Virchows Arch. Abt. A Path. Anat. 348, 309—327 (1969)

Viral Hepatitis in Subhuman Primates and its Relationship to Human Viral Hepatitis*

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Received July 24, 1969

Virushepatitis bei subhumanen Primaten und ihre Beziehung zur Virushepatitis beim Menschen

Zusammenfassung. Zahlreiche Versuche der experimentellen Übertragung der Virushepatitis von Menschen auf subhumane Primaten zwecks besseren Verständnisses und möglicher Kontrolle dieser Krankheit sind bisher daran gescheitert, daß das Virus noch nicht identifiziert ist und isoliert werden kann und daß die gebräuchlichen Versuchstiere zur Übertragung ungeeignet waren. Auf Grund von Berichten der Häufung von Hepatitiden bei Wärtern in Chimpansenkolonien wurden neue Versuche unternommen, Hepatitis von Menschen auf Chimpansen zu übertragen, die aber meist erfolglos blieben. Inzwischen gelang es anscheinend Bearcroft in Yaba, Nigerien, subhumane Primaten (Erythrocebus patas) mit Leberbrei eines menschlichen Falles von Hepatitis zu infizieren. Analysen der möglichen Gründe der Schwierigkeiten der experimentellen Übertragung dieser Krankheit ergab, daß idiopathische Hepatitis bei Chimpansen und Erythrocebus nicht selten ist, so daß die Tiere nachher immun sind; außerdem ist die Potenz des infektiösen Materials schwer festzustellen, da sie nur an Menschen geprüft werden kann.

Laboratoriumsstudien von gewissen Blutenzymen (Serum Glutamic Oxalacetic-Transaminase, Serum Glutamic Pyruvic-Transaminase und Serum Isocitric Dehydrogenase) an Chimpansen, die gerade zum Delta Primate Research Center geschickt worden waren, zeigten zufällig im Blute eines der Tiere erhöhte Werte, die gewöhnlich mit Leberentzündung einhergehen. Histologische Untersuchung von Punktionsbiopsien der Leber bestätigen den Verdacht auf Hepatitis, trotz des Fehlens erkennbarer klinischer Befunde dieser Krankheit. Im Laufe einiger Wochen erkrankten 4 andere Chimpansen nacheinander, während 5 Tiere dieser Gruppe gesund blieben. Alle Chimpansen erholten sich vollständig und zeigten keinerlei Folgeerscheinungen.

Virushepatitis kann von infizierten subhumanen Primaten auf andere empfängliche Tiere und Menschen übertragen werden, doch bleibt die Quelle dieser Erkrankung meist unbekannt, da die Tiere gewöhnlich keinerlei klinische Symptome zeigen. Nach Übertragung auf Menschen hingegen erzeugt das Virus ein Krankheitsbild, das sich in keiner Weise von einer primären menschlichen infektiösen Leberentzündung unterscheidet.

Virushepatitis konnte auf subhumane Primaten mittels Materialien von einem Patienten mit Hepatitis übertragen werden, der diese Krankheit von Chimpansen bekommen hatte; das wies hin auf eine reziproke Infektionsfähigkeit des Erregers vom Versuchstier zum Menschen und zurück zum Tiere.

* Original work referred to has been conducted under U.S. Army Contract DA-49-193-MD-244.

Primäre menschliche infektiöse Virushepatitis, wie auch Serumhepatitis konnte auf subhumane Primaten mittels oraler, subcutaner, intravenöser oder intraperitonealer Inoculierung übertragen werden, falls die Versuchstiere vorher keine infektiöse Leberentzündung überstanden hatten.

Versuche einer Reinfektion von subhumanen Primaten nach einer abgelaufenen spontanen oder induzierten Hepatitis mittels Materialien, die nachweisbar Hepatitis in Kontrolltieren erzeugen konnten, waren durchaus erfolglos.

Die vielen Fehlversuche der Übertragung von Virushepatitis vom Menschen auf subhumane Primaten sind entweder darauf zurückzuführen, daß die Versuchstiere eine frühere Infektion überstanden hatten — und daher immun waren — oder daß die Materialien zur Übertragung der Infektion ungeeignet waren.

