HEPATO-ADRENAL NECROSIS WITH INTRANUCLEAR INCLUSION BODIES *

REPORT OF A CASE

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The principal purpose of this communication is to present the description of intranuclear inclusion bodies in the parenchymal cells of the liver and adrenals of a 2 weeks old premature infant in whom the chief pathological findings were a widespread necrosis of the liver and focal cortical necrosis of the adrenals. This case was brought to my attention by Prof. S. Burt Wolbach, who had never observed similar histopathology in his wide experience in the study of diseases of infancy and childhood. So far as the writer is aware no disease of similar nature has been reported in the literature.

REPORT OF CASE

Clinical History: The patient was a 7 months premature female negro infant, 40 cm. in length, weighing 3 pounds. The mother and father were in good health and there was no history of miscarriages. The patient was the mother's first baby and was 16 hours old when admitted to the hospital.

Physical examination showed a drowsy infant who, when aroused, cried lustily. There was a large, soft, non-fluctuant swelling over the right side of the skull and the anterior and posterior fontanelles were normal. There was no jaundice. Her temperature was 97° F. The clinical diagnoses were prematurity and caput succedaneum.

During the first week in the hospital the patient voluntarily ingested increasing amounts of breast milk so that by the end of the week she was taking 35 calories per pound. The swelling over the parietal region of the skull slowly disappeared and she became less active. On the eleventh day of life it was necessary to give nourishment by gavage. Her temperature gradually rose to 100° F on the twelfth day. On the thirteenth day she was transfused with 30 cc. of citrated blood obtained from her father. Four hours later her diaper was stained with blood. A small amount of blood persisted in the feces and was visible in the food which she soon began to vomit. No unusual degree of jaundice was noted. Her temperature fell to 97° F, but she died about 12 hours after the transfusion, at 14 days of age. The discharge diagnoses were prematurity and caput succedaneum.

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Autopsy Protocol

The autopsy was performed 3 hours after death.

Heart: The heart weighed 15 gm. The patent foramen ovale measured 5 mm. in diameter. A delicate row of pale red, small, granulation-like structures were found on the mitral valve.

Lungs: The right lung weighed 27 gm. and the left 22 gm. They were uniformly pink and normally crepitant except in the dependent portions where they were dark red in color and less crepitant than elsewhere. Cross-sections of the parenchyma showed numerous small red areas, which were interpreted as foci of hemorrhage rather than of pneumonic consolidation.

Spleen: The spleen weighed 10 gm., was dark red and firm.

Alimentary Tract: The stomach was small and contracted. There were a few small mucosal hemorrhages. The ileum and colon were normal.

Pancreas: Normal.

Liver: The liver weighed 75 gm. (birth weight of the liver of a normal full term infant 78 gm.). The enlargement seemed to be confined principally to the left lobe. The consistence was diminished and the surface mottled, there being numerous, irregular, grayish yellow to pale brown areas varying from less than 1 to 4 mm. in diameter. These were separated by broader zones of reddish brown parenchyma. The grayish yellow areas usually were discrete but in several instances were confluent. In the centers of several of these scattered regions were minute hemorrhagic spots. The lesions were not elevated above the plane of the surface of the liver. No exudate was present on the surface of the overlying capsule. The interior of the liver, exposed by sectioning, showed a fairly uniform distribution of the gross lesions throughout the parenchyma. No abscesses were visible.

Gall-Bladder: The gall-bladder was filled with pale brown bile less viscid than normal. Bile was expressed easily through the cystic and common ducts into the duodenum.

Kidneys: The right weighed 10 gm. and the left 9 gm. The fetal lobulations were prominent. The capsules were not adherent to the cortical substance. The cortex, medulla and pelvis of each organ were normal except for one small hemorrhagic area in the cortex of the left kidney.

Adrenals: The adrenals, which together weighed 4 gm., were normal.

Pelvic Organs: Normal.

Brain and Spinal Cord: The brain weighed 180 gm. and was considered to be normal at the time of removal. After fixation in formalin a note was made which stated that the substance of the brain was soft and fatty. The spinal cord was normal.

Bone Marrow: The bone marrow in the vertebral bodies was bright red in color.

Bacteriology: A culture of blood removed from the right auricle showed *Bacillus coli* and *Staphylococcus albus*. The same organisms were obtained from the peritoneal cavity by cultural methods.

Microscopic Study

Heart: There is a slight interstitial infiltration with polymorphonuclear leukocytes which is localized to the perivascular tissues. In rare instances the adjacent muscle fibers show minimal evidence of necrosis. No blocks were taken through the peculiar structures on the mitral valve. They may have been small "blood cysts," which are not uncommon in infancy.

Lungs: There is evidence of immature development. Many groups of alveoli are not fully distended. Other groups are overdistended and interalveolar septa often are ruptured. Occasional bronchi contain an acellular, granular, eosinophilic débris in which are numerous bacteria of variable morphology. This débris probably represents aspirated foreign material. In several alveolar spaces are extravasated red blood cells, an albuminous precipitate, asphyxial membranes and entrapped air. There is no inflammatory reaction.

