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The Histopathology of Experimental Yellow Fever

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With 18 Figures in the Text, of which 8 in Colour

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The spread of jungle yellow fever (SOPER; WARREN) into areas of the North American continent constitutes a potential danger for extension of this dreaded disease into other parts of the world that could provide favorable conditions for its existence.

Within the jungle, the virus is transmitted from monkey to monkey by the Aedine mosquito, *Haemagogus spegazzinii*, which is ordinarily confined to the forest canopy; tree cutters who penetrate into the jungle sometimes become inoculated with the virus of jungle yellow fever by the bite of infected mosquitoes living in the foliage of the felled trees. After infection has taken place, the transmission from man to man can then be accomplished by mosquitoes of the *Aedes aegypti* species breeding in the neighborhood of human habitations. From such endemic foci, the disease could then be introduced by modern rapid air travel into other parts of the world by either infected mosquitoes or by human beings harboring the virus during the prodromal stage of yellow fever.

No reliable test for rapid diagnosis of yellow fever in its early phases has, as yet, been developed; the mouse protection test (COLLIER, SMITH) takes more than a week to evaluate, and it requires special laboratory equipment and trained personnel difficult to find in remote areas. On inquiry into the practice of needle biopsy of the liver for the diagnosis of yellow fever, it was learned that this procedure is frowned upon by most physicians because of fear of hemorrhage in the wake of liver puncture.

The clinical manifestations (KERR) of early yellow fever in human beings are too nonspecific for accurate recognition of sporadic cases. Patients taken to the hospital usually arrive *in extremis*, making an accurate clinical evaluation difficult. The condition can, however, be recognized at autopsy, or by the study of liver tissue by the viscerotome method in fatal cases.

The information gained by the clinical study of isolated cases of yellow fever is rather limited, and the autopsy fails to provide insight into the physiopathologic mechanism of the disease or into its early and significant laboratory and cytopathologic characteristics. Because of the gap in our knowledge concerning practically all phases of yellow fever, including its correct diagnosis and its histopathologic evolution, an experimental approach was decided upon (TIGERTT et al.).

Material and Methods

Macaca mulatta monkeys, of mixed sex, and weighing 2.5 kg. on the average, were chosen as the host animal, and the Asibi strain of virus was used as the infecting agent. Twenty-six animals were inoculated with 10^3 mouse intracerebral lethal doses ($MICLD_{50}$) units of plasma

virus, and pairs of experimental animals were sacrificed daily at intervals of from 12 to 18 hours. During the period of 2 weeks prior to the start of the experiments, the animals had been closely observed clinically, and blood samples had been taken for base-line serologic and hematologic examinations as well as for clinical and laboratory tests; rectal temperatures were recorded daily, chest x-ray films were made, and EKG readings were taken.

After challenge with yellow fever virus, the animals were studied clinically, rectal temperatures were taken at 6-hour intervals, EKG recordings were made on alternate days, and chest x-rays were repeated before the animals were sacrificed.

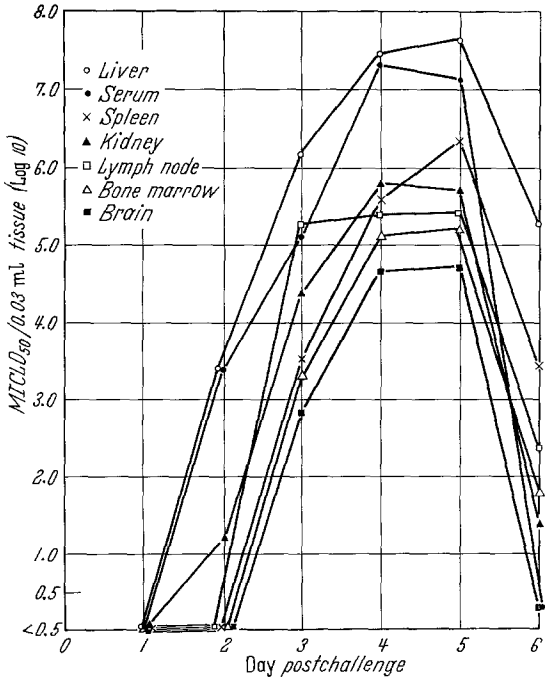

Fig. 1. Yellow fever virus content in serum and tissues of rhesus monkeys sacrificed at given intervals following intraperitoneal inoculations with 1.0×10^3 $MICLD_{50}$ of Asibi strain. [Courtesy: Trans. N.Y. Acad. Sci., Ser. II. **22**, 325 (1960) (TIGERTT et al.)]

Prior to the death of each animal, two needle biopsy specimens from the liver were taken for electron microscopic and fluorescent antibody studies (MARSHALL et al.) and for histopathologic preparations. At the same time, blood samples were secured for laboratory tests, liver function studies, and hematologic examinations. Immediately afterwards, the monkeys were killed, and a complete autopsy was performed on each animal. Imprints of most organs were secured, and tissues of all organs were fixed in buffered formalin (20 gm. calcium acetate per thousand cc. of 10% formalin); in addition, tissues were quick-frozen for viral studies.

Observations

Level of virus in blood and parenchymatous organs. Search for virus during the first 24 hours gave equivocal results. From the second day on, increasing amounts of virus were demonstrated in blood and tissues until the fifth day postchallenge, when the level of virus in the blood and in the parenchymatous organs fell rapidly, particularly in serum and brain; the liver, however, retained a significant amount of virus. In general, liver emulsions consistently contained the greatest amount of virus (THEILER), reaching a level of $10^{7.5}$ $MICLD_{50}$ units per gram of tissue on the fourth and fifth days after infection (Fig. 1). Serum antigen determination gave a remarkably uniform response, as measured by the viremia and the complement-fixing antigen. Although viremia levels varied between $10^{6.6}$ and $10^{7.4}$ $MICLD_{50}$ units — a sixfold difference — four of the sera showed complement-fixing antigen titers of 1:256, and two others showed titers of 1:512 — only a twofold variation. Hemagglutination levels varied widely from 1:10 to 1:10,240 in sera of closely similar virus titers. The results of these tests are presented in Fig. 2.

Clinical findings. The initial febrile response occurred usually on days 3 and 4 postchallenge. Blood counts revealed a moderate leukopenia; with the onset of fever the number of lymphocytes dropped, while myeloid cells showed an increase, with a shift to the left. Prior to the death of the animals, the white blood count sometimes rose to a higher level than could be explained by hemoconcentration, with a predominance of neutrophilic polymorphonuclear leukocytes.

There was no significant deviation from base-line levels in clinical chemistry studies of blood urea nitrogen, cholesterol, phosphorus, and glucose levels during the first 3 days following challenge. Cephalin flocculation and thymol turbidity values also showed no significant deviation from the normal at this time. A moderate rise of the total serum bilirubin occurred in animals sacrificed 4, 5, and 6 days after inoculation; alkaline phosphatase values were higher the third and fourth days. The most dramatic response was experienced in the increase of serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxalacetic transaminase (SGOT) on days 4 and 5, reaching values in individual cases of over 1,000 units per milliliter. The results of the clinical laboratory findings are summarized in Fig. 3.