Summary. Viral hepatitis can occur spontaneously in chimpanzees and patas monkeys, usually producing only mild clinical symptoms which are difficult, if not impossible, to recognize without adequate laboratory studies or liver biopsy. The disease, however, can be transferred to other subhuman primates as well as to human beings by contact. The source of the infection remains obscure in most cases and the virus may be of human or animal origin. After transfer of hepatitis virus from chimpanzees to human beings, it often causes a much more severe illness than in the primates; the disease then is indistinguishable from primary viral hepatitis by clinical, laboratory and histologic means.

It has been possible to transfer viral hepatitis to chimpanzees and patas monkeys with materials from a human being who had acquired the disease from chimpanzees harboring the virus of spontaneous hepatitis. A reciprocative infectivity from animals to humans and back to the animals is proved.

Primary human hepatitis of the infectious as well as of the homologous serum type has been transferred to subhuman primates by oral, subcutaneous, intravenous or intraperitoneal routes, provided that the animals had had no previous experience with this disease.

Following an attack of either spontaneous or induced viral hepatitis of the infectious or homologous serum type, the animals proved to be immune to re-infection with materials capable of producing viral hepatitis in control animals of a pilot study.

The many reported failures of transmission of viral hepatitis from human beings to subhuman primates are probably due to either a previous unrecognized infection with this virus of the animals employed — rendering them immune to the disease — or to the use of impotent infective materials.

Introduction

The numerous attempts made in the past to understand, reproduce experimentally, and possibly to control infectious hepatitis have failed because of the inability to cultivate and identify the causative agent and the lack of a reliably susceptible laboratory animal (Evans, 1954; Hepatitis Surveillance No. 26, 1966). Following the report of Hillis (1961), confirmed by several other investigators (Held, 1963; Riopelle, 1963, 1964; Hepatitis Surveillance No. 23, 1965; No. 27, 1967; No. 29, 1968; Smetana, 1965, 1967; Davenport *et al.*, 1966; Mosley *et al.*, 1967; Ruddy *et al.*, 1967), of chimpanzee-associated hepatitis among animal caretakers, hope for the successful use of these primates for the experimental study of hepatitis was again raised. These expectations were, however, only partly fulfilled (Deinhardt *et al.*, 1962; Atchley and Kimbrough, 1966; Smetana, 1969). Meanwhile, Bearcroft (1963, 1964) probed the potential susceptibility of the Erythrocebus patas monkey to human hepatitis and this suggestion was applied to a study of the effect of inoculation of material from a proven case of infectious viral hepatitis to patas monkeys (Smetana, 1965). Although this experiment was partially successful, the low percentage of the positive results was rather discouraging. In an analysis of the causes responsible for the difficulties in experimen-

tation with infectious hepatitis in subhuman primates, two factors became apparent:

1. the occurrence of clinically unrecognized "spontaneous" hepatitis in subhuman primates which renders them ineffectual for experimental transfer of this disease following such an attack;

2. the difficulties in proving the potency of infective material for successful transmission of hepatitis.

The influence of these two factors will become apparent in the several studies presented in the following sections of this report.

The diagnosis of viral hepatitis in subhuman primates and in man is based on clinical symptoms, laboratory findings, especially liver function tests, such as determinations of the activity of the serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and isocitric dehydronase (ICD), and on histologic changes seen in liver biopsy specimens (Smetana *et al.*, 1953; Smetana, 1954). While the clinical symptoms of viral hepatitis in human beings are usually quite definite, subhuman primates often show little or no evidence of clinical disease. Despite the paucity of clinical symptoms, liver function tests in adult or maturing juvenile chimpanzees usually reveal definite abnormalities. However, in infantile and young juvenile chimpanzees, there is but little increase in SGOT, SGPT or ICD activities. Jaundice in primates is difficult to detect by clinical inspection, but can be demonstrated by serum bilirubin determinations. Stress incidental to captivity and handling of newly acquired animals can cause a rise in titer of various serum enzymes (Cope and Polis, 1959). It is also important to rule out the possible effect of incidental bacteriologic, parasitic, mycologic and other viral infections.

The histopathologic changes seen in needle biopsy specimens of the liver in viral hepatitis have been repeatedly described (Smetana, 1957, 1963). In general, the alterations in human viral hepatitis are similar to those in subhuman primates, but the liver cell changes are more severe and variable in human beings than in other primates. Ballooning of hepatocytes and formation of acidophilic bodies occurs in chimpanzees and patas monkeys, but the most prominent histologic changes are periportal and sinusoidal monocytic infiltrates. Evidence of necrosis and loss of liver cells, documented in human cases by the presence of phagocytized granules of lipofuscin from destroyed hepatocytes, is not evident in chimpanzees or patas monkeys. Rapid regeneration of liver cells apparently occurs as indicated by the presence of numerous binucleated cells. Apparently the injured and destroyed hepatocytes are so rapidly and effectively replaced that distortion of the liver cell plates is held to a minimum.

