STUDIES ON THE TRANSMISSION OF THE AGENT OF INFECTIOUS HEPATITIS TO PATAS MONKEYS

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PLATES I AND II

The changes present in the livers of patas and some other species of West African monkeys after inoculation with liver suspensions from fatal human cases of infectious hepatitis have been described (Bearcroft, 1963, 1964).

These changes occurred 2–4 wk after inoculation and were thought to be caused by a relatively thermostable virus, which could be maintained in serial monkey to monkey passage by inoculation of suspensions of liver from infected into healthy animals. The hepatic lesions varied in extent; sometimes they were inapparent, more often they consisted of scattered foci of liver cell changes together with portal degeneration and infiltration. Occasionally they became widespread and were associated with death of the monkey. Because the lesions varied in extent and time of occurrence it was considered that their observation was not a sufficiently reliable indication of infection to justify the use of the patas monkey as an experimental animal for serological and other studies of the infection. Furthermore, although the hepatic changes were thought to be induced by an infectious agent present in the inoculum it was possible that they arose from other, unrelated causes.

Before attempting to use monkeys for laboratory tests, it was considered necessary to determine whether the hepatic lesions formed part of a more generalised disease process induced by an infectious agent present in the human or passage-monkey livers. In cases of infectious hepatitis in man the predominant pathological changes are a reduction in numbers of neutrophil leucocytes (Havens and Marck, 1946; Miles, 1951) and the production of liver lesions (Mallory, 1947; Smetana, 1957). Changes have also been recorded in the spleen and lymph-glands by Lucké (1944) and Wilson (1951). Lucké described changes in the gall-bladder, bone marrow and central nervous system in fatal cases of this disease. Saphir, Amromin and Yokoo (1956) found foci of necrosis in the myocardium and Conrad, Schwartz and Young (1964) found alterations in biopsy specimens of the kidneys and gastrointestinal tract. It seemed possible, therefore, that, if the liver changes recorded in monkeys formed part of a disease process, there might be changes in other tissues, especially a decrease in numbers of neutrophil leucocytes.
A detailed study has therefore been made of the tissues of patas monkeys that had been given an inoculation of, or had been fed with, a suspension of liver from a fatal human case of infectious hepatitis or from a monkey to which the infection had been passed by inoculation or feeding. Patas monkeys have also been given injections of liver from human patients dying from a variety of causes other than infectious hepatitis. Observations have been made on the frequency of occurrence and extent of the hepatic changes, and electron microscopy has been carried out to detect finer alterations in the liver cells. Because tests for liver function show abnormal values in human cases of infectious hepatitis, similar tests have been made on infected monkeys. Studies have also been made to elucidate the nature of the causative agent and to attempt to isolate it from both human and monkey sources in tissue cultures.

Materials and methods

Monkeys

For most experiments young adult patas monkeys (Erythrocebus patas) of either sex and weighing between 1100 and 2500 g were used. Some smaller animals, 600-1000 g, and a few large monkeys, 4100-6100 g, were also used. Monkeys obtained from local dealers suffered from a variety of diseases. It was necessary, therefore, before attempting experimental studies, to place animals in quarantine for 2-4 wk and treat their diseases with the appropriate medications. For pre-inoculation studies, monkeys were transferred to an "uninfected house"; later they were transferred to an "infected house" before the administration of presumably infectious material.

The animals were fed on a diet of mixed fruits twice daily and drinking water was given ad libitum. In addition, each monkey was given daily about 100 ml of reconstituted powdered milk containing chlortetracycline, which was used to avert diarrhoea caused by Salmonella enteritidis or S. typhimurium, vitamin complex containing vitamin B₁₂ and folic acid (Jones et al., 1947), and ferrous sulphate. This milk was prepared by homogenising 35 g Gitana powdered milk (Co-Op, Holland), 2 g veterinary chlortetracycline (Cyanamid of Great Britain Ltd), two capsules Concavit (Wallace Ltd, London) and two tablets Fersolate (Glaxo Laboratories, Nigeria, Ltd) in 1 litre of tap water. Attempts to remove intestinal worms (Strongyloides spp. and Trichuris trichiura), which may cause suppurative ulceration of the intestine, were made by a weekly addition of 500 mg thiabendazole (Mintezol, Merck Sharp & Dohme Ltd) to 1 litre of reconstituted milk. Fleas and lice (Linognathus spp.) were killed by dusting at weekly intervals with Vetox 5 Insecticide (Shell Chemicals Ltd). Septic conditions of the tail and eyes were treated by intramuscular injections of penicillin and the local application of chlortetracycline ointment. Fungus infections were treated by the application of Tineafax (Burroughs Wellcome & Co., Nigeria, Ltd) to the affected areas or the local administration of griseofulvin (Fulcin Forte, Imperial Chemical Industries Ltd, Macclesfield). Infections with Hepatocystis kochi were sometimes present but were not treated. A total of 367 patas monkeys was used.

Material inoculated into monkeys

Four groups of specimens of liver, which were obtained in Nigeria, were used for inoculation into monkeys.

Group 1. Seven livers showing normal morphology on histological examination. These were derived from five patients dying from causes other than infectious hepatitis and from two healthy, freshly killed patas monkeys.

Group 2. Twelve human livers showing an altered morphology on microscopic examination. The histopathological changes were cirrhosis (2 livers), secondary carcinoma (2),
moderate to marked fatty change (3), small foci of necrosis caused during eclampsia (2), marked centrolobular necrosis caused during congestive cardiac failure (2) and peripheral necrosis with fatty change (1). The last liver was derived from a clinical case of infectious hepatitis (patient KL). The extent of necrosis present in the livers of the two patients with eclampsia was about the same as that seen in biopsy specimens of the liver in non-fatal human infectious hepatitis. The necrosis in the livers of the two cases with congestive cardiac failure was comparable in extent to that seen in the human liver in fatal infectious hepatitis.

**Group 3.** Four human livers showing histopathological changes characteristic of fulminant infectious hepatitis. These were from young adult Nigerians, two males (patients JN and GA) and two females (patients EE and TK). All had a history of icterus of the sclera, dark urine and anorexia for about 1 wk before being admitted to hospital in semi-coma. Most patients showed increased serum bilirubin and alkaline phosphatase and bile pigments were present in the urine. All were diagnosed as infectious hepatitis and died within 36 hr of admission to hospital. This diagnosis was confirmed at necropsy.