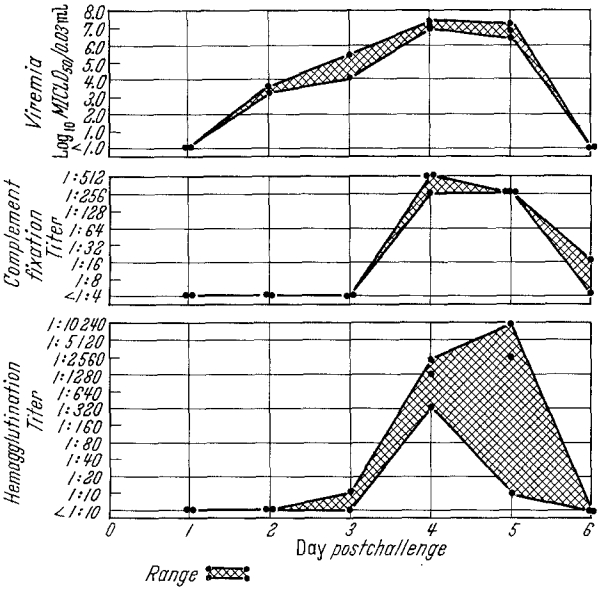

Fig. 2. Virus, complement-fixing antigen, and chick cell hemagglutinin in sera of rhesus monkeys sacrificed at daily intervals following inoculation with Asibi virus (10^3 $MICLD_{50}$). [Courtesy: Trans. N.Y. Acad. Sci., Ser. II. **22**, 326 (1960) (TIGERTT et al.)]

Histopathologic observations. No significant alterations of liver cells

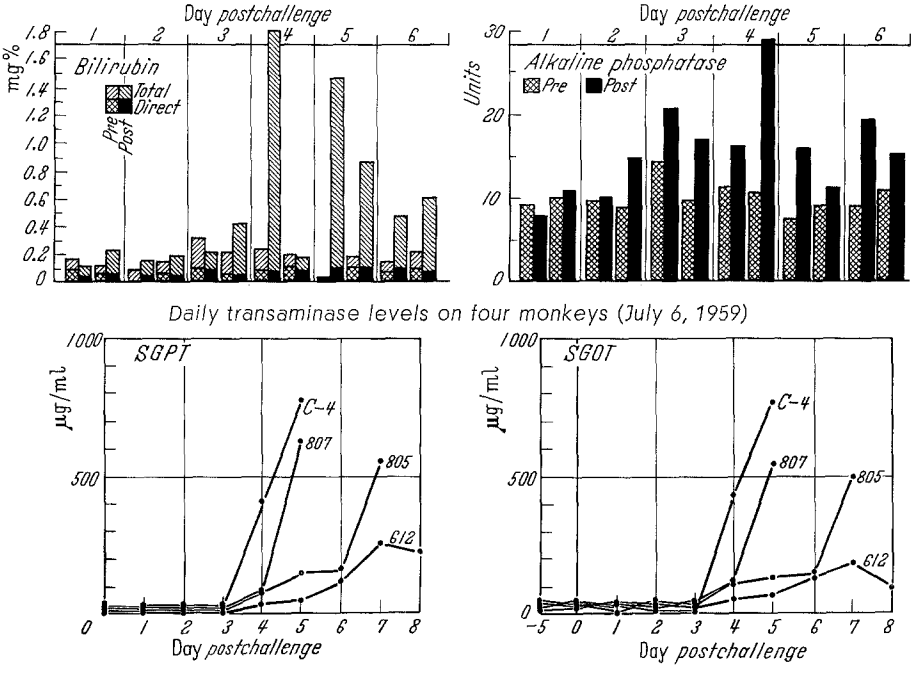

Fig. 3. Bilirubin, alkaline phosphatase, and serum transaminase determination in sera of rhesus monkeys experimentally inoculated with Asibi virus. SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxalacetic transaminase. [Courtesy: Trans. N.Y. Acad. Sci., Ser. II. **22**, 327 (1960) (TIGERTT et al.)]

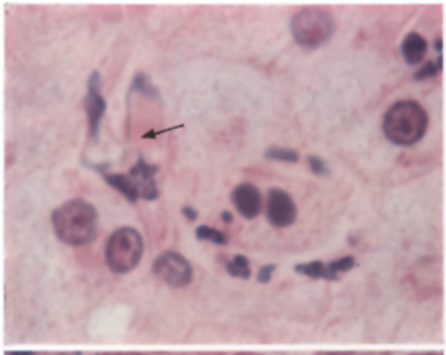

Fig. 4

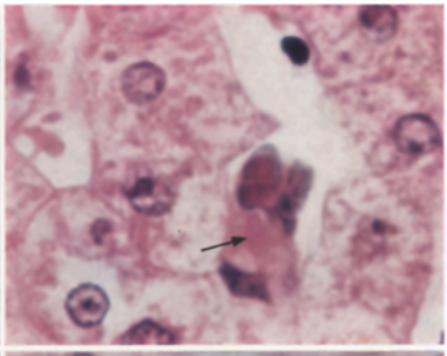

Fig. 5

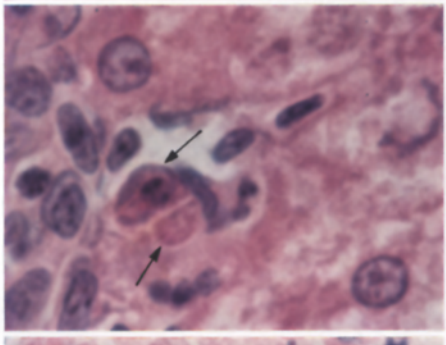

Fig. 6

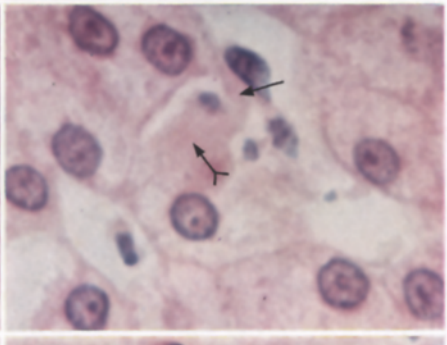

Fig. 7

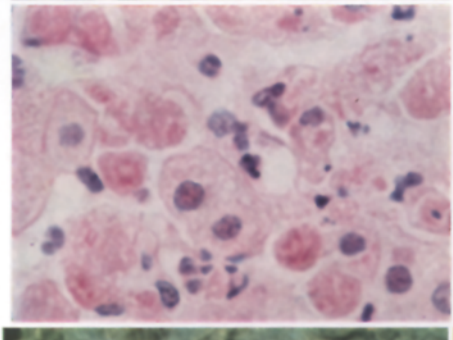

Fig .8

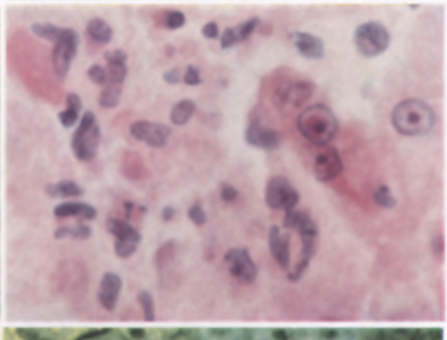

Fig. 9

Fig. 10

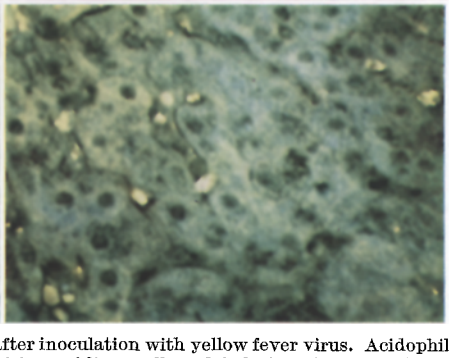

Fig. 11

Fig. 4. Section of liver of rhesus monkey 19 hours after inoculation with yellow fever virus. Acidophilic hyaline necrosis of Kupffer cell (→). Amitotic divisions of liver cell nuclei; leukocytic infiltration in neighboring sinusoids. Hematoxylin and eosin. Mag. 900 ×. AFIP Neg. 59—3775