Spleen: The follicles are small and of immature type and the sinusoids are distended with red blood cells. Small areas of hematopoiesis are composed largely of cells of the myeloid series. Numerous mononuclear phagocytes laden with hemosiderin are present. One small colony of cocci which seems to have stimulated no regional inflammatory reaction is found. Although the nucleoli of several of the lining cells of sinusoids are prominent there are no inclusion bodies similar to those which are found in the liver and adrenals.

Pancreas: Except for the presence of pancreatic tissue beneath the mucosa of the duodenum, no unusual histological findings are seen.

Ileum: Sections of the ileum disclose no lesions, but colonies of bacteria are seen in several vessels.

Kidneys: The only histological features of interest are those of immaturity and cloudy swelling and vacuolation of the cytoplasm of the convoluted tubules.

Urinary Bladder: No abnormal findings are noted.

Thymus: Normal.

Vertebra: No important changes are seen in the bone or bone marrow.

Brain and Spinal Cord: Blocks were taken from the cerebral cortex and the cerebellum but there are no lesions. The spinal cord shows a normal structure.

Liver: Blocks of liver were fixed in Zenker's fluid and formalin. Material which had been in formalin for 6 years was mordanted in Regaud's fluid for 48 hours. The tissue was embedded in paraffin and sections of from 5 to 7 microns in thickness were cut. The following stains and staining methods were used: hematoxylin-eosin, eosin-methylene blue, Wolbach's modification of the Giemsa stain, Gram-Weigert's method for the demonstration of bacteria, Ziehl-Neelson's carbol fuchsin stain for acid-fast organisms, Levaditi's technique for spirochetes, Mallory's anilin blue-acid fuchsin-orange G collagen stain, and Mallory's phosphotungstic acid hematoxylin.

All sections show an extensive acute necrosis with a few widely isolated clusters of viable liver cells remaining as a rule around the portal areas. The pale yellow areas which were noted in the gross specimen differ histologically from the intervening reddish brown tissue in that they represent zones where there is a most severe type of degeneration. In these regions the lobular architecture is indistinguishable. There are no continuous cords of liver cells and the sinusoidal system is disrupted. Remnants of liver cell cytoplasm, pyknotic fragments of nucleoplasm and degenerated cellular elements of the blood are fused into a conglomerate mass of débris which fills and obliterates the sinusoidal spaces and central veins. The portal structures often have succumbed, but they appear to be the last part of the structural unit to have undergone necrosis. Very little fibrin is present, although occasional necrotic vessels filled with remnants of fibrinous thrombi are found. There are no abscesses or significant local accumulations of leukocytes.

The friable reddish brown tissue which lies between the yellow

areas of necrosis shows a similar type of degeneration but the necrosis in these regions has not advanced to such a complete state of colliquation. The shadowy outlines of interrupted columns of liver cells separated by indistinct, though partly intact, sinusoids are visible amid the masses of cellular débris. Numerous extravasations of blood cells are present but most of the blood seems to be confined in the irregular, distorted sinusoidal spaces. Even though the major portion of the parenchyma is necrotic, the red blood cells appear to be much more viable in the reddish areas than in the yellow zones which have been described above. An occasional central vein is detected. These veins rarely are dilated or filled with fibrin networks in which partially degenerated blood cells are enmeshed. Portal structures and periportal connective tissue usually can be recognized but often they are involved by the necrotizing process, which seems to spread from the parenchyma, invade the periportal connective tissue and destroy, first the collagen, secondly the ducts, and finally the blood vessels. The portal veins often are dilated and, occasionally, when involved by the spread of the primary process, they are filled with fibrinous thrombi which blend at their margins with the indistinct outlines of the swollen, homogeneous, degenerated vascular wall. The reaction to this injury is very slight. The polymorphonuclear leukocytes are slightly increased in number but there is no proliferation of bile ducts or connective tissue cells.

Study of the widely separated small islands of viable liver cells and the gradual transition through various stages of degeneration in the zones bordering on the areas of necrosis discloses the most significant histological findings. These clusters of cells comprise about one-tenth of the total volume of the liver. They usually lie adjacent to the portal areas and in no instance is there a wholly intact lobule. As a rule, the architecture is almost normal, either in the center of the groups of cells or in that part which is in apposition to the periportal connective tissue. By arbitrary reconstruction of the process it would seem that the disease in the beginning must have affected the central and midzonal regions of the primary lobule.

The description of a typical viable remnant of hepatic parenchyma (Fig. 13), as studied in the sections fixed in Zenker's solution and stained by Wolbach's modification of the Giemsa stain, may suffice to exemplify a fairly uniform picture. The portion of this irregular, poorly demarcated island of cells which is adjacent to the periportal

connective tissue shows an orderly arrangement of liver cells and sinusoids. As one progresses toward the central veins an increasing number of structural alterations becomes apparent, until finally the disintegrating columns of abnormal liver cells and distorted sinusoids blend imperceptibly into the surrounding necrotic mass of liver structure.