**Group 4.** Livers from monkeys that had been given injections of, or had been fed with, livers from patients with infectious hepatitis (group 3) and livers obtained by serial monkey to monkey passage of infected livers from these animals. The livers obtained from monkeys infected with the liver suspensions of patient EE were passed serially seven times in further monkeys. Livers from monkeys infected with the livers of patients JN and GA were passed only four and two times respectively, whilst the liver of patient TK has been recently received and has not been passed.

**Methods of inoculation**

All livers were emulsified in sterile physiological saline, containing 100 units of penicillin and 50 µg streptomycin per ml, to make 10 per cent. suspensions. The suspensions were clarified by centrifugation at 1000g for 30 min., and the supernatant fluids were stored at −70°C or at +4°C until required. Suspensions of livers from groups 1 and 2 were injected intraperitoneally into monkeys in 5-ml amounts. Suspensions of livers from groups 3 and 4 were given to monkeys in 1–5 ml amounts by the oral or intraperitoneal route. The livers of patients JN and GA were administered to groups of monkeys by both routes, but the liver of patient EE was given by injection only and that of patient TK by feeding only. Feeding was carried out either by pipetting the liver suspension directly into the mouth or by mixing it with crushed banana. To determine the effects of heat on the infectivity of livers in groups 3 and 4, some suspensions were heated at 56°C for 30 min. and others at 56°C for 5 hr before feeding or injecting into monkeys.

**Necropsy**

Unless they died earlier, the monkeys were killed under ether anaesthesia 6–8 wk after the administration of liver suspensions. At necropsy, samples were taken from varying regions of the liver and from a variety of other tissues, fixed in Carnoy's 6 : 3 : 1 fluid, sectioned and stained with haematoxylin and eosin for histological examination. The remainder of the livers of animals in groups 3 and 4 were stored at 4°C. Those livers showing parenchymal and stromal changes suggesting a virus infection were then emulsified for passage to healthy animals.

**Electron-microscope studies**

These were carried out mainly on patas monkeys, but some mona, putty-nosed and tantalus guenons were also studied. Animals were given an injection of a suspension of infected patas monkey liver (group 4). Liver biopsies were carried out between 3 and 6 wk after inoculation. Biopsies were made also on the livers of 10 healthy, uninoculated monkeys, 4 of which were patas, 3 mona and 3 tantalus guenon. All liver specimens were divided into two portions. One of these was immediately cut into small fragments, fixed in ice-cold 1 per cent. osmium tetroxide, buffered at pH 7.4, passed through ascending strengths of ethanol and embedded in a 1 : 4 mixture of methyl and n-butyl methacrylates. The other portion was briefly fixed in Carnoy’s 6 : 3 : 1 fluid and conventional paraffin wax sections prepared. These were
stained with haematoxylin and eosin, and by the PAS method, with amylase extraction, for glycogen. After examination by light microscopy, 16 infected and 10 uninfected livers were selected for study by electron microscopy. From infected animals, biopsies were chosen that showed moderate to marked parenchymal and stromal changes and either absence or marked reduction of cytoplasmic glycogen.

Sections were cut on a Si-Ro-Flex ultramicrotome, fitted with a diamond knife and stained with lead hydroxide (Lever, 1960). Examinations were carried out with an AEI EM6 electron microscope at instrumental magnifications between ×4000 and ×50,000; 592 photographs were taken.

**Leucocyte counts**

Most experiments were made with groups of 3–6 animals. A total of 219 monkeys was examined. In most cases the leucocyte counts were made over a period of 2 wk before feeding or injecting the liver suspensions and, unless the monkeys died earlier, for periods of 6–8 wk thereafter. In some cases such detailed pre-inoculation counts were not carried out. Counts were usually made thrice weekly, but in some instances they were carried out daily and in others twice weekly. The numbers of leucocytes were estimated in blood from an ear vein. About three-quarters of the total white cell counts were made by counting all cells in the four corner squares of two Neubauer haemacytometer chambers. The remainder were made with a Coulter Model “D” electronic blood cell counter.

Differential white cell counts were made by counting 200 cells in a Leishman-stained blood film using the “battlement” method (MacGregor, Richards and Loh, 1940). The numbers of each cell type for each monkey were expressed in absolute figures. These figures were then added and the mean determined for each cell type in each group of monkeys. Graphs were then prepared showing the absolute and mean variations in the number of cells during the period of investigation. A total of 6130 leucocyte counts was made.

Suspensions of livers were given to monkeys after completion of blood counts on the day of inoculation. To avoid haemorrhage or the introduction of pathogenic bacteria, which might affect the numbers of leucocytes, liver biopsies were never carried out.

**Biochemical tests of liver function**

Liver function tests were made on the sera of uninfected patas monkeys and sera were collected from 44 monkeys after inoculation of human infectious hepatitis or passage monkey liver suspensions. Animals surviving the infection were killed 8–12 wk after inoculation and evidence of hepatitis was obtained by histological examination of the liver after necropsy. To avoid trauma of the liver, which might affect the results of the biochemical tests, pre- and post-inoculation biopsies were not carried out. The inocula consisted of suspensions of either liver from a fatal human case of infectious hepatitis or livers from passage monkeys. Samples of serum were taken from the femoral blood vessels before and at intervals of 3–7 days after inoculation. The following tests were carried out on the day of collection of the serum.

**Serum alkaline phosphatase.** Levels were estimated by the enzymic release of phenol from Folini and Ciocalteu’s reagent and expressed in King-Armstrong units per 100 ml serum; 156 pre-inoculation and 340 post-inoculation sera were studied.

**Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT).** Levels were determined with prepared substrates and colour reagents (Sigma Chemical Company) and readings recorded in Frankel units per ml serum. Studies on both enzymes were made on 100 pre-inoculation and 386 post-inoculation sera.

**Bromsulphthalein (BSP) retention.** Studies were carried out by injecting sodium bromsulphthalein (G. T. Gurr Ltd), 10 mg per kg body weight, into a saphenous vein. Blood samples were taken 20 and 45 min. after injection. Forty-two tests were made on uninoculated monkeys and 34 on infected monkeys. The results are expressed as the percentage of dye retained at the times of bleeding.

