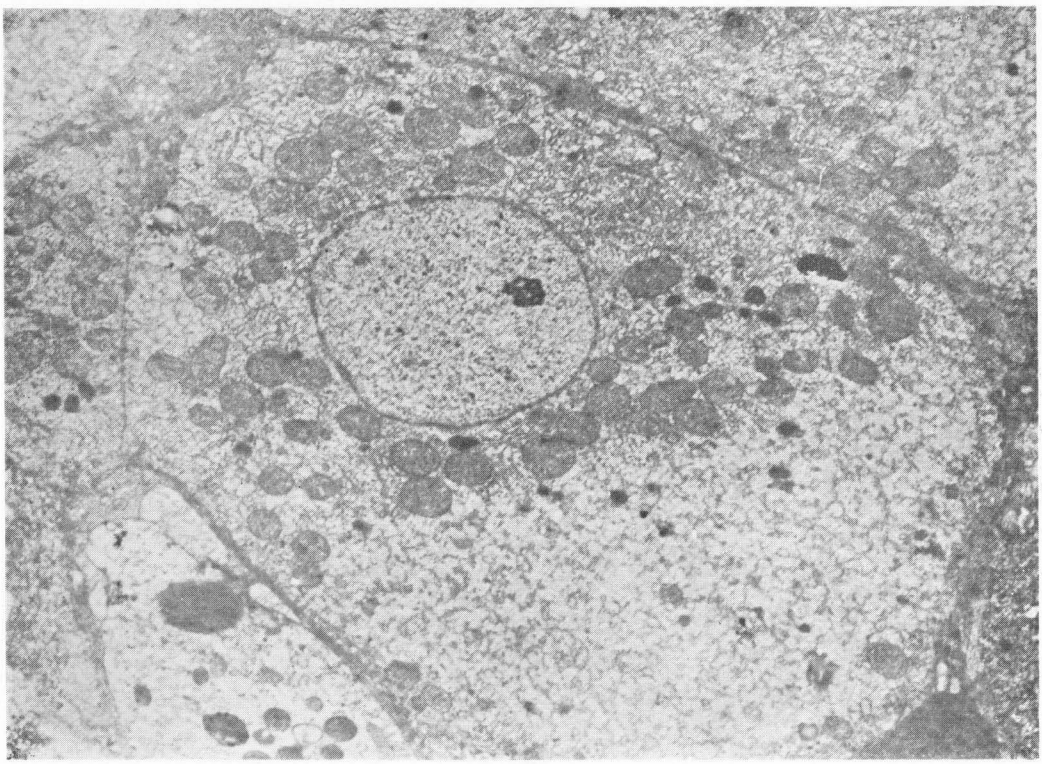

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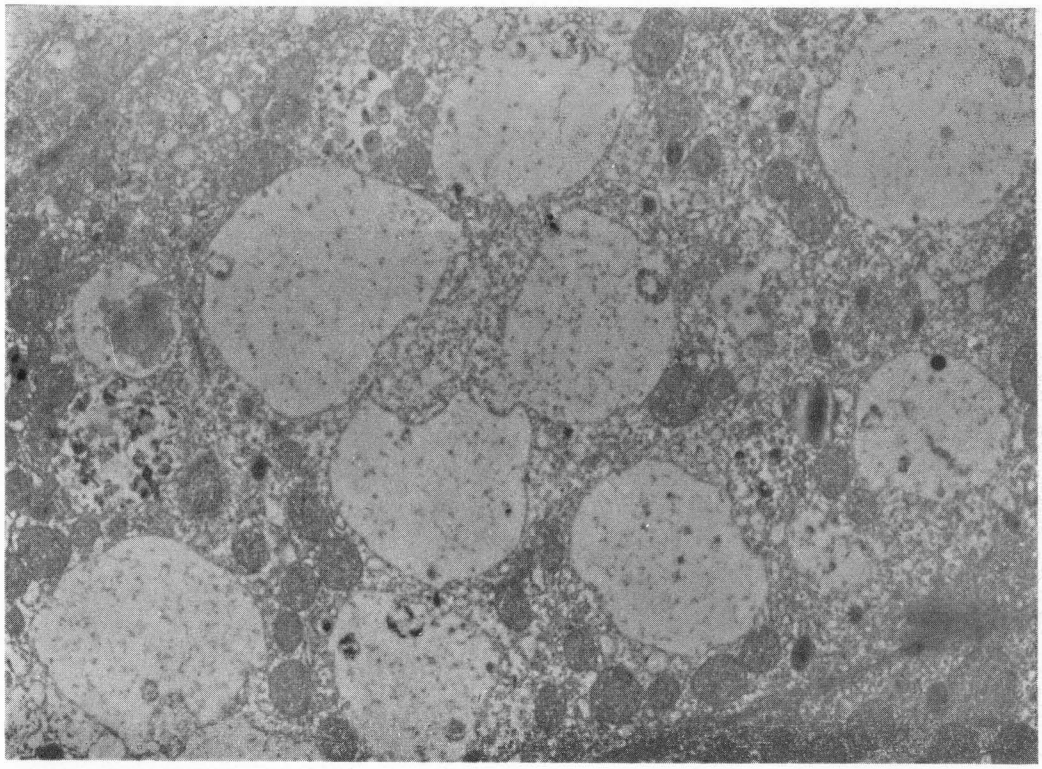