Spontaneous Viral Hepatitis in Chimpanzees

(Apparent Transmission of the Disease from an Infected Animal to Other Chimpanzees of the Colony).

Three weeks after the arrival of a number of juvenile chimpanzees at the Research Center from an animal importing company, one of the animals (chimp 1858) showed increased activities of SGOT, SGPT and ICD as well as an increased total serum bilirubin, accompanied by histopathologic evidence of active hepatitis on needle biopsy of the liver. After two and three weeks respec-

tively, two other chimpanzees in succession came down with hepatitis, while four other animals housed in the same compound remained unaffected. Two of three juvenile chimpanzees of a later shipment from the same importing company developed abnormal liver function tests five and seven weeks respectively, after their arrival at the Center and histologic evidence of active viral hepatitis was apparent in needle biopsies of the liver. The pertinent laboratory data from the affected animals and from one unaffected chimpanzee of each series are presented in Table 1 (Perrin, 1968). A photomicrograph of a liver biopsy from one of the chimpanzees (Fig. 1) demonstrates the changes characteristic of all members of the affected group. None of the five affected chimpanzees showed any characteristic or significant clinical symptoms during the entire 11 week period of close observation.

Two episodes of spontaneous hepatitis, presumably of viral origin in chimpanzees, followed by attacks of hepatitis in their human caretakers were described previously (Smetana, 1965, 1967). In both instances there was substantial reason to assume a direct relationship between the hepatitis in the animals and that in the handlers.

Table 1. *Spontaneous viral hepatitis in chimpanzees. Laboratory tests and results of liver biopsies on newly acquired animals*

Chimpanzee	Weeks after arrival								
		3	4	5	6	7	8	9	12
	Tests	LB 1	LB 2	LB 3	LB 4	LB 5	LB 6	LB 7	LB 8
1858	SGOT	180[a]	70[a]	21[b]	39	39	39	24	13
Juvenile	SGPT	126	150	48	32	34	38	30	30
female	ICD	2,532	570	600	678	845	564	192	501
	T. Bi.	1.1	2.1	0.5	0.2	0.3	0.3	0.3	0.4
1857	SGOT	29	150[a]	44[a]	21[b]	29	34	34	18
Juvenile	SGPT	56	280	380	50	42	38	76	34
male	ICD	513	1,065	735	345	240	360	294	420
	T. Bi	0.4	1.2	1.2	0.6	0.4	0.4	0.4	0.3
1854	SGOT	39	34	21	150[a]	180[a]	66[b]	39	24
Juvenile	SGPT	24	34	38	130	465	250	69	42
male	ICD	489	390	336	2,652	2,652	648	252	234
	T. Bi.	0.3	0.5	0.2	0.4	1.0	0.5	0.4	0.2
		LB 1		LB 2		LB 3		LB 4	LB 5
1895	SGOT	44	—	150[a]	—	34[b]	—	29	29
Juvenile	SGPT	24	—	310	—	46	—	21	26
male	ICD	450	—	755	—	705	—	165	294
	T. Bi.	0.1	—	0.4	—	0.4	—	0.4	0.2
1894	SGOT	21	—	70	—	78[a]	—	21[b]	29
Juvenile	SGPT	24	—	56	—	83	—	26	26
female	ICD	486	—	588	—	1,260	—	90	390
	T. Bi.	0.1	—	0.2	—	0.5	—	—	0.4

[a] Active viral hepatitis.

[b] Subsiding hepatitis. SGOT, SGPT, ICD activities in units per ml. T. Bi = Total serum bilirubin, milligrams per cent. LB = Liver biopsy.