**Total protein, albumin and globulin.** Levels were estimated by the biuret method. Studies
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were made on 70 pre-inoculation and 44 post-inoculation sera. All colorimetric observations were carried out with a Klett-Summerson photoelectric colorimeter.

Thymol turbidity. Determinations on 50 pre-inoculation and 135 post-inoculation sera were made by the method of Maclagan and the results expressed in Maclagan units.

Colloidal gold and colloidal red. Tests were made on 71 pre-inoculation and 106 post-inoculation sera by the methods of Maclagan and Ducci and the degree of precipitation was recorded as from “0” to “5+”.

Cephalin-cholesterol flocculation. Tests were done on the sera of 98 un inoculated patas monkeys with Bacto cephalin-cholesterol antigen (Difco Laboratories) and the results recorded as from “0” to “4+” flocculation.

Electrophoresis of serum proteins. Electrophoretic patterns were made on 5 x 36 cm strips of Whatman no. 1 chromatography paper in a vertical tank (Shandon Scientific Company). With constant current, 1.25 mA were applied to each strip by a Vokam power unit for 17-18 hr. After drying at 80°C for 30 min. the strips were stained with lissamine green, dried and scanned with an EEL scanner (Evans Electroelenium Ltd). Examinations were made on 123 sera from uninoculated patas, 24 sera from uninoculated monkeys of other West African species and 82 sera taken from patas monkeys between 21 and 84 days after inoculation.

About 2650 biochemical tests of all types were carried out. Because there is never evidence of jaundice, studies were not made on serum bilirubin.

RESULTS

Clinical findings in infected monkeys

Although the disease usually runs a non-fatal course, animals may die during the second or subsequent weeks after administration of liver suspensions by the oral or intraperitoneal route.

Non-fatal cases. In non-fatal cases there are no clinical manifestations of disease. Although anorexia is rarely apparent, some animals show a loss in weight of from 100 to 200 g. Icterus is never observed in the conjunctivae and no significant alterations are noted in the colour of the blood serum, urine or faeces. The temperature fluctuates from day to day within the normal limits of 38.3°C and 39.9°C. There is no evidence of involvement of the central nervous system after intracerebral inoculation of infectious material.

Fatal cases. In monkeys in which the disease ends fatally, sickness is first noticed 2-4 days before death. The earliest sign is listlessness; the animals prefer to sit quietly in a corner of the cage using the walls for support. They become fully active when disturbed. During the next 24-48 hr listlessness increases and the animals sit with their heads resting on the upper part of the chest. At the time anorexia is apparent and the temperature falls rapidly to reach about 35.5°C and listlessness passes in to semi-coma. At this time the animals lie motionless on the floor of the cage. Coma ensues and death occurs within a few hours. There is no jaundice or other evidence of liver disease.

Pathological changes

Liver

Hepatic changes are induced in patas and some other species of West African monkeys after the oral (fig. 1) or parenteral administration of suspensions of liver from human patients with infectious hepatitis or from passage
monkeys, but their extent and the time and frequency of their occurrence are very variable. In many monkeys no hepatic changes can be found in either biopsy or necropsy specimens. In other animals the changes are minimal and often difficult to recognise. In a few animals they become widespread and are associated with death of the animal. Although an insufficient number of monkeys has been examined to permit accurate assessment, the available results suggest that hepatic changes occur with about equal frequency whether the animals are fed with infectious material or it is injected. Though the severity of these changes does not appear to be related to the sex of the monkey, it is influenced by weight; the earliest and most pronounced alterations occur in animals of 600–1000 g. Thus, of eight small patas monkeys given injections of human infectious hepatitis or passage-monkey liver, seven showed liver changes and two died. Limited biopsy and necropsy studies on large monkeys have shown that liver changes are not marked and that death from hepatitis does not occur. The severity of the liver changes appears to increase on passage of the agent through patas monkeys. Thus, after the administration of liver suspensions from fatal human cases of infectious hepatitis to 56 patas, 32 (57.1 per cent.) of the animals showed liver changes and two (3.6 per cent.) of them died. Though there was a slight increase in the frequency and extent of the liver changes between the first and subsequent passages, a review of all passage monkeys showed that 78 of 95 (82.1 per cent.) had liver changes and 21 (22.1 per cent.) died. In not all cases, however, were the hepatic changes sufficiently marked to account for death of the animal. The earliest recorded time of death attributable to the infection was 11 days; the latest time was 56 days and the mean 28.5 days.

At necropsy there were no alterations visible to the naked eye, and never any discoloration of the tissues suggesting jaundice. The liver appeared normal in size, colour and consistency, and neither the surface nor the cut section gave any indication of the extent of parenchymal or stromal changes. In histological section the changes vary from occasional small foci to widespread parenchymal change. The foci consist of liver and Kupffer cells with increased cytoplasmic basophilia and enlarged nuclei and nucleoli. Although necrosis is rare, mitotic figures are present. The foci and the portal tracts are infiltrated with inflammatory cells. These changes have been described in detail (Bearcroft, 1964). The electron-microscope appearance of healthy, uninoculated patas monkey livers has also been recorded (Bearcroft, 1962). Electron microscopy of the livers of monkeys after injection of passage-monkey livers shows a variety of morphological changes. The liver cell nucleus is often irregular in shape and the nuclear membrane is undulated. The nucleoplasm is usually diffusely filled with granular material suggesting an increase of nucleoprotein. In the cytoplasm the mitochondria are often increased in numbers from about 40 per cell section in uninoculated monkeys up to 200 per cell section in specimens where there is advanced liver cell change. Forms that appear to be in division are only occasionally seen. Frequently the mitochondria are swollen and gaps may be present in the limiting membranes. The endoplasmic reticulum is almost always dilated to form large irregular sacs and many of the ribosomes appear
to be absent from the rough components. Ribosomes are, however, seen scattered throughout the cytoplasmic matrix either singly or in aggregates of up to eight. Glycogen granules are almost always absent. The lysosomes do not appear to be altered in number, but sometimes contain a variety of internal structures, which vary from multiple parallel membranes to cyst-like bodies. Often gaps are present in the lysosome membranes through which the internal contents appear to be passing into the cytoplasmic matrix. The Golgi complex is often greatly hypertrophied in both the liver and Kupffer cells. The cisternae of these organelles are swollen into sac-like structures, which are surrounded by large numbers of vesicles. No structures that might be interpreted as virus particles have been seen. Changes, similar to the above, are present in the livers of the other species of monkeys examined.