Fig. 5. Section of liver of rhesus monkey 24 hours after inoculation with yellow fever virus. Acidophilic hyaline necrosis of Kupffer cell (→). Normal liver cells. Hematoxylin and eosin. Mag. 900 ×. AFIP Neg. 59—3772. [Courtesy: Trans. N.Y. Acad. Sci., Ser. II. **22**, 328 (1960) (TIGERTT et al.)]

were seen during the first 3 days following the inoculation of yellow fever virus into the monkeys. During the first 24 hours postchallenge, however, occassional individual Kupffer cells exhibited a peculiar swelling and acidophilic hyalinization of the cytoplasm, accompanied by karyolysis or karyorrhexis of the nucleus. During this period, the liver cells appeared unaltered, and their columns and lobular arrangement remained intact. Within some of the sinusoids — especially in the neighborhood of necrotic Kupffer cells — small groups of poorly preserved polymorphonuclear leukocytes were seen (Figs. 4 and 5). Liver cells adjacent to necrotic Kupffer cells sometimes exhibited signs of reaction in the form of amitotic nuclear division. Fluorescent antibody studies on liver tissue suggested the presence of specific antigen in occasional Kupffer cells (Fig. 10).

During the period from 24 hours to 48 hours after challenge, progress of this process was hardly discernible except perhaps by the increase in the number of affected Kupffer cells and a slight monocytic response within some of the sinusoids (Fig. 6). The liver cells appeared quiescent, as were the portal triads and the efferent veins. Intranuclear inclusion bodies were not seen in liver cells at that time.

During the period between 2 and 3 days postchallenge, the number of affected necrobiotic Kupffer cells increased greatly, while liver cells remained essentially unaltered except for rare, ill-defined, acidophilic intranuclear inclusions. A number of acidophilic ghost cells whose actual origin was difficult to discern could be seen within the liver lobules. Fluorescent antibody preparations made at this stage revealed quite a number of cells located within sinusoids, apparently Kupffer cells, whose cytoplasm exhibited a yellow-green fluorescence (Fig. 11).

Approximately 67 to 72 hours postchallenge, a gradual change occurred that was characterized by an apparent extension of the hyaline necrosis of Kupffer cells to adjacent liver cells (Fig. 7). In general, the transition of this process from Kupffer cells to liver cells appeared rather subtle but was unmistakable when both types of cells were in one plane. Up to this time following challenge, most of the liver tissue had remained unaffected, except for the necrotic and hyalinized Kupffer cells, and there was practically no evidence of any inflammatory exudative reaction in any portion of the liver lobules. Occasional liver cells showed suggestions

Fig. 6. Section of liver of rhesus monkey 48 hours after inoculation with yellow fever virus. Necrosis and necrobiosis of Kupffer cells (→) accompanied by moderate monocytic inflammatory response. Normal liver cells. Hematoxylin and eosin. Mag. 900 ×. AFIP Neg. 59—3785

Fig. 7. Section of liver of rhesus monkey 67 hours after inoculation with yellow fever virus. Partial necrosis of cytoplasm of liver cell (↠) adjacent to an affected Kupffer cell (→). Hematoxylin and eosin. Mag. 900 ×. AFIP Neg. 59—3787

Fig. 8. Section of liver of rhesus monkey 92 hours after inoculation with yellow fever virus. Necrobiosis of numerous liver cells and presence of Councilman bodies. Hematoxylin and eosin. Mag. 550 ×. AFIP Neg. 59—3801. [Courtesy: Trans. N.Y. Acad. Sci., Ser. II. **22**, 330 (1960) (TIGERTT et al.)]

Fig. 9. Section of liver of rhesus monkey 6 days after inoculation with yellow fever virus. Granulomatous reaction, with giant cell formation about Councilman bodies. Hematoxylin and eosin. Mag. 900 ×. AFIP Neg. 59—3802. [Courtesy: Trans. N.Y. Acad. Sci., Ser. II. **22**, 331 (1960) (TIGERTT et al.)]

Fig. 10. Section of liver of rhesus monkey 19 hours after inoculation with yellow fever virus. Fluorescence in occasional Kupffer cell (→). Normal liver cells. Fluorescein isothiocyanate fluorescent antibody Mag. 350 ×. AFIP Neg. 59—3779 (a)

Fig. 11. Section of liver of rhesus monkey 72 hours after inoculation with yellow fever virus. Yellow-green fluorescence in many Kupffer cells. Normal liver cells. Fluorescein isothiocyanate fluorescent antibody technique. Mag. 260 ×. AFIP Neg. 59—3779 (b)

of the presence of acidophilic intranuclear inclusions; only rarely could such inclusions be seen in liver cells that showed no obvious cytoplasmic alterations.

The number of involved parenchymal liver elements increased explosively during the fourth day (72 to 96 hours) following infection. By then the cytoplasm of the majority of liver cells had become necrotic and was composed of coarsely granular and finely vacuolated acidophilic material; the nuclei showed pyknosis, karyolysis, or karyorrhexis (Figs. 8, 12, 13). During this period, necrotic Kupffer cells were very difficult to differentiate from necrotic liver cells, even in reticulum

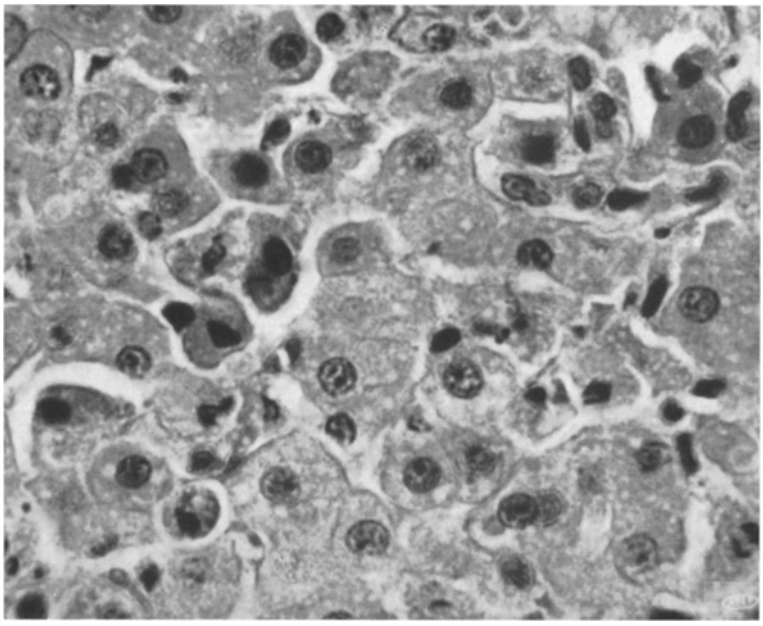

Fig. 12. Section of liver of rhesus monkey 75 hours after inoculation with yellow fever virus. Scattered necrobiotic Kupffer cells and early necrosis of liver cells (Councilman bodies). Occasional acidophilic inclusions in nuclei of liver cells. Hematoxylin and eosin. Mag. 800 ×. AFIP Neg. 61—4472

preparations. Intranuclear acidophilic inclusions were commonly seen in relatively well-preserved liver cells. A number of polymorphonuclear leukocytes were present within the sinusoids, usually situated near affected liver cells. The majority of the severely injured liver cells were confined to the intermediate zone, between the efferent vein and the portal canals, in a distribution similar to that in advanced cases of yellow fever in human beings. The portal fields exhibited remarkably little reaction to the profound damage of the liver parenchyma.