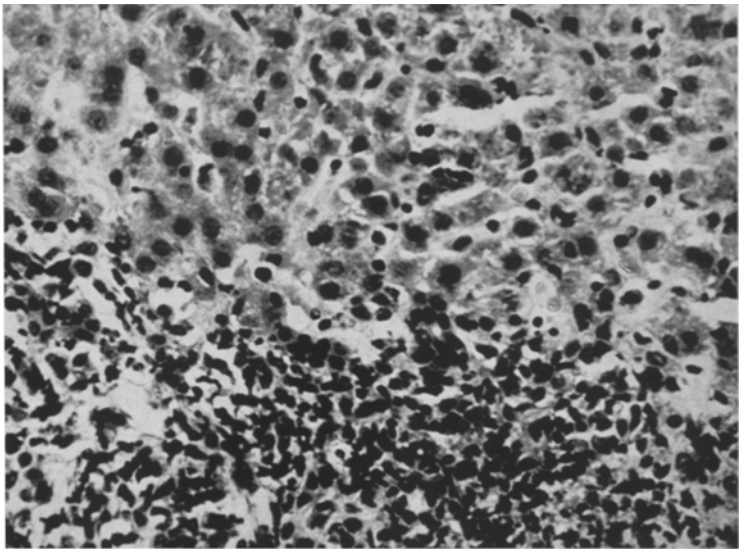

Fig. 1. Spontaneous viral hepatitis, chimpanzee No. 1894. Section of liver, taken 7 weeks after arrival of animal at Center, 2 weeks after discovery of spontaneous hepatitis of chimpanzee 1895 (see Table 1). The hepatocytes show moderate irregularities in size, shape and staining characteristics and there is some disruption of hepatic cell cords. Massive inflammatory infiltrates occupy the stroma of most portal canals and are also present in some of the sinusoids; these are composed of monocytic cells and of polymorphonuclear leukocytes. Hematoxylin and Eosin; × 250

Spontaneous Viral Hepatitis in Patas Monkeys

Although transfer of infectious viral hepatitis from patas monkeys to humans has as yet not been documented, there is good reason to believe that spontaneous hepatitis occurs in this species. Three animals employed as controls in our study of experimental transfer of serum hepatitis with Thrombin (Cutter)[1], developed

Table 2. *Spontaneous viral hepatitis in patas monkeys. Laboratory tests and results of liver biopsies of animals, inoculated with control materials*

Control animals	Materials used and route of administration	Average values of 50 control tests			Findings in biopsy of liver
		SGOT	SGPT	ICD	
		52	33	398	
Patas 270 (Male)	Albumin and saline, orally	192	41	1,170	Acute viral hepatitis
Patas 592 (Male)	Albumin and heparin, subcutaneously	108	69	1,290	Acute viral hepatitis
Patas 821 (Female)	Albumin and Saline, intraperitoneally	950	410	528	Acute viral hepatitis

SGOT, SGPT, ICD activities expressed in units per milliliter.

1 See below in chapter: Experiments on Patas Monkeys with Materials from Human Cases of Homologous Serum Hepatitis.

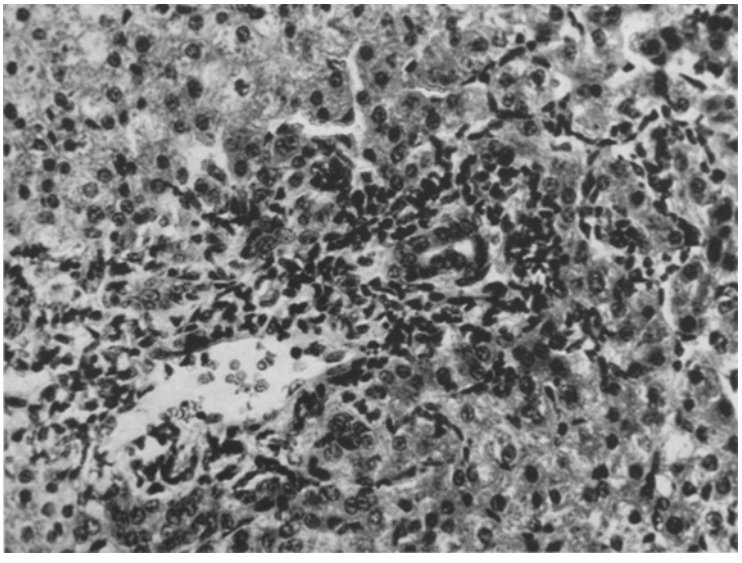

Fig. 2. Spontaneous viral hepatitis, control patas monkey 821 (see Table 2). Section of needle biopsy of liver showing moderate irregularities of hepatocytes about the portal areas and rather massive inflammatory infiltrates composed of polymorphonuclear leukocytes, histiocytes and monocytes in the stroma of the portal area and extending into sinusoids. Hematoxylin and Eosin; × 250

laboratory and histologic signs of viral hepatitis. The laboratory data are presented in Table 2 and the histopathologic alterations seen in biopsy specimens of the liver are compatible with those seen in spontaneous hepatitis of chimpanzees. There were never any clinical signs of disease in any of these animals. Histologic changes in the liver are pictured in Fig. 2.