Spleen and mesenteric lymph-glands

The spleen varied greatly in size and appearance in uninoculated monkeys. Necrosis was never seen by light microscopy. The spleens of most inoculated monkeys show degeneration of cells in both the red and white pulp. Sometimes, degeneration may be more extensive and cause complete necrosis of the germinal centres (figs. 2 and 3). Necrosis of the spleen is almost always present in fatal human cases of infectious hepatitis in Lagos (fig. 4). The mesenteric lymph-glands show degeneration of cells in the germinal centres.

The leucocytes

Pre-inoculation findings

The total number of leucocytes and the numbers of different types of white cells varied considerably from monkey to monkey. Day-to-day counts on individual animals often revealed marked fluctuations in the numbers of neutrophils and lymphocytes, and smaller fluctuations in those of other types of cells. In most animals, the neutrophils varied between 2500 and 7000 per c.mm., occasionally descending below 2000 per c.mm. Sometimes the numbers were increased through inapparent causes, although ulceration of the intestine resulting from infestation with Strongyloides sp. and septic conditions of the tail were frequently responsible. Occasionally animals were found with consistently low (<2000 per c.mm.) neutrophil counts or monkeys with normal or slightly raised counts showed a transient fall to a low level. Although the state of "neutropenia" has not been defined for monkeys, in the present studies it is regarded as indicating a mean neutrophil count of less than 3000 per c.mm. After a period in captivity of two or more months, some monkeys showed a slow but progressive decrease in the numbers of neutrophils. Animals with neutrophil counts that remained above 7000 or below 2000 per c.mm. were rarely inoculated. The number of lymphocytes was about the same as that of the neutrophils and usually showed similar fluctuations. Although the number of monocytes was always estimated, it was often difficult to differentiate between these cells and lymphocytes. It was, therefore, found convenient to class the monocytes with lymphocytes and record the total numbers of mononuclear
cells. Other cells, which do not appear to be either lymphocytes or monocytes, were occasionally seen. These cells varied in size and had a single large nucleus and basophilic cytoplasm. Their count rarely exceeded 50 per c.mm. and they have been classed with the lymphocytes and monocytes. The numbers of eosinophils showed very great variation. On receipt at the laboratory, monkeys usually had about 500 eosinophils per c.mm., but figures as high as 4000 were recorded. Over periods of 4–8 wk in captivity some animals showed a progressive rise in the numbers of these cells, and rarely figures as high as 10,000 were recorded. The cause of this eosinophilia was not determined. It may have been the result of infestation with intestinal worms. The basophils were the least numerous of the leucocytes and showed only small fluctuations in numbers. The numbers of the different types of leucocytes found in the blood of 100 apparently healthy monkeys are shown in table I.

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of cells per c.mm. of blood (and percentage of total leucocyte count)</th>
<th>Range</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leucocytes</td>
<td></td>
<td>4875–19,850 (100)</td>
<td>10,296 (100)</td>
<td>± 3537</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td>1045–11,800 (7.5–77.5)</td>
<td>4603 (46.7)</td>
<td>± 2465</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td>1390–14,750 (14.0–88.0)</td>
<td>4528 (44.0)</td>
<td>± 2498</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td>38–2390 (9.5–13.5)</td>
<td>499 (4.9)</td>
<td>± 365</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td>0–3975 (0–28.0)</td>
<td>505 (5.0)</td>
<td>± 695</td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td>0–788 (0–6.0)</td>
<td>152 (1.4)</td>
<td>± 148</td>
</tr>
</tbody>
</table>

Post-inoculation findings

To condense the results, the absolute and mean numbers of cells for several groups of monkeys, given injections of, or fed with, similar suspensions have been pooled and represented as a single graph (figs. 6–9). For illustration, however, the graph for a group of monkeys given an injection of sixth monkey-passage liver is shown. Graphs showing the absolute and mean numbers of neutrophils are sometimes modified by leucocytosis caused through bacterial infections arising in one or more animals after the administration of liver suspensions. Such infections were treated with the appropriate medications.
FIG. 1.—Scattered parenchymal foci of altered liver and Kupffer cells and portal infiltration in a patas monkey 56 days after it had been fed with 4 ml liver suspension of patient JN in crushed banana. Carnoy. Haematoxylin and eosin. ×250.
Fig. 2.—Germinal centre in spleen of healthy patas. Carnoy. HE. ×550.

Fig. 3.—Necrosis of germinal centre in spleen of patas dying 18 days after injection of passage monkey liver. Carnoy. HE. ×550.

Fig. 4.—Necrosis of germinal centre in fatal human infectious hepatitis. Formaldehyde-saline. HE. ×550.

Fig. 5.—Part of one of many large foci of altered liver cells with portal tract (above) and healthy liver cells (right). There is no necrosis and levels of serum enzymes were within the limits of normal. Patas, 56 days after injection. Carnoy. HE. ×225.
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Livers not from infectious hepatitis (groups 1 and 2)

The only change noticed after the intraperitoneal inoculation of 5 ml of a 10 per cent. suspension of "normal", carcinomatous, cirrhotic, fatty or necrotic human liver or "normal" monkey liver was an apparent increase in the numbers of neutrophils (fig. 6). This increase, which took place 2-4 days after injection, was rarely marked and did not occur in all monkeys. The mean number of neutrophils returned to pre-inoculation levels by the 7th day. The other types of leucocytes were unaffected. Histological examination of necropsy specimens showed a varying amount of portal infiltration in 24 per cent. of the livers, but parenchymal lesions suggesting a virus infection were never seen. Six monkeys given injections of the liver of patient KL showed no marked alterations in the number of neutrophils and no hepatic changes.

Livers from fatal cases of infectious hepatitis (group 3)

Both the intraperitoneal and the oral administration of liver suspensions from the four fatal cases of infectious hepatitis caused a depression of the neutrophil count and the production of hepatic changes. There was great variation in the response of individual animals, but graphs of the mean neutrophil levels for groups of monkeys showed a characteristic pattern, that for intraperitoneal inoculation differing in certain features from that for oral infection.