By the end of the fifth day postchallenge, most of the surviving animals appeared very sick, but none of these were left to die. Moribund monkeys were sacrificed in order to prevent distortion of the changes by postmortem autolysis. The clinical signs at that stage were those of listlessness, anorexia, jaundice, and evidence of hepatic insufficiency. Clinical laboratory studies showed a moderate increase in serum bilirubin and in the alkaline phosphatase, but a rather spectacular rise in the values of the serum transaminases on the fifth day (Fig. 3), as well as an increased number of white blood cells, with prominence of neutrophilic polymorphonuclear leukocytes.

Histologic studies of sections of the liver of animals sacrificed during the period of 96 to 120 hours postchallenge showed necrosis of the majority of liver cells, with formation of characteristic Councilman bodies (Fig. 14). Most of the nuclei of former liver cells had disappeared, but those that were present exhibited distortion, with pyknosis, karyolysis, or various stages of karyorrhexis. The cytoplasm was occupied by masses of acidophilic, coarsely granular material, often containing small vacuoles. The distinction between necrotic Kupffer cells and liver cells became increasingly difficult and often impossible. At this time many

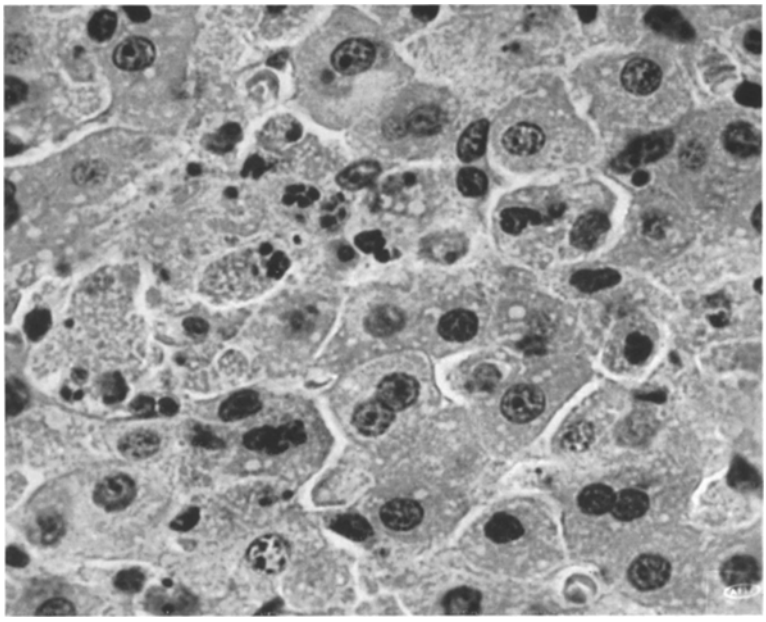

Fig. 13. Section of liver of rhesus monkey 79 hours after inoculation with yellow fever virus. Numerous necrobiotic Kupffer cells and Councilman bodies in liver cells. Increasing difficulty of differentiation between liver cells and Kupffer cells. Acidophilic inclusions in nuclei of liver cells, with occasional rupture of nuclear membrane. Hematoxylin and eosin. Mag. 800 ×. AFIP Neg. 61—4473

of the nuclei of apparently preserved liver cells contained prominent acidophilic inclusions, sometimes with rupture of the nuclear membrane.

Histopathologic studies of all the parenchymatous organs during the incubation period, as well as during the clinical phase of yellow fever, showed no significant primary lesions except hyaline necrosis of some of the reticuloendothelial cells in the spleen and lymph nodes; occasional glial cells of the central nervous system were similarly affected.

Sections of the liver of monkeys 6 days following the infection with yellow fever virus showed significant changes in the character of the histopathologic lesions. The number of Councilman bodies seemed to be decreasing compared to the number seen at the peak of the disease, 4 days postchallenge. The inflammatory cellular reaction, consisting predominantly of histiocytes, showed a tendency to assume a granulomatous character within the sinusoids of the liver (Figs. 15, 16). In a number of places, irregularly shaped giant cells were associated with other reactive elements of apparently histiocytic origin. Many of the liver cells were

large and showed evidence of regeneration with a tendency to restoration of cell plates. The nucleoli of the liver cells were prominent and often exhibited acidophilia. This granulomatous reaction following the extensive destruction of liver tissue appeared to focalize the subsiding injury caused by the yellow fever virus preparatory to the process of healing and restoration (Fig. 9). The appearance of antibodies in the blood stream at this stage constituted confirmatory evidence of the fundamental change in the nature of the tissue response to the infection.

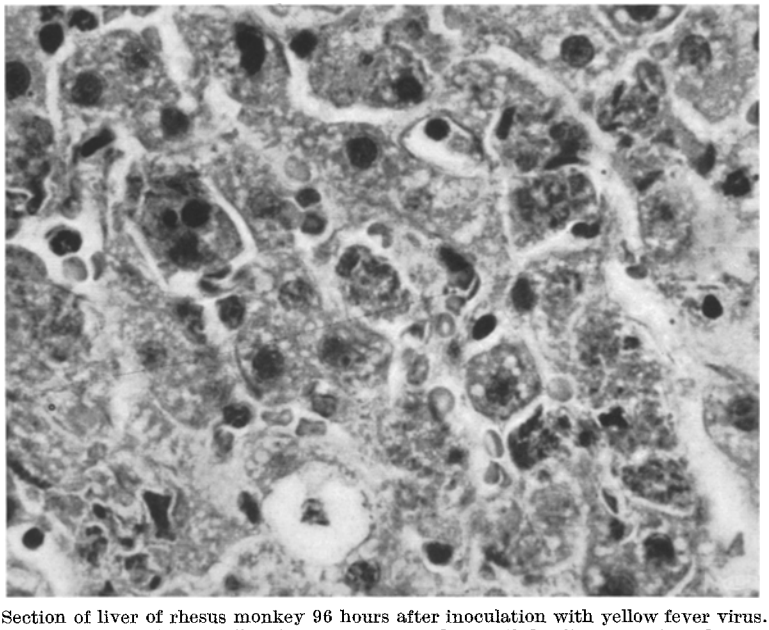

Fig. 14. Section of liver of rhesus monkey 96 hours after inoculation with yellow fever virus. Transformation of practically all liver cells into Councilman bodies, with hyaline necrosis and vacuolization of cytoplasm, karyolysis or karyorrhexis of nuclei, and hyaline acidophilic intranuclear inclusions. The differentiation between Kupffer cells and liver cells has become almost impossible. Hematoxylin and eosin. Mag. 800 ×. AFIP Neg. 61—4476

Some of the infected animals survived the acute phase of the infection and gradually recovered. A study of the changes in the liver of these monkeys, after different periods of time of survival, showed progressive stages of healing and restoration. Seven days after challenge, only occasional liver cells showing hyaline acidophilic necrosis remained. These were surrounded by massive granulomatous infiltrates composed of monocytes and located within the sinusoids. It was very difficult to determine at this time whether these elements originated from affected Kupffer cells or liver cells, but they seemed to form the center of each individual granuloma. Many of the liver cells were large, angular, and exhibited the appearance of regenerating cells, forming irregular plates. Evidence of mitosis was rarely seen, however, but amitotic division was not uncommon (Fig. 17).