The appearance of the individual liver cells, and especially their nuclei, is of principal interest in this study. In the periportal zone many cells are normal in size and shape. The cytoplasm is pale bluish pink, normal in structure, and the nuclei are round or oval. Delicate chromatin networks ramify throughout the nucleoplasm. The nucleoli usually are slightly eccentric in position. Among these cells, which are apparently normal, there are a few cells in which only the nuclei show abnormalities. In the first place the nuclear membranes are slightly irregular or undulate. Secondly, the chromatin networks are indistinct in the center of the nucleus and there is a definite tendency toward an accumulation of chromatin in the zone which lies adjacent to the nuclear membrane. Thirdly, most nucleoli are situated adjacent to the nuclear membrane. In these cells the relative homogeneity of normal cytoplasm is changed to a delicately granular or reticular substance. The granules are slightly acidophilic and are more deeply stained than the background of pale cytoplasm.

As one progresses from the relatively normal periportal zone of liver cells an increasing number of cytological abnormalities becomes apparent. Adjacent to the necrotic parenchyma almost all the cells exhibit the peculiar changes which characterize this malady. The unique histopathology is restricted to the nuclei, although as a rule there are attendant cytoplasmic changes of variable nature.

The nuclei for purposes of description may be divided into two groups: first, those in which there are acidophilic intranuclear bodies and, secondly, those that are characterized by abnormal basophilic intranuclear structures. A certain number of nuclei serve to exemplify the morphological and tinctorial gradations between these two principal groups.

The nuclei which contain acidophilic bodies are more numerous than the other types. The smallest bodies are found not infrequently in cells which otherwise appear to be normal. More commonly there are detectable cytoplasmic and nuclear changes. The cytoplasm

often is swollen, granular and delicately vacuolated. In the early stages the nuclei are appreciably enlarged, the chromatin networks are altered and the nucleoli are either eccentric in position or are in apposition to the nuclear membrane. In such cells minute pink granules of irregular contour appear between the partially disrupted strands of deeply basophilic chromatin which seems to be maintained distinctly apart from the acidophilic bodies (Fig. 1). The strands of chromatin gradually disappear in the center of the nucleus and preceded by the nucleolus the remnants of chromatin retreat toward the nuclear membrane, leaving a central area into which the acidophilic granules migrate (Fig. 2) so as to fuse eventually into a single, pale pink, amorphous, irregular mass of a slightly deeper red tint than the elementary bodies of which it is comprised (Fig. 3). Occasionally, heavy strands of chromatin retain their position and traverse the diameter of the nucleus in such a manner as to maintain barriers between the agglomerating unit bodies. In such instances two and rarely three distinct acidophilic masses become segregated in the divided zones (Fig. 4). As a rule a single amorphous mass occupies the center of the nucleus. As it condenses, it becomes more homogeneous and the nucleolus and chromatin retreat farther and farther so that eventually they become aligned along or intimately fused with the nuclear membrane (Fig. 5). During this stage the nucleus usually decreases in size and the nuclear membrane becomes at first serrate, and then undulate, thickened and crumpled. Finally, the fully formed inclusion body, which is deeply acidophilic, homogeneous, and well circumscribed with a sharply defined margin, lies in the center of the nucleus surrounded by a clear halo that separates almost its entire circumference from the thickened, undulate, nuclear membrane (Fig. 6).

The second variety of changes in the nuclei is almost as common as the developmental sequence which has been described above. In a few respects the two processes are similar and it is impossible to determine whether certain intranuclear bodies are a part of the first or the second theoretical sequence of changes.

In the following description an attempt has been made to reconstruct the steps in the development of the second type of intranuclear bodies. The well preserved periportal parenchymatous cells contain a few examples of the early stages. As one progresses toward the less viable central zones a great many nuclei are affected and the more

advanced stages become the most interesting feature of the histology. The earliest stage seems to be preceded by an increase in the size of the nucleus. The strands of chromatin become interrupted, chromatin material loses its affinity for basic dyes and the chromatin as well as the nucleolus seems to disappear as if by lysis. Tiny round, and often sharply defined, regularly spaced, pale blue granules appear and in almost every instance seem to fill the entire nucleus (Fig. 7). Rarely there are persistent remnants of chromatin and nucleoli which are marginated along the nuclear membrane. These delicate granules, which can be resolved definitely into distinct unit structures, occasionally are amphophilic or lightly acidophilic, but even when acidophilic their uniform punctate appearance tends to segregate them from the small, irregular pink granules which seem to form the elementary units of the inclusion bodies of the first type. Nevertheless, there are certain nuclei in which the elementary bodies of the second type are surrounded by a clear zone which partially separates them from the nuclear membrane (Fig. 8). In these nuclei one can imagine a series of gradations through which a typical inclusion body of the first type might have been formed. However, this is not apparent. There is a tendency for these small round granules to maintain their basophilic or lightly acidophilic nature and to fade into a structureless homogeneous nucleoplasm which varies from pale pink to dark blue and almost invariably fills the entire nucleus (Figs. 9 and 10). As one approaches the zone of necrosis gradual dissolution of parenchymatous cells supervenes and the homogeneous nuclei become dark blue to purplish red (Fig. 11). The nuclear membrane, at first delicate and distended, in the progressive stages becomes thickened and irregular or serrate. Finally it seems to fuse with the intranuclear plasm as the liver cell shows evidence of disintegration (Fig. 12). The cytoplasm of the liver cells that exhibit this peculiar general type of intranuclear morphology is usually pale, delicately granular, slightly vacuolated and swollen in the early stages. The cytoplasm in the later stages becomes less granular, more homogeneous and more intensely basophilic or acidophilic. In the marginal areas of necrosis where deeply basophilic and acidophilic nuclei are abundant the cytoplasmic membranes are no longer detectable and groups of liver cells seem to have flowed together to form irregular cytoplasmic masses containing several closely approximated, circular or elliptical, basophilic or

lightly acidophilic bodies, which represent the remains of the nuclei and their homogeneous content (Fig. 12). Eventually, there is complete dissolution, often preceded by a loss of differential staining reactions.