These experiences with chimpanzees and patas monkeys purchased from import companies suggest that viral hepatitis is not uncommon among such animals and that the infection may be transferred from animal to animal as well as from animal to man.

Induced Infectious Viral Hepatitis in Subhuman Primates

Attempts were made to induce viral hepatitis in primates by administering a 10% suspension of stools of animal caretaker P. M. by various routes to experimental animals (Smetana, 1967).

This infectious material was obtained during the acute phase of viral hepatitis of this man; it was prepared in Eagle's basal medium with 2% calf serum and 50 mg each of Kanamycin®, Fungizone® and Achromycin® as well as with 1,000 units of Penicillin and 500 micrograms of Streptomycin per milliliter (ml). This suspension was centrifuged at 2,000 rpm at 2 to 4° C, for 10 min. The supernatent fluid was then centrifuged again in a Beckman L ultracentrifuge at 10,000 rpm at 2° C, for 30 min. Bacterial cultures were negative and attempts to isolate viral agents from the supernatant fluid in human embryonic kidney, vervet kidney, WI 38, BSC-1 and LCMK-2 cell lines were not successful.

Suspensions of this material, which was kept in the Revco freezer at minus 70° C, were prepared under identical conditions — and were used in this and several other experiments. This material will be referred to subsequently as "P. M. stool suspension".

Experiments with Chimpanzees

1. Five ml aliquots of "P. M. stool suspension" were inoculated intraperitoneally into 2 infantile and 2 young juvenile chimpanzees (392, 397, 1528, 1530); one animal (1526) was not inoculated but was kept as a control albeit in the same room as the experimental animals. All four inoculated animals developed laboratory evidence of slightly abnormal liver function. Liver biopsies 4 to 8 weeks after inoculation showed histologic alteration compatible with mild viral hepatitis.

Nine to 11 weeks after the start of the experiment the control animal (1526) showed laboratory and histologic evidence of infectious viral hepatitis, presumably acquired secondarily from one of the inoculated animals housed in the same room. Table 3 presents the pertinent data of this experiment and Figs. 3 and 4 show the corresponding histologic alterations seen on liver biopsy.

2. Two infantile, laboratory-born chimpanzees, male 1640 and female 1665 were inoculated orally and intraperitoneally with 5 ml each of "P. M. stool suspension" following the establishment of baseline hematologic and serum enzyme tests as well as examination of sections of needle biopsy specimens of the liver

Table 3. *Experimental hepatitis in chimpanzees results of inoculation of P. M. Stool suspension*

Chimpanzee	Amount and route of inoculum	Weeks	SGOT	SGPT	ICD	Degree of changes in liver[a]
			Mean control activities			
			24	24	476	
392	5 ml	0	27	21	195	None
Infantile	Intraperitoneal	6	70	103	660	Viral hepatitis
male		10	19	15	375	Subsiding hepatitis
397	5 ml	0	13	25	186	None
Infantile	Intraperitoneal	7	16	41	414	Viral hepatitis
female		18	8	18	291	Subsiding hepatitis
1528	5 ml	0	22	25	186	None
Juvenile	Intraperitoneal	6	78	69	645	Viral hepatitis
male		18	48	29	450	Subsiding hepatitis
1530	5 ml	0	16	37	255	None
Young	Intraperitoneal	4	22	41	645	Early hepatitis
juvenile		10	27	41	525	Viral hepatitis
male		18	10	12	309	Subsiding hepatitis
1526[b]	None	0	19	18	195	None
Young		9	32	56	495	Early hepatitis
juvenile		11	55	205	621	Viral hepatitis
male		17	10	15	231	Normal

[a] Histologic changes in sections of serial needle biopsies of the liver.

[b] Control animal caged in the same room as the experimental animals. SGOT, SGPT, ICD activities expressed in units per ml.