Intraperitoneal inoculation (fig. 7). During the 1st wk after inoculation there was no constant pattern of change in the leucocyte counts. Some graphs showed a marked depression during the first 4 days, but others showed normal or even slightly raised levels. In most of the latter cases, however, there was a transient fall on or about the 7th day. An occasional low count was recorded during the 2 wk before inoculation, but an increased number of monkeys showed absolute values of 2000, or fewer, cells per c.mm. between days 2 and 56. During the 1st wk, therefore, the over-all picture was that of a slight depression of the mean neutrophil count to about 3000 cells per c.mm. During the 2nd wk many monkeys showed an increase in the number of neutrophils. This increase reached a maximum by the 10th day and thereafter subsided rapidly so that the mean count was about 2000 by day 16. Between days 16 and 56 the mean count fluctuated between 2000 and 3000, with transient increases to about 4000. Concurrent bacterial infections did not greatly affect the level of neutrophils during the 1st wk, but abscess formation caused considerable increases in some animals in the subsequent weeks. Because of these intercurrent infections it was difficult to ascertain the length of time for which the neutrophil count remained depressed, though many monkeys still showed counts of 2000 or less on day 56. The mean neutrophil level for all groups of monkeys showed a decrease in neutrophils after the end of the 2nd wk and only 1 out of 21 monkeys did not show neutropenia at any time.

Oral administration (fig. 8). The earliest change was a rapid fall in the number of neutrophils. This fall started on the 2nd day after infection and the counts reached their lowest values by the 4th day, when the mean neutrophil
FIG. 6.—Means of neutrophil counts in monkeys receiving (arrow) either suspensions of livers from human patients and monkeys not having infectious hepatitis, or heated suspensions of infected liver.

- - - - Means for 77 monkeys given intraperitoneal injections of “uninfected” human or monkey liver (groups 1 and 2).

- - - - - Means for 12 monkeys given intraperitoneal injections of liver from two human patients with congestive cardiac failure.

- - - - - - Means for 14 monkeys after inoculation by feeding with human infectious hepatitis and passage monkey livers (groups 3 and 4) after these had been heated at 56°C for 5 hr.

FIG. 7.—Individual (-) and mean (—) counts of neutrophils in the blood in monkeys given intraperitoneal injections of 4.5 ml of a suspension of human infectious hepatitis liver (group 3).
counts in the different groups of animals were between 2000 and 2500. Only 1 out of 18 monkeys did not show this decrease. Subsequently the mean neutrophil counts for most groups remained at about the same low level until the 56th day. In a few groups, however, there was a moderate increase in neutrophils during the 2nd, 3rd and 4th wk followed by a return to neutropenia. In such cases there was often evidence of sepsis in one or more animals.

Livers from passage monkeys (group 4)

Neutropenia and hepatic lesions were also produced by feeding or injecting suspensions of liver from monkeys showing hepatic changes after the administration of liver from human infectious hepatitis or passage monkeys. The liver lesions increased in severity and frequency of occurrence and may have been associated with death of the monkey.

Intraperitoneal inoculation (fig. 9). Although there was variation in response between different groups of monkeys, most groups showed a moderate to large fall in the number of neutrophils during the first 7–9 days after inoculation (fig. 10). There was some evidence that this depression began within 24–48 hr, but that in occasional animals increases in the count took place in the middle of the 1st wk. After the initial fall in the count most groups showed a transient leucocytosis with mean neutrophil levels of about 7000 per c.mm.; this usually took place 10–12 days after inoculation. During the 3rd wk there was a rapid reduction in the numbers of neutrophils to less than 3000 cells per c.mm. Except in a few groups the mean neutrophil counts were about 2500 per c.mm. until day 56. The over-all picture, therefore, was similar to that after intraperitoneal inoculation of human infectious hepatitis liver suspensions except that there was a greater reduction in neutrophils during the 1st wk. Although neutropenia occurred in all of 22 monkeys, in 5 there were transient rises in the count, possibly through bacterial infections.

Oral administration (fig. 9). During the first 48 hr after infection there was a rapid reduction in the numbers of neutrophils to a mean of about 2500 per c.mm. This early fall was constant for all groups of monkeys and took place in animals showing a moderate pre-inoculation leucocytosis resulting from sepsis. Except for transient increases resulting from secondary bacterial infections, the mean numbers of neutrophils remained below 3000 per c.mm. until the 56th day. Except for the earlier initial fall in the count the over-all picture resembled that produced after the oral administration of human infectious hepatitis liver suspensions.

Mononuclear cells. In the majority of monkeys there was no apparent alteration in the numbers of mononuclear cells in the blood during the 8 wk after injection or feeding of livers from fatal human cases of infectious hepatitis or from passage monkeys. The mean count usually lay between 5000 and 5500 cells per c.mm. Rarely, however, in some monkeys dying with extensive liver changes, there was a progressive decline in the counts to less than 2000. No constant abnormality was detected in the morphology of the lymphocytes and monocytes, although sometimes there were marked irregularities at the periphery.
**Fig. 8.**—Individual (·) and mean (——) counts of neutrophils in monkeys fed (arrow) with a suspension of human infectious hepatitis liver (group 3).

**Fig. 9.**—Means of neutrophil counts in monkeys given (arrow) suspensions of passage monkey liver: ·—·, after inoculation by feeding, and ·—·—·, after inoculation by intraperitoneal injection.
of the cytoplasm, suggesting fragmentation. Similar abnormal cells have been noted in uninoculated monkeys and are, therefore, thought to be non-specific. There was no marked increase in the numbers of large basophilic mononuclear cells.

_Eosinophils and basophils._ There was no detectable difference in the numbers of these cells in monkeys given injections of, or fed with, liver from human infectious hepatitis or passage monkeys compared with those in monkeys given injections of other liver suspensions.

![Graph](https://example.com/graph.png)

**Fig. 10.**—Means of leucocyte counts in a group of three monkeys given, on day 0, an intraperitoneal injection of a sixth-monkey-passage liver suspension that had been heated to 56°C for 30 min.: 
- total leucocytes, 
- neutrophils, 
- mononuclears.