The various special stains are of no significant value, but it seems worthwhile to record briefly the staining reactions of the intranuclear bodies in tissues fixed in Zenker's fluid. In general, all granules and the fully formed acidophilic inclusions are colored pale red by Mallory's anilin blue-acid fuchsin-orange G collagen stain. The homogeneous variety of the second sequence of nuclear change is pale red to dark maroon (Figs. 10 and 11). After hematoxylin and eosin the Gram-Weigert method for the demonstration of bacteria stains the typical large intranuclear inclusions dark red or maroon (Figs. 5 and 6). The punctate granules, as described in the second series of changes, are pale purple (Figs. 7, 8 and 9), while those filled with the homogeneous substance are magenta (Fig. 11). Mallory's phosphotungstic acid hematoxylin stains the abnormal intranuclear granules a pale purple. Not infrequently they exhibit a slight orange tinge. The typical intranuclear inclusions are dark purple (Figs. 5 and 6). The homogeneous nuclei vary from pale purple to almost black (Figs. 10 and 11). The inclusions do not retain the Ziehl-Neelson carbol fuchsin stain for acid-fast organisms. The sections stained with hematoxylin-eosin and eosin-methylene blue are comparable to those which are stained with Giemsa. The intensity and sharpness of detail, as obtained by Wolbach's modification of the Giemsa stain, make these sections most satisfactory for study.

An attempt was made to demonstrate bacteria. The Ziehl-Neelson carbol fuchsin stain discloses no acid-fast organisms. The tissues which were treated by Levaditi's method for impregnation of spirochetes contain no demonstrable treponema. The Giemsa and eosin-methylene blue stains disclose rare clumps of bacilli in the liver and adrenals. They are Gram-positive, and are not situated specifically in areas of necrosis. Similar organisms unaccompanied by necrosis are found in the lungs, spleen and vessels of the wall of the ileum.

Adrenals: In the peripheral subcapsular portions of the cortex of each adrenal gland there are numerous small focal areas of necrosis. The fundamental changes in these areas are similar to those in the broad fields of degeneration in the liver. The lesions are so small that many can be included in a high dry microscopic field. All ne-

croses are acute. The primary histological changes suggest an autolytic type of parenchymal degeneration followed by disruption of sinusoids and consequent extravasation of red blood cells. In the more advanced lesions an agglomeration of the necrotic parenchymal elements and blood cells into structureless masses is characteristic. No significant inflammatory reaction is present.

One's attention, here, as well as in the liver, is attracted by the peculiar morphological alterations in the parenchymal cells. The great advantage in the study of the small early lesions is that the first stages of cellular degeneration and the progressive changes in the nuclei and cytoplasm are more clearly defined than in the liver. Intranuclear bodies of varied character which are identical with those in the liver cells are always present in the lesions. The morphological variations in the nuclear chromatin seem to precede or accompany the development of the intranuclear structures. The changes in the cytoplasm of most of the cells appear to follow the changes in the nuclei, because many cells, especially at the periphery of the lesions, have prominent intranuclear bodies without detectable abnormalities of the cytoplasm. The earliest evidence of cytoplasmic change consists of swelling, diminution in affinity for acid stains, reticulation and vacuolation. The cytoplasmic membrane is distended and the cell tends to be circular in outline. This is not accompanied by any apparent local increase in vascularity or significant disturbance of the general structural relations of the various cortical elements. In the more advanced lesions the cytoplasm is disintegrated and the cytoplasmic membrane often is disrupted. The nuclei and intranuclear bodies are almost indistinguishable. The vascularity is increased. The most severe lesions are characterized in their central portions by a fusion of the necrotic parenchymal elements and extravasated blood cells into structureless acidophilic masses. Peripherally, the progressive stages of cellular degeneration are found. In all instances the almost complete absence of leukocytic infiltration in the involved areas and the scant evidence of crystallized fibrin are consistent and inexplicable findings.

Discussion

It is not within the scope of this presentation or within the range of the writer's experience to enter at great length into the spirited

polemics that have characterized the dissertations of morphologists and bacteriologists concerning the nature and significance of "inclusion bodies." We know that there are certain viruses which have many of the properties of living matter, that these agents are ultravisible in size, that they may pass through the pores of filters which withhold ordinary bacteria, that they are capable of producing disease and that the pathology of the disease is characterized by the presence, singly or in combination, of intranuclear or intracytoplasmic masses which are called "inclusion bodies." The similarity of structure and mode of formation of inclusion bodies in different filtrable virus diseases often make it difficult to distinguish the type of virus disease by a microscopic study of the inclusion bodies. Nevertheless, there frequently are certain histological differences which may enable one to classify the virus on the basis of the morphology of the inclusions which are associated with it. These differences need not be considered fully in this presentation.