None of the parenchymatous organs showed evidence of a granulomatous process attributable to healing lesions caused by the yellow fever infection.

Sections of the liver of a monkey that had survived 15 days after inoculation showed an essentially normal parenchyma with mild portal infiltrates consisting

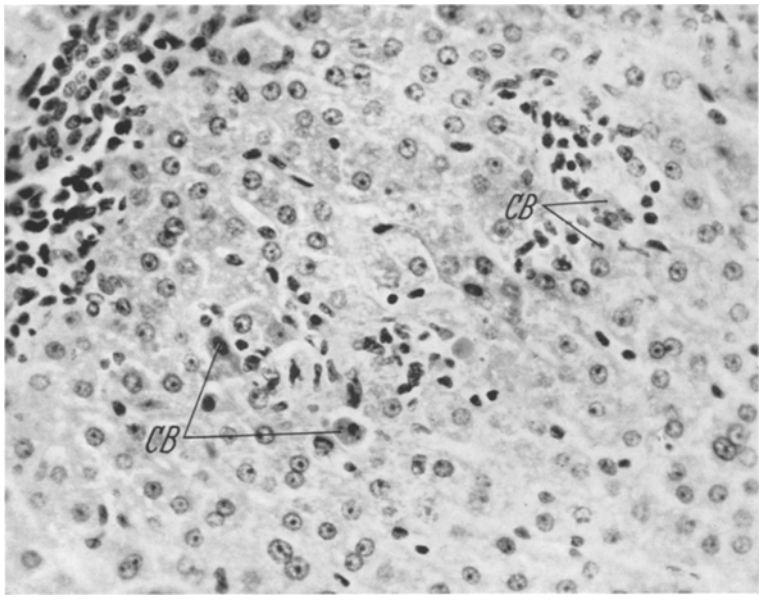

Fig. 15. Section of liver of rhesus monkey 139 hours after inoculation with yellow fever virus. Focal monocytic infiltrates about degenerating Councilman bodies (*CB*), forming granulomata within sinusoids. Hematoxylin and eosin. Mag. 350 ×. AFIP Neg. 59—3767

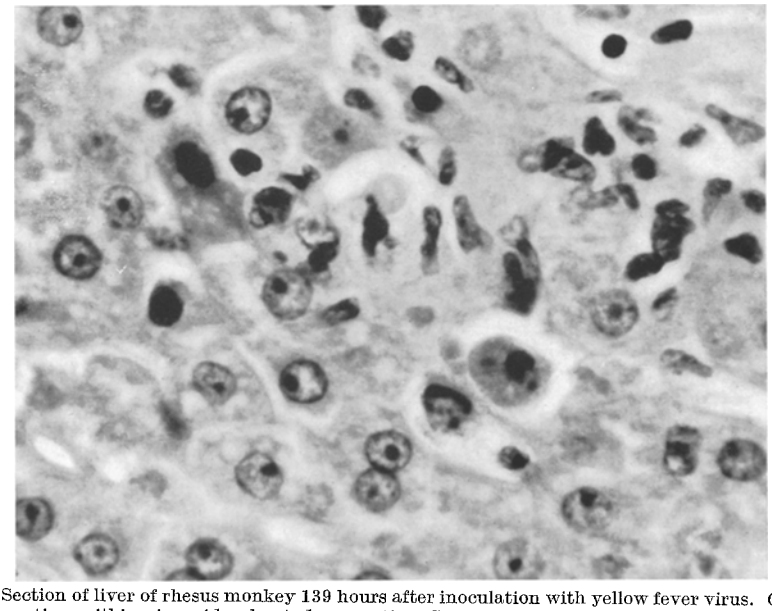

Fig. 16. Section of liver of rhesus monkey 139 hours after inoculation with yellow fever virus. Granulomatous reaction within sinusoids about degenerating Councilman bodies. Hematoxylin and eosin. Mag. 900 ×. AFIP Neg. 59—3766

of monocytes and occasional eosinophils. There was no scarring, and the architecture of the liver cell plates and lobules appeared within normal limits.

Sections of the liver of a monkey that survived 28 days postchallenge presented a few focal infiltrates in normal-appearing liver lobules composed of regular plates and accessory structures.

Sixty days after the infection, occasional portal infiltrates were all that remained of the reaction to the infection with yellow fever virus. The architecture of the lobules was normal, as were the plates, sinusoids, and Kupffer cells. Scars

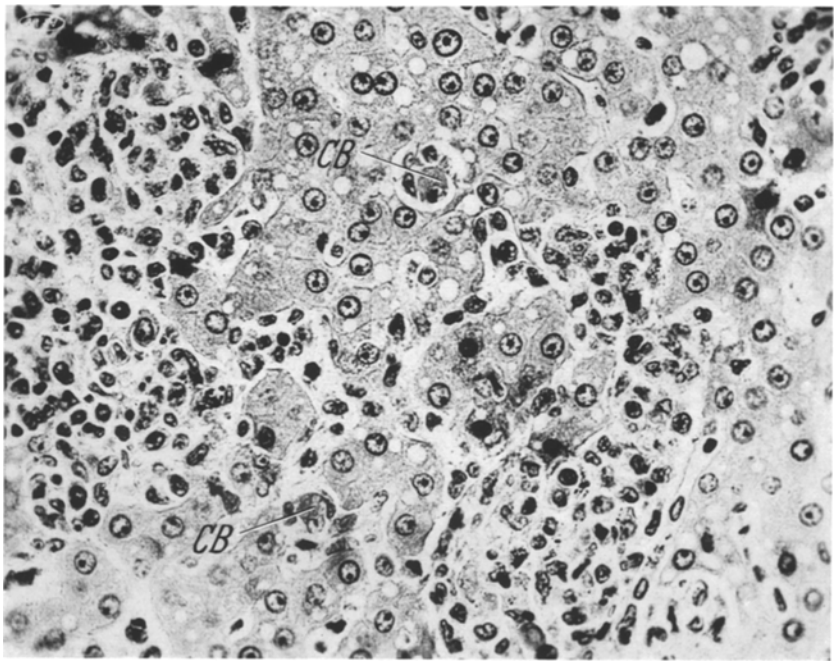

Fig. 17. Section of liver of rhesus monkey 7 days after inoculation with yellow fever virus. Occasional Councilman bodies (*CB*) surrounded by massive histiocytic infiltrates within sinusoids. Regenerating liver cells exhibiting vesicular nuclei and prominent nucleoli forming bulky liver cell plates. Hematoxylin and eosin. Mag. 350 ×. AFIP Neg. 61—2715

were not present, and there was no evidence of postnecrotic collapse (Fig. 18). Sections of all the organs of the many animals in this group, including the central nervous system, showed no significant changes or residues of the effects of the yellow fever virus.

The host factor of yellow fever infection was explored in a parallel study employing Cynomologus monkeys, instead of rhesus monkeys, as experimental animals. Sixteen monkeys were infected with 10^3 $MICLD_{50}$ units of plasma virus, Asibi strain, and one animal was sacrificed daily for 5 days. Daily liver needle biopsies were performed upon three other animals on 5 successive days.

No clinical symptoms of disease were recognized between days 1 and 5 postchallenge. One monkey became moribund after 5 days, two after 6 days, three after 7 days, and one after 8 days following inoculation. Four animals survived and were sacrificed 15 days postchallenge. In this series the incubation period was prolonged, lasting 5 days, as compared with 3 days in *Macaca mulatta* monkeys under identical experimental conditions. Fatal clinical disease followed in seven

animals (44%), comparable to the number of similarly affected rhesus monkeys (46%). Four Cynomologus monkeys (25%) survived, as compared to four surviving rhesus monkeys (17%) of 26 experimental animals used in the first series.