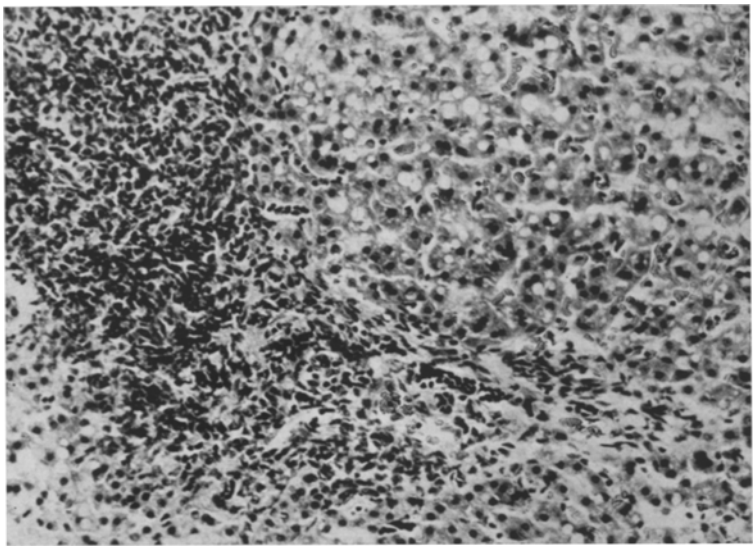

Fig. 3. Experimental viral hepatitis, infantile chimpanzee No. 392 (see Table 3). Section of needle biopsy of liver 6 weeks after intraperitoneal inoculation of "P. M. stool suspension". The hepatocytes exhibit moderate irregularities in size, shape and staining reactions and there is vacuolozation of their cytoplasm. Massive inflammatory infiltrates are present in the stroma of the portal canals, sometimes extending into sinusoids. These infiltrates are composed mainly of monocytes. Hematoxylin and Eosin; × 160

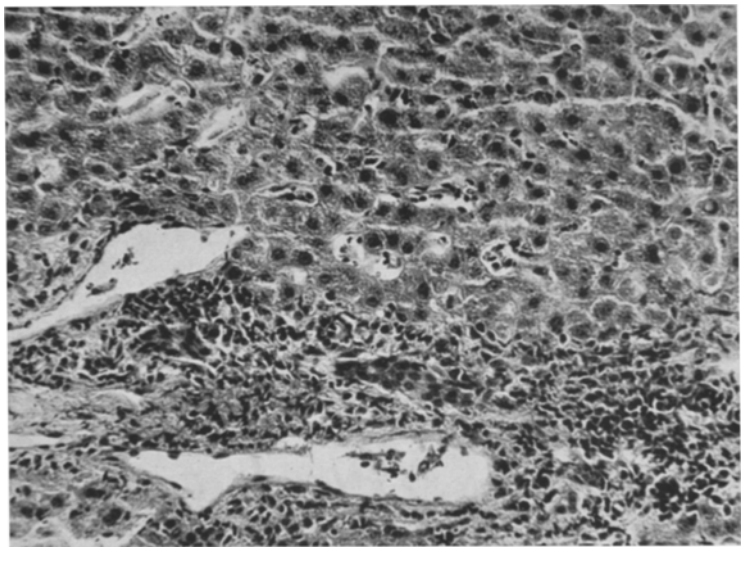

Fig. 4. Secondary viral hepatitis, control chimpanzee No. 1526. Section of needle biopsy of liver, taken 11 weeks after intraperitoneal inoculation of "P. M. stool suspension" of chimpanzees 392, 397, 1528 and 1530 (see Table 3). There are moderate irregularities in size, shape and staining reactions of hepatocytes with some disruption of hepatic cell cords. Dense inflammatory infiltrates, composed of monocytic cells and polymorphonuclear leukocytes occupy the stroma of the portal canals. Hematoxylin and Eosin; × 200