**Additional post-inoculation findings**

Monkeys with initially high (>15,000 per c.mm.) neutrophil levels, as may occur in septic conditions, showed a rapid decline in the number of these cells during the 1st wk, although neutropenia might not occur until the 2nd wk after injection. On the other hand, monkeys with initially low (2000 or fewer) neutrophil counts did not show significant alterations in the counts after injection. The results for monkeys with initially high or low neutrophil counts have been omitted from fig. 9. Although hepatic changes occurred in conjunction with neutropenia, the frequency of occurrence and the extent of the changes bore no relation to the pre-inoculation neutrophil levels. Thus, monkeys showing very high pre-inoculation levels did not show any difference in the time or frequency of occurrence or the extent of hepatic changes as compared with animals with normal or low pre-inoculation levels. The liver lesions did not appear to be modified by secondary bacterial infections arising during the course of the disease. An attempted titration by intraperitoneal inoculation of one liver (patient GA) suggested that more than 1 ml of liver suspension was required to induce the changes.
Stability of the infective agent

Suspensions of liver from human infectious hepatitis or passage monkeys heated at 56°C for 30 min. retained their ability to induce neutropenia (fig. 10) and liver changes after feeding or intraperitoneal injection. When, however, suspensions were heated at 56°C for 5 hr they did not induce neutropenia (fig. 6); the animals given this material developed neutropenia.

Table II

Results of ‘‘t’’ test on the neutrophils of monkeys fed on, or given intraperitoneal injections of liver from human infectious hepatitis, passage monkeys and ‘‘control’’ subjects

<table>
<thead>
<tr>
<th>Liver material inoculated and method of inoculation</th>
<th>Parameter</th>
<th>Value of parameter at day after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Human infectious hepatitis, intraperitoneal</td>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>2.216</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.05</td>
</tr>
<tr>
<td>Passage monkey, intraperitoneal</td>
<td>n</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>2.383</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.05</td>
</tr>
<tr>
<td>Human infectious hepatitis, oral</td>
<td>n</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>2.190</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.05</td>
</tr>
<tr>
<td>Passage monkey, oral</td>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>4.176</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.01</td>
</tr>
<tr>
<td>Heat-inactivated human infectious hepatitis and passage monkey, oral</td>
<td>n</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>...</td>
</tr>
<tr>
<td>‘‘Control’’ subjects (patients with congestive cardiac failure), intraperitoneal</td>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>...</td>
</tr>
</tbody>
</table>

* n = Degrees of freedom, t = ‘‘t’’ number, P = probability;
... = ‘‘t’’ test not carried out as mean raised above pre-inoculation mean;
+ = ‘‘t’’ test not carried out because many monkeys were fed with unheated human infectious hepatitis or passage monkey livers at day 42.

and hepatic changes when subsequently fed with unheated suspensions. Livers or liver suspensions maintained at +4°C for 6 mth or at −70°C for 8 mth retained their ability to induce leucocytic and hepatic changes in monkeys. The infective agent was not inactivated by exposure to 10 per cent. ether for 24 hr at 4°C.

Statistical evaluation of results

To test the significance of the differences of the mean neutrophil levels in the different groups of monkeys, paired groups of absolute neutrophil numbers were submitted to the
"t" test. Because counts were not always made over a 2-wk period before inoculation, post-inoculation day groups were compared with day "0" groups. The results are given in table II. They show that significant decreases in neutrophil count were produced by the oral or parenteral administration of suspensions of liver from human infectious hepatitis or passage monkeys, but not by the injection of suspensions of liver from cases of congestive cardiac failure or by the oral administration of human infectious hepatitis or passage-monkey liver previously heated to inactivate the agent.

**Biochemical tests of liver function**

*Pre-inoculation findings*

The results of biochemical tests carried out on the sera of uninoculated patas monkeys are recorded in table III. Of the 98 sera used in the cephalin-cholesterol flocculation test, 57 showed 1+ to 4+ flocculation. The electrophoretic pattern of the serum proteins was similar in all species of West African monkeys tested and was characterised by the absence of \( \alpha_1 \) globulin.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>9-58 units</td>
</tr>
<tr>
<td>SGOT</td>
<td>24-72 units</td>
</tr>
<tr>
<td>SGPT</td>
<td>8-35 units</td>
</tr>
<tr>
<td>Total protein</td>
<td>4.7-7.1 g</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.0-4.1 g</td>
</tr>
<tr>
<td>Globulin</td>
<td>1.7-3.7 g</td>
</tr>
<tr>
<td>Albumin:globulin ratio</td>
<td>1:0-0.6 to 1:1.75</td>
</tr>
<tr>
<td>Bromsulphthalein (per cent.)</td>
<td>0-3.5 at 20 min.,</td>
</tr>
<tr>
<td>retention</td>
<td>0-1.5 at 45 min.</td>
</tr>
<tr>
<td>Colloidal gold</td>
<td>0-0</td>
</tr>
<tr>
<td>Colloidal red</td>
<td>0-0</td>
</tr>
<tr>
<td>Thymol turbidity</td>
<td>0-1.36 units</td>
</tr>
</tbody>
</table>
| * The number of monkeys examined by each test is given under Materials and methods as number of "pre-inoculation sera".

*Post-inoculation findings*

Although the quality of alkaline phosphatase in the serum in infected patas monkeys varied during the course of the disease, there was no evidence that the quantity was significantly increased. The highest figure recorded was 58 King-Armstrong units. The amounts of glutamic-oxaloacetic and glutamic-pyruvic transaminases in the serum (SGOT and SGPT) also varied throughout the infection. Although the level of SGOT always remained above the level of SGPT neither enzyme showed a marked increase suggestive of liver damage (fig. 5). Even in rapidly fatal cases both levels remained within normal limits. The highest figures recorded were SGOT, 75 Frankel units, and SGPT, 35
Frankel units. Sera from infected monkeys showed no increased precipitation in either the colloidal gold or colloidal red test, and caused no increase in the degree of thymol turbidity. The amounts of total protein, albumin and globulin remained unaltered and the albumin:globulin ratio was unaffected. No difference was observed between the electrophoretic pattern of the serum proteins in the uninfected and infected monkeys. The results of limited studies on bromsulphthalein excretion suggest that in animals not dying from the disease the rate at which the dye was removed from the serum was unaltered. In one monkey sick at 15 days after inoculation with infectious material 35 per cent. of the dye was retained at 20 min. and 28 per cent. at 45 min. At necropsy this monkey showed marked liver changes. Because the sera from most uninfected patas monkeys cause flocculation with cephalin-cholesterol antigen, this test was not applied to infected animals.