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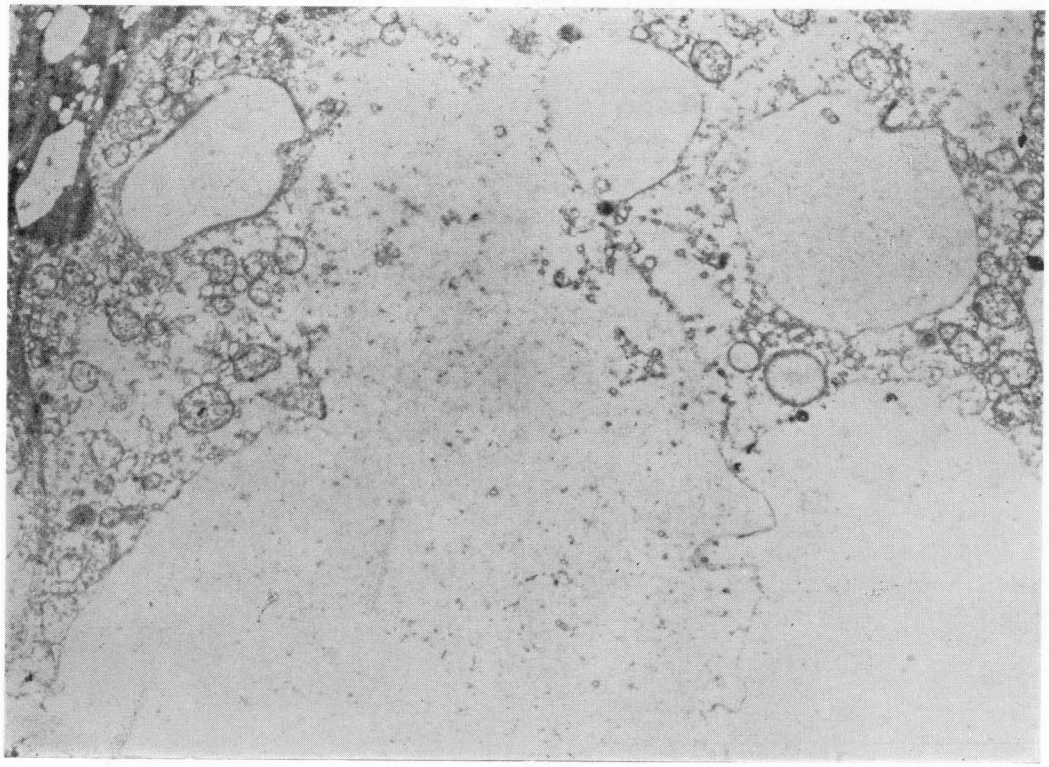

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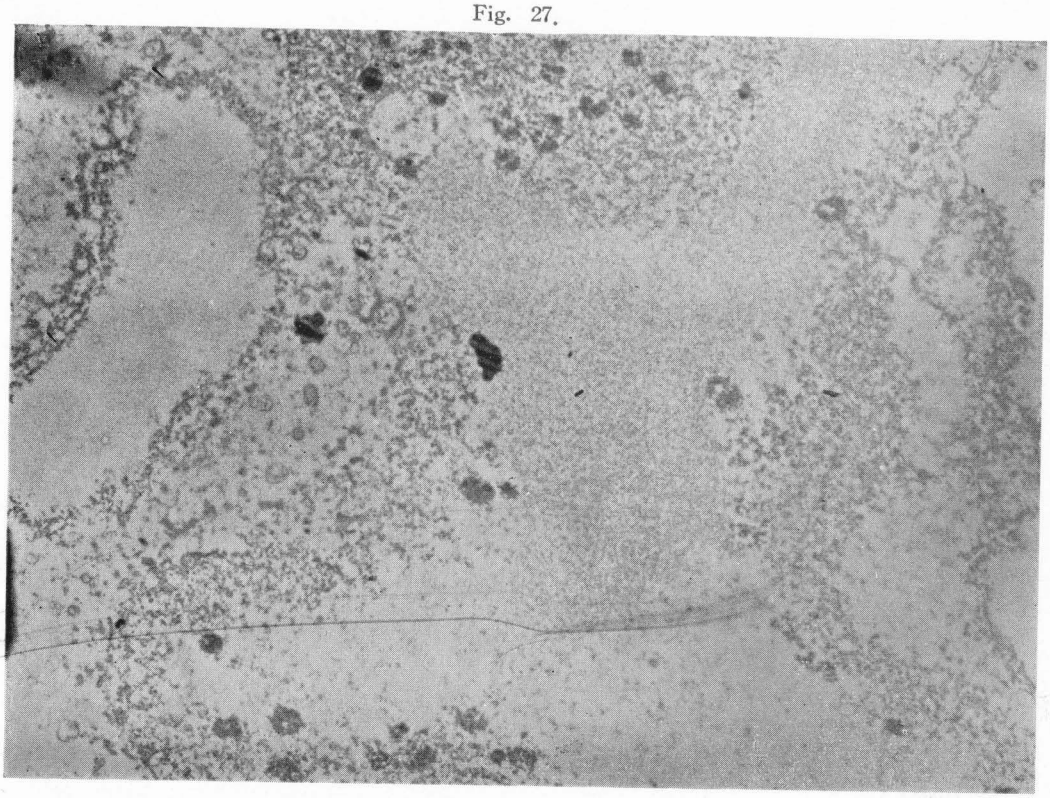

Fig. 28.