The histologic lesions were identical, however, in both groups. The reason for the apparent greater resistance of the dog monkeys to the yellow fever virus infection has not been determined.

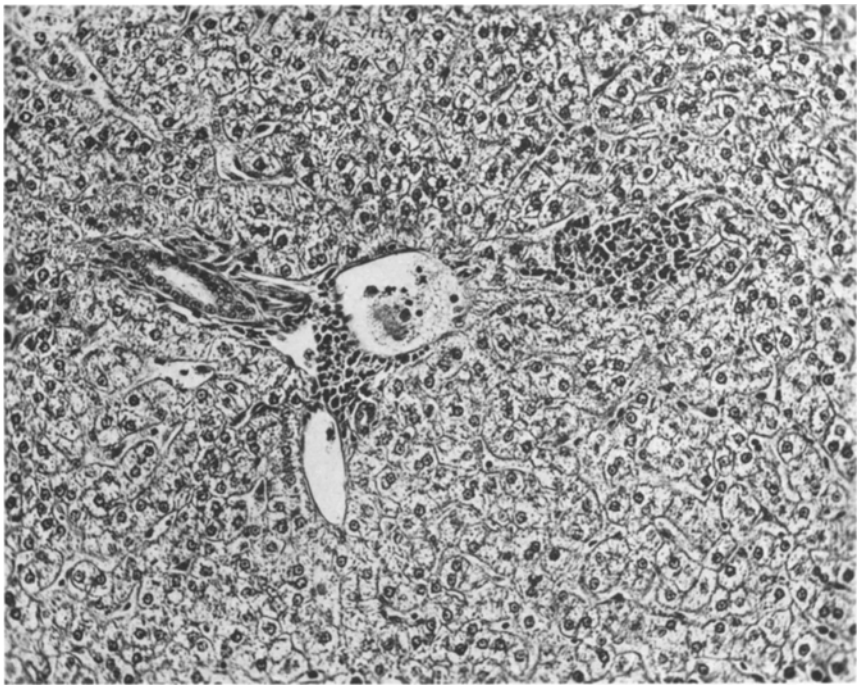

Fig. 18. Section of liver of rhesus monkey 60 days after inoculation with yellow fever virus. Mild residual portal infiltrates composed mainly of lymphocytes. Complete restoration of liver cell plates. Hematoxylin and eosin. Mag. 180 ×. AFIP Neg. 61—2716

Discussion

The histopathologic sequence of events that take place in the organs, particularly in the liver, following infection with yellow fever represents stages in the development of the lesions that are characteristic of this disease. The first step concerns the reaction of the host to the virus following its introduction either by the bite of an infected mosquito or by artificial transmission. The results of model animal experiments (THEILER) indicate that there occurs a rapid diminution in the amount of virus that had been introduced, due to the dilution of the inoculum as well as to the clearing action of the reticuloendothelial cells. Although the reticuloendothelial system is spread widely through the various viscera and tissues, the consequences following the phagocytosis of the virus by these cells are best observed in the liver because of the affinity of the viscerotropic agent for this organ. It appears that multiplication of the virus can take place only intracellularly, even if its survival is possible in blood or serum.

After infection of a nonimmune individual, a certain amount of time elapses before the virus produces its expected action on liver cells. This period is presumably

necessary for adaptation of the virus to the host environment, and for multiplication before it is able to overcome the blood-cell barrier and reach the target — the liver cell. The duration of this incubation period varies somewhat, depending on factors such as the amount of virus and its manner of introduction, its virulence, the host resistance and degree of immunity. The variation in the reaction time of the host to different means of introduction of the virus was first observed during the fundamental experiments conducted by the members of the Walter Reed Commission on Yellow Fever (REED et al.): Following the subcutaneous injection of blood from a patient suffering from yellow fever into a healthy human volunteer, the incubation period lasted $67^1/_2$ hours; following the bite of mosquitoes that had fed on yellow fever patients, it was 94 hours before symptoms of yellow fever developed. The 67-hour length of the incubation period compares well with that observed in our experiments following the intraperitoneal introduction of yellow fever virus into Macacus rhesus monkeys (TIGERTT et al.). Differences in the length of the inoculation period and in the degree of host resistance were brought out by employing the Cynomologus strain of experimental monkeys in our study.

The earliest noticeable effect of inoculation with yellow fever virus was seen in occasional Kupffer cells, which showed acidophilic necrosis several days before the parenchymal liver cells exhibited any significant alterations. This observation was made even in the first animal sacrificed, 19 hours after challenge. The number of affected Kupffer cells increased in animals subsequently sacrificed up to approximately 72 hours postchallenge, when liver cells showed unmistakable signs of the development of Councilman bodies.

During the first 3 days following the inoculation of the virus there were no significant clinical signs or symptoms of disease, and the laboratory findings and results of liver function tests remained within acceptable normal limits.

The suspicion that the phagocytosed virus was the cause of the necrobiosis of the Kupffer cells was supported by the results of fluorescent antibody studies (MARSHALL et al., TIGERTT et al.) on sections of liver obtained by needle biopsy during the first few days following infection. The number of cells exhibiting specific fluorescence increased during the first 3 days, after which time fluorescence became rather diffuse.

Intranuclear inclusion bodies were rarely found during the first 3 days postchallenge, but were rather commonly observed during the later stages of the disease, as described by COWDRY and KITCHEN (1,2); GOODPASTURE and TEAGUE; TORRES.

After the third day postchallenge, acidophilic necrosis of individual liver cells (COUNCILMAN) became increasingly prominent; the early alteration of individual liver parenchymal cells often appeared related to an adjacent necrotic Kupffer cell, but this was not always evident. All stages, from a partial cytoplasmic involvement with preservation of the nucleus to the complete necrosis of the cell and its nucleus after 4 days postchallenge, could be found; and small fat vacuoles, presumably the result of dissociation of cytoplasmic contents, made their appearance in this advanced period [BUGHER; KLOTZ and BELT (1)]. At this time it was increasingly difficult to distinguish necrotic Kupffer cells from liver cells, as the cell plates became disorganized and disrupted. Many of the affected liver cells, both necrotic or fairly well preserved, exhibited acidophilic intranuclear inclusions during this period.

Clinically, this stage (ca. day 4) was characterized by the development of signs of liver disease with moderate fever, jaundice, increase in myeloid cells in the bone marrow, shift to the left, listlessness, prostration, and coma. The laboratory tests indicated a rise in the serum alkaline phosphatase, the indirect fraction of bilirubin, and particularly, increase in both the serum glutamic-oxalacetic and serum glutamic-pyruvic transaminase.

In brief, the earliest histopathologic indication of the effect of the yellow fever virus was the progressive acidophilic necrosis of occasional Kupffer cells, followed by the gradual involvement of liver cells, with formation of Councilman bodies, and widespread injury and destruction of parenchymal cells. These alterations appeared more conspicuous in the intermediate zone of the lobules [da ROCHA-LIMA (1, 2)] and were accompanied by a profound disorientation and disruption of the cell plates, as well as of the lobular architecture.