The morphology of the intranuclear bodies in the present case will allow, within the limits of our knowledge, but one conclusion. Here we must be dealing with a disease which was produced by a hitherto unknown virus, filtrable in nature and of small physical dimensions, or by a known virus which has selected unusual sites for localization and which has exhibited its pathogenicity in an unique manner. There is no justification for assuming that the unit structures, which at times could be resolved as tiny granules, were actual single microorganisms. Neither is there any justification for assuming that they are not the virus bodies or clusters of those bodies, which must have been instrumental in the production of the extensive hepatic and adrenal necrosis. In this regard it may be said that certain rickettsiae, such as the *Dermacentroxenus rickettsii* of spotted fever, have been accepted as pathogenic microörganisms. These may inhabit the nuclei of cells in ticks (Wolbach[1]). They have been cultivated in tissue cultures in the nuclei of infected mammalian mesenchymal cells (Pinkerton and Hass[2]). These microörganisms are often no larger than the elementary bodies, especially those of basophilic nature, in the nuclei of the parenchymatous cells of the liver and adrenal of the present case. The same may be said in regard to the elementary bodies of many other virus diseases. In Zenker-fixed tissues there is an undeniable resemblance between the intranuclear microörganisms of spotted fever and the intranuclear structures in

Figure 7. Not only is this true but it is quite apparent that the inclusion bodies which are formed by intranuclear masses of spotted fever rickettsiae, as demonstrated in tissue cultures, are similar to certain intranuclear inclusion bodies in various filtrable virus diseases.

It has been contended by certain authors that the acidophilic nature of inclusion bodies in general militates absolutely against the belief that they are composed of microörganisms. This does not seem entirely valid because in the present instance, as well as in herpes and experimental spotted fever, the inclusions and their constituent parts may be basophilic, amphophilic or acidophilic. Neither can such a simple criterion as the staining reaction be depended upon to indicate whether or not the virus inhabits the sphere of the inclusion body. It seems that the restrictions of microscopic vision will not allow the student to transgress the barrier, which arbitrarily has been thrown up between visible viruses which are recognized as intracellular inhabitants, and the ultravisible viruses which are characterized by the presence of intracellular inclusion bodies. A few workers, notably Goodpasture,[3] have produced evidence that the ultravisible filtrable virus may be intimately associated with the inclusion body. A further important bond of similarity is that the continued cultivation of so-called filtrable viruses, as well as the rickettsiae, depends upon the presence of living cells in the medium. It is difficult to draw hard and fast lines between the two classes of pathogenic agents, one ultravisible and characterized by inclusion bodies and the other visible and characterized by intercellular masses of microörganisms similar to inclusion bodies.

Let us compare briefly the present disease with those virus diseases that give rise to intranuclear inclusion bodies in the liver of man or animal, and with those instances in which inclusion bodies have been found in the liver independent of any established cause.

Yellow fever is a disease which presumably is caused by a filtrable virus that gives rise to necrosis of the liver and intranuclear inclusion bodies in the parenchymal cells. The inclusion bodies are very infrequent in human cases but are commonly found in the livers of monkeys in which the disease has been produced experimentally (Klotz and Belt,[4] and Cowdry and Kitchen [5]). A comparison of the inclusions of the present case with those of experimental yellow fever in monkeys revealed superficial resemblances between a few

selected inclusion bodies. On the whole the morphological changes were unlike those of yellow fever.

Intranuclear and intracytoplasmic inclusion bodies have been noted from time to time in the liver, lungs, pancreas, thyroid, adrenals, kidneys and salivary glands of infants. A group of 25 cases was reported by Farber and Wolbach.[6] The inclusions in these cases apparently were identical with those which various authors, especially Goodpasture and Talbot [7] and VonGlahn and Pappenheimer,[8] have reported and collected from the literature. A comparison of the present case with the material studied by Farber and Wolbach, and with the descriptions of the collected cases, yielded no similarities which would confuse the "protozoan-like" cells and their inclusions with the inclusion bodies of the present case. Neither has any pathogenic importance been attached to the "protozoan-like" cells with intranuclear inclusions, while it seemed reasonable to believe that the injury to the liver and adrenals of the case under discussion was due to specific localization of a virus.

McCordock and Smith [9] listed a series of infants in which there were intranuclear inclusions. Case 3 of Group 1 had foci of necrosis in the liver and suprarenal glands. There was no detailed description of the histology. It is possible that their case may have much in common with the malady described in this report.

VonGlahn and Pappenheimer [8] described intranuclear inclusion bodies in the intestine, liver and lungs of an adult who had a hepatic abscess and ulcerations of the cecum. They stated that the inclusions were identical with those that have been described in the viscera of infants. Dr. William VonGlahn has permitted the writer to study a section of the liver of their case. Neither the structure of the inclusion bodies nor the large "protozoan-like" cells which contained them was similar to the findings in the present case.

Rift Valley fever is a non-fatal virus disease which is characterized by focal necrosis in the liver of certain susceptible animals, such as sheep, goats, rats, squirrels, voles and wood mice. The inclusion bodies are restricted to the nuclei of the parenchymatous cells of the liver. The intranuclear inclusions are similar to those of yellow fever (Findlay [10]). The writer is indebted to Dr. G. M. Findlay for a section of the liver of a mouse with Rift Valley fever. A comparison with the liver of the present case disclosed similarities between many inclusion bodies. However, the resemblances were not sufficient to

admit the acceptance of close relationship or identity of the two processes.