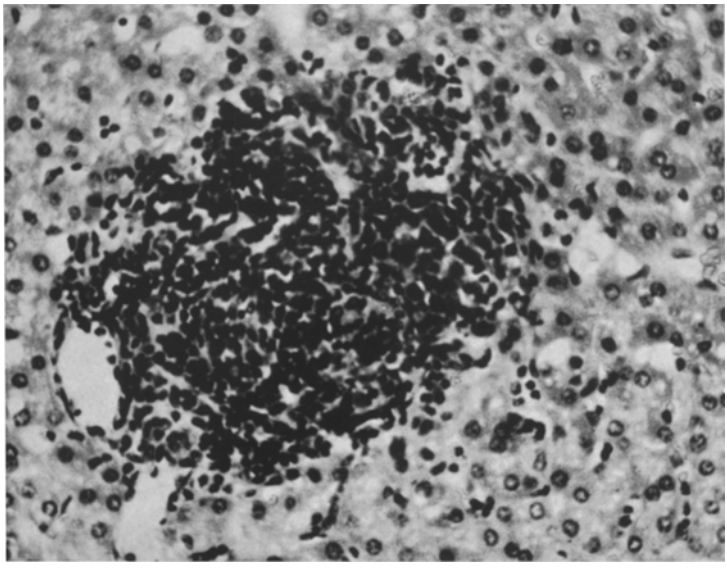

Fig. 5. Experimental viral hepatitis, infantile chimpanzee No. 1665 (laboratory born). Needle biopsy of liver, 6 weeks after oral and intraperitoneal onoculation of "P. M. stool suspension". Dense portal inflammatory infiltrates occupy the stroma of the portal canals and some extend into sinusoids; they are mainly composed of monocytic leukocytes. The hepatocytes appear normal and the hepatic cell cords show only minimal distortion. Hematoxylin and Eosin; × 250

prior to the experiment. Serial laboratory studies showed a mild rise of the activities of SGOT, SGPT and ICD, and sections of the biopsy specimens revealed evidence of liver injury, compatible with that seen in viral hepatitis. These alterations occurred about 6 weeks after the inoculation of the infectious material and are presented in Fig. 5.

Experiments with Erythrocebus Patas

1. Results of inoculation in a patas monkey of serum from a proven case of infectious viral hepatitis, tested for infectivity in human volunteers, were reported previously (Smetana, 1965). The laboratory findings in this animal (67) as well as the histopathologic alterations of the liver presented the closest resemblance to the pathognomonic picture of acute viral hepatitis seen in man among all the animals studied during this project.

2. Oral and intraperitoneal administration of 5 ml each of "P. M. stool suspension" was used in 4 male and 5 female patas monkeys and 3 animals were kept as controls. Six of the experimental animals developed minor elevations of the serum enzymes activities and mild histologic changes in sections of serial needle biopsies of the liver. Three of the inoculated animals showed significant lesions in the liver from 8 to 10 weeks afterwards and an increase of the activities of SGOT from an average level of 30 to 107 units, SGPT from 33 to 126 units and ICD from 398 to 1230 units. The total serum bilirubin increased from 0.1 to 0.5 mg per 100 ml. Representative histologic changes are depicted in Fig. 6.

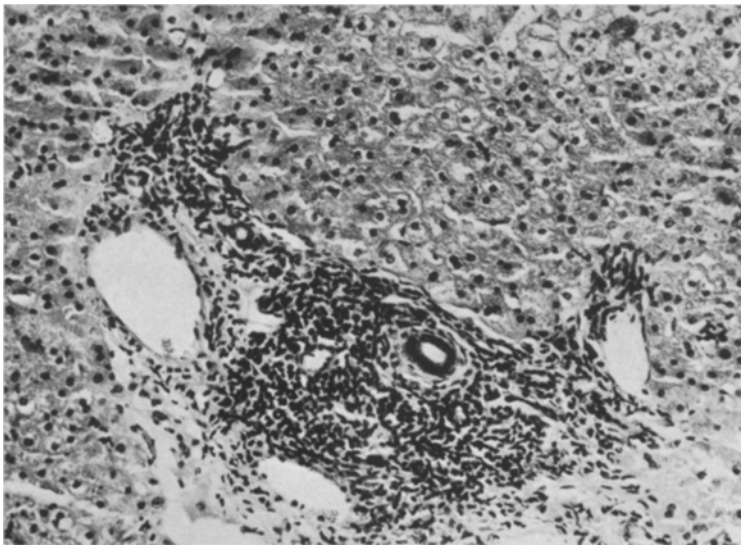

Fig. 6. Experimental viral hepatitis, patas monkey No. 1819. Needle biopsy of liver, 9 weeks after oral and intraperitoneal administration of 5 ml. "P. M. stool suspension". There is moderate irregularity of hepatocytes as to their size, shape and staining reaction. Massive inflammatory infiltrates, mainly composed of monocytic cells are present in the stroma of the portal canals, and occasionally extend into sinusoids. Hematoxylin and Eosin; × 150

Experiments on Patas Monkeys with Materials from Human Cases of Homologous Serum Hepatitis