The silent period of incubation is of great interest, particularly concerning the problem of the whereabouts of the virus, its place of multiplication, recognition of its presence, and its possible infectivity. These questions have been raised previously: In his study of the "Course of Infection in Rhesus Monkeys Inoculated with Viscerotropic Strains" THEILER followed the fate of measured amounts of yellow fever virus introduced into an experimental host by titration and demonstrated its rapid disappearance during the first 5 hours after inoculation. Twenty-four to 48 hours postchallenge, however, virus reappeared, and its concentration in the blood stream, as well as in the parenchymatous organs, increased steadily, albeit always being highest in the liver. He assumed, from the results of these experiments and on the basis of the postmortem histopathologic studies of KLOTZ and BELT (1), that the virus, during the incubation period, probably multiplies within parenchymal liver cells. In view of the tremendous increase of virus during the first 3 days following challenge (Fig. 1) without clinical signs or symptoms of liver disease and without significant laboratory findings (Figs. 2, 3), as well as lack of evidence of cellular damage of parenchymal liver cells, this interpretation is certainly questionable. As soon as liver cells exhibit signs of injury, however, the clinical picture changes immediately, and fever, jaundice, and clinical and laboratory evidence of hepatic damage become apparent.

Although alterations of the Kupffer cells have been described previously [BEARCROFT (1, 2); BUGHER; COUNCILMAN; KLOTZ and BELT (1); SEIDELIN; THEILER; TURNBULL; VILLELA], the early and progressive necrobiosis of these reticuloendothelial elements has apparently not impressed any of the observers. In this respect it is particularly regrettable that the studies by THEILER were not accompanied by histopathologic examinations of liver biopsies, since postmortem changes [KLOTZ and BELT (1)] often alter the lesions. Despite the exacting and comprehensive histopathologic studies by BEACROFT (1) and their electron microscopic extensions to later phases of yellow fever [BEACROFT (2)], details of the earliest stages of the infection and its transition from infected Kupffer cells to the formation of Councilman bodies in liver cells had not been explored. Necrosis of reticuloendothelial elements in the spleen and lymph nodes was described in later stages of the disease [BUGHER; KLOTZ and BELT (2); KLOTZ and SIMPSON (1, 2); STOKES et al. (1, 2); THEILER]. In our material these were rather difficult to recognize and never assumed the proportions of changes seen in the Kupffer cells.

The phenomenon of a latent clinical period following viral infection is by no means limited to yellow fever. Many of the common viral diseases — e.g., variola, measles, poliomyelitis, and others — pass through a period of noncharacteristic prodromal symptoms during which clinical diagnosis is difficult, if not impossible; the nature of most of these conditions becomes clinically evident only after the appearance of specific lesions. Might it not be that the incubation period of these infectious diseases represents a stage of adaptation of virus to the host environment during which multiplication of the virus takes place within Kupffer cells, prior to reaching the final target cell? This concept is not solely of academic interest but may constitute a basic consideration in attempts at reaching the virus, soon after infection within the reticuloendothelial system, by therapeutic means; attempts to influence the progress of the disease after the parenchymal cells have been invaded and destroyed would seem to be futile.

The role of the reticuloendothelial cells as hosts for infective organisms has been recognized in a number of diseases. In kala-azar, the Leishman bodies seem to thrive in reticuloendothelial cells; in some of the generalized fungus infections, in tuberculosis, and in typhus fever, the specific organisms causing these diseases have been seen in the cells of the reticuloendothelial system. Similarly, BAMARAPRAVATI has recently described lesions in Kupffer cells and liver cells in cases of hemorrhagic fever of Thailand that closely resemble lesions of yellow fever. Results of experimental studies of viral hepatitis in mice by RUEBNER also indicate that Kupffer cell changes precede any parenchymal cell alterations. It appears possible, therefore, that the reticuloendothelial system can provide shelter for the survival of organisms and furnish ribonucleic proteins for the formation of virus particles [BEARCROFT (2)].

The Councilman body is without doubt the most characteristic and pathognomonic histopathologic lesion of yellow fever. It represents evidence of damage and possible death of affected liver cells and is comparable to the Mallory body of nutritional cirrhosis, as well as to the acidophilic body of infectious hepatitis. The number of such bodies provides a gauge concerning the intensity of the infection, since regeneration of parenchymal cells from necrotic or necrobiotic elements is impossible.

The histopathologic study of the livers of animals that survived the infection showed gradually diminishing numbers of Councilman bodies, surrounded by monocytic cells. Formations of such granulomatous reactions were seen in monkeys 5 days after challenge, but were much more prominent and massive in animals 7 days after inoculation. The necrotic cells, presumably representing residues of degenerated Councilman bodies, were surrounded by monocytes and histiocytic giant cells forming rather definite granulomas, and these changes were accompanied by extensive regeneration of liver cells. This process resulted in systematic repair of the liver cell plates, with gradual restoration of the lobule. For several weeks following the recovery, focal accumulations of monocytes and lymphocytes were evident in the liver tissue, particularly in the stroma of the portal canals, and then they too became insignificant. The liver parenchyma had regained its usual appearance, and only minor traces of the former damage remained; no scars were evident [KLOTZ and BELT (1)].

The time of the appearance of the granulomatous reaction coincided with the time of appearance of antibodies to yellow fever virus in the blood stream, beginning approximately 6 days postchallenge. Similar observations and explanations were reported by BEARCROFT (2) and CASALS.

Intranuclear inclusions of liver cells, pathognomonic of yellow fever in human beings and experimental animals, have been described by TORRES and by COWDRY and KITCHEN (1, 2) and have been compared with those produced experimentally by GOODPASTURE and TEAGUE, with herpes virus. Presence of intranuclear inclusion bodies in yellow fever has been noted by KLOTZ and BELT (1) in about one-fourth of their human cases and in 14 out of 19 monkeys infected with yellow fever virus, but the diagnostic significance of these inclusions is questioned by these authors. To judge from our own material, the time of the appearance of intranuclear inclusions in the course of yellow fever is too late for diagnostic evaluation. Intricate details of the histopathologic and electron microscopic evolution of the inclusion bodies are given by BEARCROFT (1, 2). According to these studies, the intranuclear changes consist of an enlargement of the nucleolus by aggregation of amorphous material. At the time of their development the cytoplasm shows swelling and distortion of mitochondria, loss of membranous elements of the endoplasmic reticulum, and accumulation of lipid droplets. Spherical particles, presumably representing virus and measuring 55 to 61 mμ, are formed from rosettes of aggregated particles in the cytoplasmic matrix; these granules are related to the passage of ribonucleic protein, synthetized within the nucleolus, to the cytoplasm. These intracellular alterations result, then, in the distortion of the liver cells and rupture of liver cell plates and their bile canaliculi. Virus particles have never been demonstrated within the nucleus in yellow fever.

Summary and Conclusions

1. In the prodromal phase of experimental yellow fever, the acidophilic hyaline necrosis of Kupffer cells appears to represent the earliest histopathologic evidence of the presence of infection.

2. During the clinical stage of experimental yellow fever, the histopathologic diagnosis is based on the presence of Councilman bodies in the liver, which are pathognomonic of this condition. In advanced cases, these bodies are distributed mainly through the midzonal region of the lobules, but neither the periphery nor the central area are exempted.

3. The granulomatous reaction indicates subsidence of active injury and introduces the healing phase of yellow fever; its pathogenetic origin may be difficult to recognize without reference to previous stages of the lesions because of the lack of specific criteria.

4. Coinciding with the appearance of Councilman bodies, clinical symptoms of liver damage become apparent, and are often coupled with evidence of gastrointestinal, renal, and cerebral involvement, as well as with disturbances of the clotting mechanisms of the blood.

5. In experimental animals surviving the yellow fever infection for 1 week or longer, the liver exhibits a granulomatous reaction about remains of Councilman bodies, accompanied by extensive regeneration of liver cells.