Pacheco's parrot virus gives rise to characteristic intranuclear inclusion bodies, which may be found in the liver and other organs of parrots and parrakeets (Pacheco and Bier,[11] Rivers and Schwentker[12]). The inclusion bodies are not unlike those that have been found in yellow fever and Rift Valley fever. Although hepatic necrosis occurs in the susceptible avians, the pathogenicity of this virus for humans has not been demonstrated. The author is indebted to Dr. Thomas Rivers for a section of the liver of a parrakeet which died of this disease. The similarity between a few of the inclusions in the present case and those of the parrakeet disease did not distract from the great dissimilarity of the majority of the inclusions.

Findlay[13] described intranuclear bodies in a strain of Clacton mice. These bodies, which in many respects resembled hypertrophied nucleoli, appeared in the liver cells of Banbury mice that had been inoculated with a suspension of liver tissue of Clacton mice. Evidence was brought forward to suggest that the intranuclear bodies were caused by a filtrable virus of low pathogenicity.

Cowdry and Scott[14] described intranuclear inclusion bodies in the livers of dogs. Covell[15] found intranuclear inclusions in livers of monkeys. In each instance no ultramicroscopic virus was demonstrated.

The possibility of a localization of the herpes virus in the liver and adrenals of the present case must be considered seriously. The inclusion bodies in many respects were similar to those that arise in herpetic (herpes simplex) infections. The nature of the development of the inclusions, their morphology, their staining reactions, the presence of basophilic granules, the concurrence of basophilic and acidophilic material in several inclusions, the margination of chromatin and nucleoli, the "halo" around many of the typical well developed bodies, and other features which have been given in detail in the microscopic description, revealed an undeniable resemblance to and frequent identity with the morphology of the inclusion bodies of herpes. By no means, because of the similarity of intranuclear inclusion bodies in many diverse virus diseases, could one state that the present case illustrated an instance of herpetic infection of the liver and adrenals. However, if one were forced to select the etiological agent from the group of well established filtrable viruses, the

herpetic virus would be favored as the most probable causative factor in this singular disease. Goodpasture and Teague [16] were able to demonstrate intranuclear inclusion bodies in the parenchymatous cells of the liver and adrenals of rabbits in those areas where they had injected the herpes virus. Cowdry and Kitchen [5] obtained the same results by injection of the herpes virus into the livers of monkeys.

The portal of entry and the route of infection in the present case were not determined. It was possible that the umbilical cord may have served as the site of the primary infection, although no local lesion was demonstrated. It seemed possible that the virus may have been introduced into the infant by transfusion with the father's blood. The peculiar persistence of viruses in the tissues and fluids of humans and animals long after the disease process has subsided is well recognized. Therefore, the apparent healthy condition of the father would not militate strongly against the transmission of a virus by transfusion. There was another possibility which may be considered in the light of our knowledge of virus III infections of rabbits. This latent agent apparently lies dormant in the testes of normal rabbits. It attains virulence and produces disease only after repeated passage through the testes of rabbits. It is possible, but very unlikely, that a similar dormant species virus may have inhabited the blood of the father. As has been stated in the clinical record, the infant failed rapidly after the transfusion and died 12 hours later. This deserves repetition and emphasis only because the clue to this disease remains obscure. A careful study and analysis of similar cases may afford some inquisitive person the opportunity to transmit the disease to animals and identify the pathogenic agent. For the present the description and interpretation of the pathology of this singular malady must suffice.

Summary and Conclusions

1. A case of a 7 months premature infant, who was afflicted with a fatal disease characterized by hepato-adrenal necrosis and intranuclear inclusion bodies in the parenchymatous cells of the liver and adrenal cortex, is described.