Transmission and tissue culture studies

Attempts were made to isolate a pathogenic bacterium from the livers of the infected monkeys by culture in a variety of aerobic and anaerobic media, but all were unsuccessful. No micro-organism was seen in Levaditi- or Giemsa-stained sections. A Millipore GS (0.22 μm) filtrate of a suspension of one human liver (patient JN) induced liver changes in patas monkeys. The infective agent was found to induce liver changes, similar to those seen in patas monkeys, in Cercopithecus and Cercocebus monkeys, but not in baboons, drills, pottos or galagos. No clinical manifestations of disease occurred in infant or adult white mice, white rats, guinea-pigs or rabbits after intracerebral or intraperitoneal inoculation of suspensions of liver from fatal human cases of infectious hepatitis or from infected patas monkeys. Attempts to isolate the agent from both human and monkey sources in a line of human embryo lung cells (Davis, 1961) were unsuccessful. The material for these studies consisted of 143 specimens taken from monkeys between 4 and 60 days after injection with liver suspensions. About half of the specimens were faeces and the remainder were liver, spleen, serum or heparinised whole blood. Fifty specimens of liver, spleen, bile, serum or faeces were obtained from 3 fatal and 22 non-fatal human cases of infectious hepatitis. A few attempts to culture the agent in primary cultures of green-monkey (Cercopithecus aethiops tantalus) kidneys were unsuccessful.

DISCUSSION

During the last four decades numerous unsuccessful attempts have been made to induce clinical and pathological manifestations of infectious hepatitis in a variety of primates other than man (Colbert, 1948–49; MacCallum, 1951; Evans, 1954). Recently, however, Deinhardt et al. (1967) have recorded hepatic changes and abnormal liver function tests in marmosets after the injection of acute phase serum from infectious hepatitis patients. During the last few years evidence that both apes and monkeys are susceptible to the agent of infectious hepatitis has been provided by epidemiological studies in the United States of
TRANSMISSION OF INFECTIOUS HEPATITIS

America (WHO Expert Committee on Hepatitis, 1964; National Communicable Disease Center Report no. 27, 1967). Small outbreaks of the disease have been caused in man by contact with young animals (Paul, 1962) that have shown no clinical evidence of disease and appear to have acquired the infection from man before export from the tropics. It is apparent, therefore, that whilst monkeys are susceptible to the agent of infectious hepatitis, difficulty lies in determining the features by which the disease may be recognised in them.

In present and past studies (Bearcroft, 1963), patas and other species of West African monkeys have shown no clinical signs that might suggest infectious hepatitis and, when liver changes have occurred, these have been most marked in small, presumably young, animals. Because of the absence of a clinically recognisable disease, reliance must be placed on laboratory examinations to detect abnormalities in the infected animals. The present study, therefore was undertaken to ascertain whether the hepatic lesions observed in monkeys after inoculation with liver suspensions from fatal human cases of infectious hepatitis formed part of a specific disease process induced by the inoculation of infectious hepatitis material or whether they arose from other unrelated causes, such as the inoculation of liver, regardless of its origin. Human cases of infectious hepatitis show changes in a variety of tissues. Among these changes are alterations in the numbers and morphology of the leucocytes, and neutropenia is the most constant finding (Havens and Marek, 1946). It seemed possible, therefore, that, if the hepatic changes described in monkeys formed part of a disease process induced by the inoculation of human infectious hepatitis, the neutrophil count would be decreased in these animals.

Neutropenia and characteristic hepatic lesions were found to be produced by the intraperitoneal or oral inoculation of liver from four fatal human cases of infectious hepatitis. The hepatic lesions varied considerably in extent and frequency of occurrence, but the neutropenia occurred with regularity. Neutropenia became evident within 4 days after the oral administration of human infectious hepatitis liver, but it did not develop until the end of the 1st wk, in the 2nd wk or early in the 3rd wk after intraperitoneal inoculation. This difference in time may, in part, be accounted for by the effect of the injection of large quantities of liver debris. The intraperitoneal injection of control liver suspensions produced a neutrophil leucocytosis during the first 4 days after injection. In these "control" animals, however, neutropenia did not occur in the 3rd or subsequent weeks, and liver changes, similar to those in monkeys receiving infectious hepatitis liver, were not seen. It is apparent, therefore, that the introduction of healthy or abnormal liver cell debris into the abdominal cavity has no depressant effect on the number of neutrophils.

Feeding animals with suspensions of livers from monkeys showing leucocytic and hepatic changes, or injecting them, caused similar changes in further groups of animals. These changes were reproduced on serial monkey to monkey passage up to the seventh pass. The hepatic lesions were produced with greater frequency by inoculation of passage livers than by that of human infectious hepatitis livers. The neutropenia occurred earlier and was usually apparent on the 2nd day after feeding animals with passage monkey liver suspensions.
The changes produced in the passage monkeys cannot result from the oral or intraperitoneal inoculation of monkey liver debris, since the intraperitoneal inoculation of healthy monkey livers caused only a transient leucocytosis and the injection of passage-monkey liver suspensions that had been heated at 56°C for 5 hr, or feeding animals with them, did not cause either neutropenia or hepatic lesions. The causative agent of human infectious hepatitis is believed to be a virus that is relatively resistant to heat (Paul et al., 1945) and may be transmitted serially from man to man by the ingestion of faecal material or injection of serum (Havens et al., 1944; Findlay and Willcox, 1945; Neefe, Stokes and Reinhold, 1945). It is probable that the same virus is present in the human liver in this condition. Although final proof of the identity of the agent causing the hepatic and leucocytic changes in monkeys is lacking, the available evidence suggests that it is the same as that present in human cases of infectious hepatitis.