6. Recovery from yellow fever infection is followed by complete healing of lesions and restoration of the architecture of the liver lobules without permanent residues or scars.

7. The presence of intranuclear inclusion bodies in liver cells is not a reliable diagnostic criterion of the infection during the early stages of experimental yellow fever.

8. The evaluation of the histopathologic changes seen in needle biopsies of the liver, in conjunction with fluorescent antibody studies, might offer a possibility of the recognition of human yellow fever infection during the incubation period.

9. The clearing mechanism, representing the phagocytic removal by reticuloendothelial cells of virus particles introduced in the blood stream, initiates the incubation period during which multiplication of the virus takes place. There are no significant clinical symptoms and laboratory findings at this stage.

10. It is suggested that the virus of yellow fever, introduced into a nonimmune individual, is taken up by reticuloendothelial cells serially $\frac{1}{M}$ particularly by Kupffer cells — and multiplies within these cells until it becomes adapted to the host enviroment and reaches optimal concentration for infecting parenchymal liver cells.

11. There are indications that this process of incubation of organisms in reticuloendothelial cells, prior to specific alterations of parenchymal cells, may also obtain in certain infections other than yellow fever.

Zusammenfassung

In der Prodromalphase des Gelbfiebers bilden die acidophilen hyalinen Nekrosen der Kupfferschen Sternzellen die frühesten histopathologischen Zeichen der Infektion. Während der klinisch manifesten Phase des experimentellen Gelbfiebers stützt sich die histopathologische Diagnose auf die Anwesenheit von Councilman-Körperchen in der Leber, welche für die Gelbfieberinfektion pathognomonisch sind. Die granulomatöse Gewebsreaktion leitet die Heilphase ein. Eine Spezifität kommt ihr nicht zu. Die Versuchstiere, welche die Gelbfieberinfektion für eine Woche und mehr überleben, zeigen neben der Granulombildung um die Reste der Councilman-Körperchen eine intensive Regeneration der Leberzellen mit vollständiger Wiederherstellung der Leberarchitektur.

In der symptomlosen Inkubationsperiode werden die im Blute zirkulierenden Gelbfieberviren von den Zellen des reticuloendothelialen Systems phagocytiert und vermehren sich innerhalb derselben. Es ist wahrscheinlich, daß erst nach entsprechender Anreicherung der Viren, insbesondere in den Kupfferschen Sternzellen, die Leberparenchymzellen infiziert und geschädigt werden.

Dieser Inkubationsprozeß trifft wahrscheinlich auch auf andere Infektionskrankheiten zu.

References

BAMARAPRAVATI, NATTH: The pathologic changes in the liver in cases of hemorrhagic fever of Thailand. To be published. Personal communication, 1960.

BEARCROFT, W. G. C.: (1) The histopathology of the liver of yellow fever-infected rhesus monkey. J. Path. Bact. **74**, 295—303 (1957).

— (2) Electron microscope studies on the liver cells of yellow fever-infected rhesus monkeys. J. Path. Bact. **80**, 421—426 (1960).

Bugher, J. C.: The pathology of yellow fever. In: Yellow fever, J. D. Strode, edit., p. 137—163. New York: McGraw-Hill Book Co. 1951.

Casals, J.: Cited by Bearcroft (1), J. Path. Bact. **74**, 302 (1957).

Collier, W. A., H. de Roever-Bonnet and J. Hoedstra: A new intracerebral yellow fever mouse protection test. Trop. geogr. Med. **11**, 167—173 (1959).

Councilman, W. T.: In G. M. Sternberg, Report on etiology and prevention of yellow fever. U.S. Marine Hospital Service. Publ. Hlth. Bull. (Wash.) No 2, p. 151—159. 1890.

Cowdry, E. V., and S. F. Kitchen: (1) Intranuclear inclusions in yellow fever. Science **61**, 252—253 (1929).

— — (2) Intranuclear inclusions in yellow fever. Amer. J. Hyg. **11**, 227—299 (1930).

Goodpasture, E. W., and O. Teague: Experimental production of herpetic lesions in organs and tissues of the rabbit. J. med. Res. **44**, 121—138 (1923).

Kerr, J. A.: The clinical aspects and diagnosis of yellow fever. In: Yellow fever, J. D. Strode, edit., p. 385—425. New York: McGraw-Hill Book Co. 1951.

Klotz, O., and T. H. Belt: (1) The pathology of the liver in yellow fever. Amer. J. Path. **6**, 663—687 (1930).

— — (2) Pathology of spleen in yellow fever. Amer. J. Path. **6**, 655—662 (1930).

—, and W. Simpson: (1) Jaundice and the liver lesions in West African yellow fever. Amer. J. trop. Med. **7**, 271—278 (1927).

— — (2) The spleen in West African yellow fever. Amer. J. Path. **3**, 483—488 (1927).

Marshall, J. D., W. C. Eveland and C. W. Smith: The superiority of fluorescein isothiocyanate (Riggs) for fluorescent antibody technic with a modification of its application. Proc. Soc. exp. Biol. (N.Y.) **98**, 898—900 (1958).

Reed, W., J. Carroll and A. Agramonte: Experimental yellow fever. Amer. Med. (Philad.) **2**, 15—23 (1901).

Rocha-Lima, H. da: (1) Zur pathologischen Anatomie des Gelbfiebers. Verh. dtsch. path. Ges. **15**, 163—182 (1912).

— (2) Zur pathologisch-anatomischen Diagnose des Gelbfiebers. Arch. Schiffs- u. Tropenhyg. **16**, Beih. 1, 192—199 (1912).

Ruebner, B. H.: The Kupffer cell reaction in experimental and human viral hepatitis. To be published. Personal communication 1961.

Seidelin, H.: Histology of the liver in yellow fever. Liverpool School of Tropical Medicine, Yellow Fever Bur. Bull. **3**, 269—298 (1913—1915).

Smith, K. C.: Immunology. In: Yellow fever, J. D. Strode, edit., p. 165—227. New York: McGraw-Hill Book Co. 1951.

Soper, F. L.: In bibliography of: Yellow fever, J. D. Strode, edit., p. 669—670. New York: McGraw-Hill Book Co. 1951.

Stokes, A., J. H. Bauer and N. P. Hudson: (1) The transmission of yellow fever to Macacus rhesus (preliminary note), J. Amer. med. Ass. **90**, 253—254 (1928).

— — — (2) Experimental transmission of yellow fever to laboratory animals. Amer. J. trop. Med. **8**, 103—164 (1928).

Theiler, M.: The Virus. In: Yellow fever, J. D. Strode, edit., p. 39—136. New York: McGraw-Hill Book Co. 1951.

Tigertt, W. D., T. O. Berge, W. S. Gochenour, C. A. Gleiser, W. C. Eveland, C. Vorder Bruegge and H. F. Smetana: Experimental yellow fever. Trans. N.Y. Acad. Sci. **22**, 323—333 (1960).

Torres, C. M.: Intranuclear inclusions in experimental yellow fever. Mem. do Inst. Oswaldo Cruz Suppl. **6**, 69—71 (1929).

Turnbull, H. M.: Report on the examination of organs from case L 26. Great Britain Yellow Fever Commission 1, p. 197, 1913.

Villela, E.: Histology of human yellow fever when death is delayed. A.M.A. Arch. Path. **31**, 665—669 (1941).

Warren, A. J.: Landmarks in the conquest of yellow fever. In Yellow fever, J. D. Strode, edit., p. 1—37. New York: McGraw-Hill Book Co. 1951.

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