2. It is assumed that the unique lesions must have been produced by a filtrable virus.

3. No similar case has been found in the literature.

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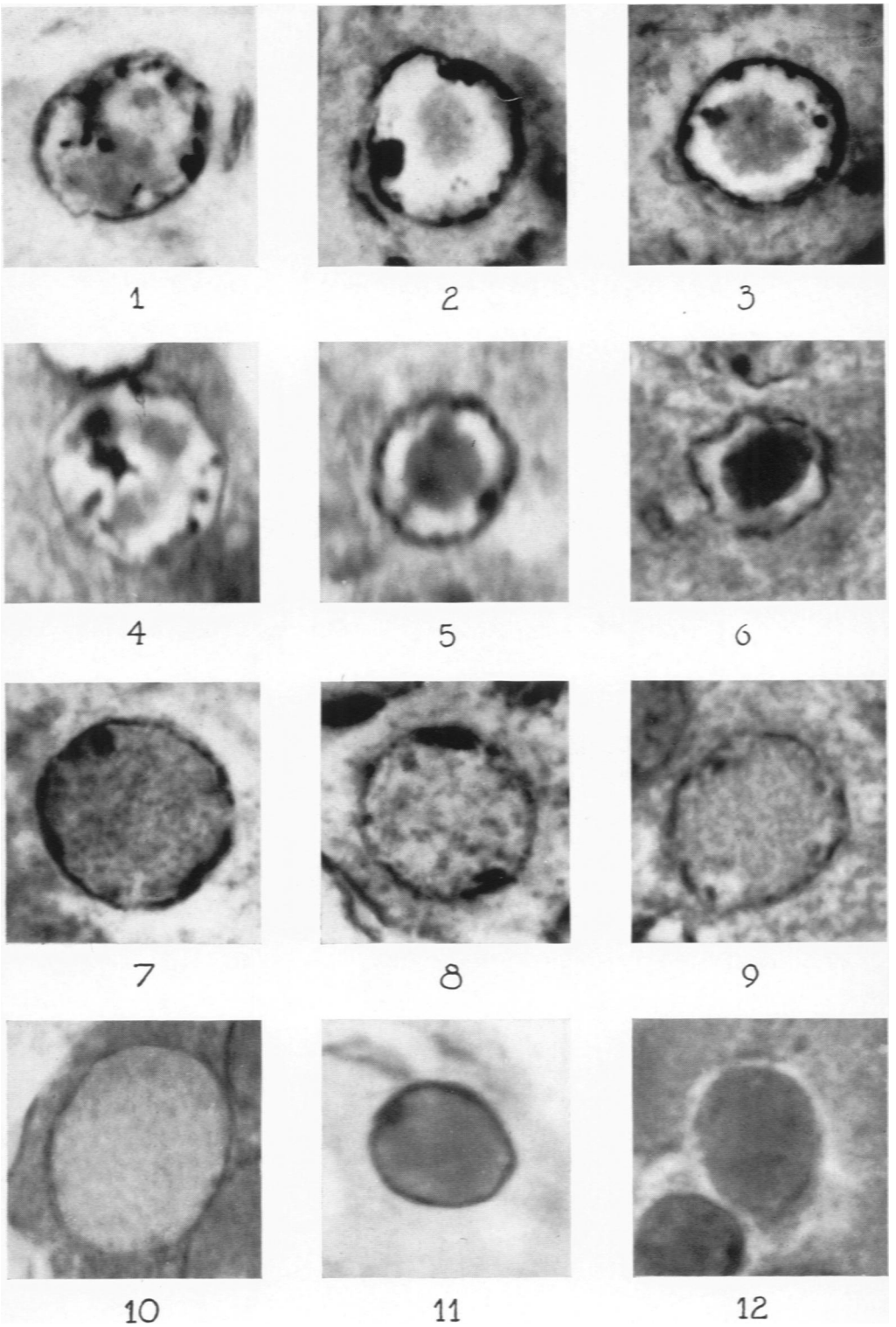
DESCRIPTION OF PLATES

PLATE 15

FIGS. 1–12. Photomicrographs of representative cell nuclei of the hepatic parenchyma. These contain "inclusion bodies" of various types, as described in detail in the microscopic descriptions. Similar intranuclear "inclusion bodies" were found in the focal necroses of the adrenal cortex. × 2800.