The leucocytic changes in human patients have been described in detail by Havens and Marck (1946) and by Miles (1951). In their patients there was no significant alteration in the number of leucocytes in the first few days after infection. During the pre-icteric stage, however, both the neutrophils and lymphocytes were decreased and abnormal mononuclear cells frequently encountered. All cells returned to normal levels during the icteric stage. The leucocytic changes occurring in monkeys differ, however, from those occurring in man. The most constant change in the former is the rapid decrease in the number of neutrophils in the first few days after feeding with human or passage-monkey liver. This change is followed by neutropenia which is most pronounced in the third and subsequent weeks.

Although neutropenia occurs in human cases of infectious hepatitis, it is not specific for this disease. A decreased number of neutrophils occurs in some virus infections (Hickling, 1925; Holbrook, 1941; Wolf, 1954; Hillenbrand, 1956) and other conditions. It is possible, therefore, that in monkeys, also, the neutropenia is not specific for infectious hepatitis. In most animals the lymphocytes remain unchanged in number and morphology and only rarely is there a slight increase in large basophilic mononuclear cells. These findings suggest that the infective agent exerts its influence, in some as yet unexplained way, on the bone marrow or the circulating neutrophils. In man infectious hepatitis appears to be a generalised disease, but laboratory diagnosis rests on pathological findings associated with liver disturbances. Among these findings are an increase in serum bilirubin, serum transaminase and alkaline phosphatase, and imbalances in the relative quantities of serum proteins which cause alterations in the colloidal gold and colloidal red tests and in thymol turbidity. Although all elements of the human liver are affected, the most pronounced changes occur in the parenchyma and result in swelling and necrosis of liver cells. These inhibit the excretion of bilirubin and are responsible for leakage of intracellular enzymes into the blood serum. In patas monkeys, however, swelling of liver cells is never marked and necrosis is rare. It is apparent, therefore, that these cellular alterations are insufficient to cause detectable abnormalities in liver function tests.
Electron-microscope studies revealed morphological alterations in the liver cells in the infected monkeys that confirmed some of the changes visualised by light microscopy, but none of the changes was considered to be a specific indication of virus infection. The most prominent features were an increase in the number of mitochondria and hypertrophy of the Golgi complex. The former change occurs under conditions of increased cell activity (Dempsey, 1956). Hypertrophy of the Golgi complex has been noted by Miyai, Slusser and Rubeber (1963) in the livers of mice infected with mouse hepatitis virus, but it occurs in other conditions also (Weisblum, Herman and Fitzgerald, 1962; Goldfischer et al., 1962). No bodies that might be interpreted as virus particles were seen in any biopsy. During the last two decades several reports have been published describing virus-like particles, which range in size between 12 and 180 nm, in the liver and other tissues (Essen and Lembke, 1949; Braunsteiner et al., 1958; Cossel, 1959; Rightsel et al., 1961; Taylor et al., 1961; Laszlo et al., 1962). The rosettes described by Bearcroft (1962) are morphologically similar to aggregates of glycogen and may be composed of this material. The absence of these rosettes from infected monkey livers, in which there is a partial or complete disappearance of glycogen, is in keeping with this view.

Some of the infected monkeys died 2–8 wk after inoculation, but the cause of their death is unknown. Histological examination did not always reveal marked hepatic changes, and necrosis of liver cells was rare. No lesions were found in other tissues that might account for death. It is apparent, however, that the disease is more severe in small monkeys that have received passage-monkey liver suspensions than in large animals receiving similar material.

The evidence from the present studies suggests that the agent of infectious hepatitis in Nigeria causes a subclinical disease in patas and some other species of West African monkeys. It is emphasised, however, that nothing is known of the response of monkeys to other strains of this agent, if such exist, which may occur outside Nigeria. Furthermore, little is known of the quantity of either human infectious hepatitis or passage-monkey livers required to induce the leucocytic and hepatic changes. One attempted titration on the liver of patient GA suggested that in this particular case, an amount greater than 1 ml of a 10 per cent. suspension was required for infection by the intraperitoneal route. On the other hand, liver changes have been induced in monkeys by the injection of small pools of liver biopsies taken from infected animals.

At present the diagnosis of the disease in monkeys is difficult. It depends partly on observation of the changes in the liver, which are often slight and not easy to recognise, but more especially on observation of the rapid induction of neutropenia. Difficulties in diagnosis are increased by concurrent infections, particularly bacterial ulceration of the intestine associated with worm infestations. Furthermore it is possible that some monkeys have experienced a previous attack of infectious hepatitis, acquired by contact with man. Liver biopsies carried out on 458 apparently healthy patas, freshly received at this laboratory, have shown minor liver changes in 12 animals that might be attributed to infectious hepatitis or an allied agent. To avoid possible monkey-to-monkey spread of enteric and other infections and the acquisition of human disease, it
seems essential that animals are kept under sanitary conditions from the time of capture, that they are isolated from each other and that they are in contact with man for the shortest possible period of time.

**Summary**

Oral or intraperitoneal inoculation of suspensions of liver from fatal human cases of infectious hepatitis in Nigeria was found to cause a subclinical disease in patas and some other species of West African monkeys; a few animals became ill and a very few died. The disease was maintained in serial monkey-to-monkey passage by the oral or intraperitoneal inoculation of infected monkey livers. It appeared to increase in severity on passage. The most frequently observed signs of the disease were neutropenia and liver lesions. The neutropenia was rapid in onset after the oral, but slower after the intraperitoneal inoculation of infected liver. It was modified when concurrent bacterial infection occurred in the 2nd and subsequent weeks. The liver lesions varied in severity and frequency of occurrence. Usually they consisted of small foci in which the liver and Kupffer cells showed an increase in nucleolar and cytoplasmic basophilia. Necrosis was rare. These foci and the portal tracts were infiltrated with inflammatory cells. In some animals the changes were absent and in others they were slight and difficult to detect. In a few animals they became widespread throughout the liver. Because there was neither swelling nor necrosis of liver cells, tests for liver function showed values within the limits of normal. Electron microscopy of the liver showed irregularity of the nuclear membrane, increased numbers of mitochondria, hypertrophy of the Golgi complex and internal structures within the lysosomes. No virus-like particles were seen. The infective agent was stable to heating at 56°C for 30 min. and exposure to ether and freezing. It passed through bacteria-stopping filters. It was not isolated in artificial media or tissue cultures and did not cause clinical disease in laboratory animals other than monkeys.

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