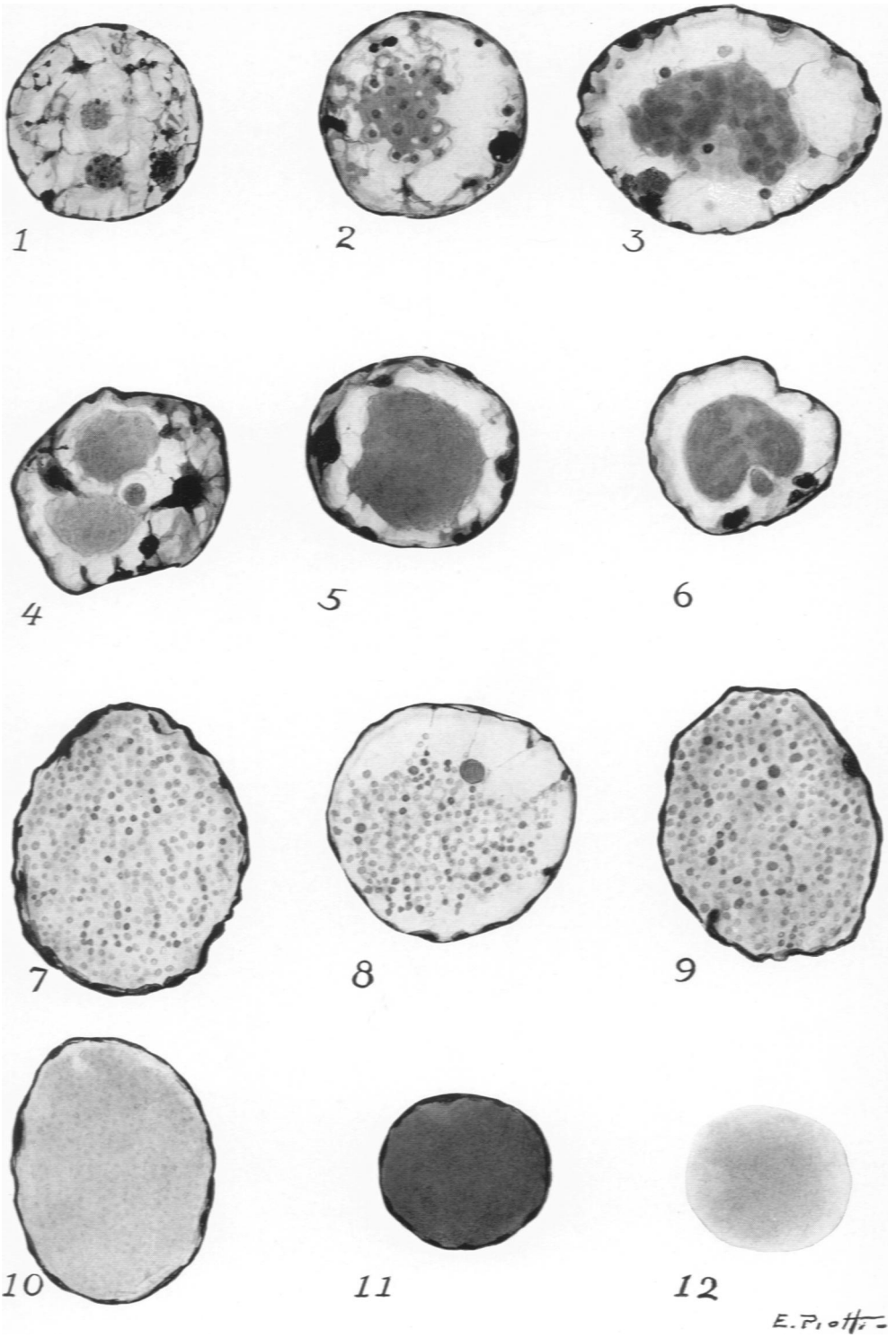


Haas

Hepato-Adrenal Necrosis

PLATE 16

FIGS. 1–12. Camera lucida drawings of nuclei of hepatic parenchymal cells. The "inclusion bodies" which are contained in these nuclei are of the same type as those in Plate 15. Corresponding nuclei have the same numbers in the two plates (Figs. 1–12). × 3200.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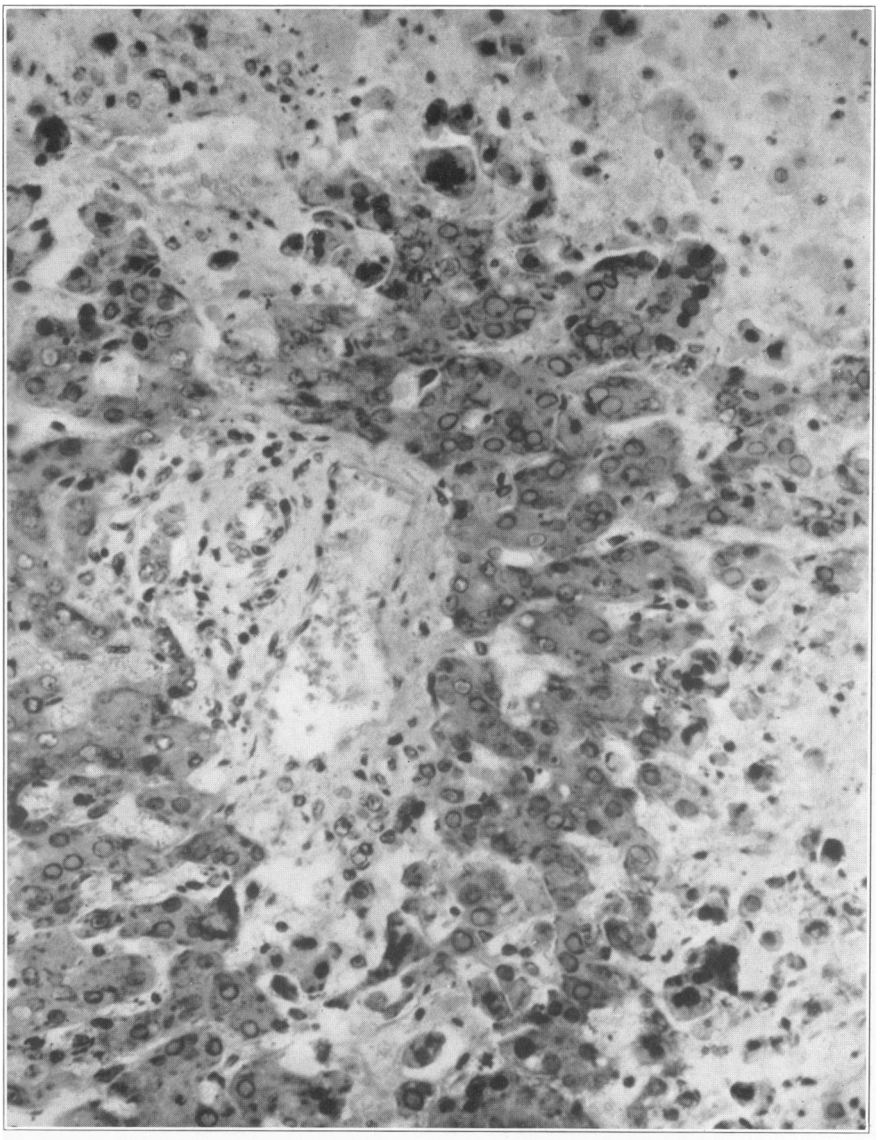
E. Pilotti

Plate 17

Fig. 13. A photomicrograph of a representative periportal area such as was considered fully in the description of the histopathology of the liver. × 150.



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