1. Thrombin (Cutter), Lot G-60581[2] *(Murray, 1954)*

Thrombin prepared from human plasma by the Cutter Laboratories, Berkeley, California was used for the inoculation. (Medication with this material for hemorrhagic or bleeding tendencies in human patients had produced homologous serum hepatitis in a significant number of cases (Porter, *et al.*, 1953). Patas monkeys were given 500 units of this material by different routes. Five units of heparin were combined with the thrombin and 2 ml phosphate saline buffer of pH 7 for intravenous and subcutaneous injection to prevent clotting, whereas saline without heparin and buffer was employed as a diluent for oral and intraperitoneal administration. Groups of 4 animals each were used for oral, subcutaneous, intravenous and intraperitoneal administration respectively. Four pairs of patas monkeys were used as controls; they received the same solutions as the experimental animals except that 1% normal human albumin was substitued for the thrombin. All animals were examined clinically, subjected to liver function tests (SGOT, SGPT and ICD) and serial needle biopsies of the liver. Four weeks after the inoculation and every week thereafter, the serum enzyme tests were repeated and needle biopsies of the liver performed. Two of the experimental patas monkeys revealed significant increases in the values of the serum enzymes as well as histologic changes in the liver 11 and 16 weeks after the intravenous

2 This portion of the study was carried out in collaboration with K. A. Hok, Ph. D. and R. Nieman from the Cutter Laboratories, Berkeley, California.

and subcutaneous administration of thrombin. Nine additional animals showed mild hepatic histologic lesions and an upward trend of the activities of the serum enzymes SGOT, SGPT and ICD. The five remaining experimental monkeys showed no significant laboratory or histologic changes. The significance of the outcome of this experiment was, however, greatly reduced by the fact that 3 control animals came down with viral hepatitis which was interpreted as being spontaneous in nature. (See above under section "Spontaneous Viral Hepatitis in Patas Monkeys".)

2. Experiments with Hyland Laboratory Plasma

Sixteen patas monkeys were inoculated with Hyland Laboratory Plasma (CDC, Atlanta, Georgia, Lot No. 200 m/25, known to be infectious (Aach and Aronson, 1965) to human beings. Four of 8 experimental animals received 1 ml of Hyland Laboratory Plasma to which 5 ml of the animals' own plasma were added (autoplasma), by intravenous routes respectively, and four of the other 8 experimental animals were inoculated with 5 ml autoplasma and 1 ml Hyland Laboratory Plasma subcutaneously or intraveneously. Four of the 8 control animals received 6 ml of autoplasma each by either subcutaneous or intravenous routes. Two of the experimental animals (648 male and 657 female) developed increases activities of SGOT and SGPT, 16 weeks after the subcutaneous inoculation of a mixture of autoplasma and Hyland Laboratory Plasma; the level of the ICD did not rise significantly. Patas 648 had received 1 ml of Hyland Laboratory Plasma and 5 ml autoplasma subcutaneously. Patas 657 was inoculated subcutaneously with 5 ml of Hyland Laboratory Plasma and 1 ml of autoplasma. Needle biopsies of liver showed histologic alterations of the parenchyma compatible with those seen in viral hepatitis. Several of the other experimental animals showed only mild biochemical and histolgic alterations. Pertinent

Table 4. *Experimental viral hepatitis in patas monkeys with materials from human serum hepatitis*

Patas No.	Amount and route of inoculum	Weeks	SGOT	SGPT	ICD	Degree of changes in liver[a]
517	Thrombin (Cutter)	9	48	206	1,110	Early hepatitis
Juvenile	2 ml	11	86	252	1,520	Viral hepatitis
female	intraveneously	13	27	90	819	Subsiding hepatitis
808	Thrombin (Cutter)	14	192	29	2,148	Early hepatitis
Juvenile	2 ml	16	150	21	1,392	Viral hepatitis
male	subcutaneously	18	78	18	1,194	Subsiding hepatitis
648	Hyland lab. plasma	14	48	37	532	None
Juvenile	1 ml	16	120	250	533	Mild hepatitis
male	subcutaneously	18	37	56	510	Subsiding hepatitis
657	Hyland lab. plasma	14	48	50	489	Mild hepatitis
Juvenile	5 ml	16	150	210	561	Subsiding hepatitis
female	subcutaneously	18	150	145	585	Residual hepatitit

[a] Histologic changes in sections of serial needle biopsies of liver. SGOT, SGPT, ICD activities expressed in units per ml.

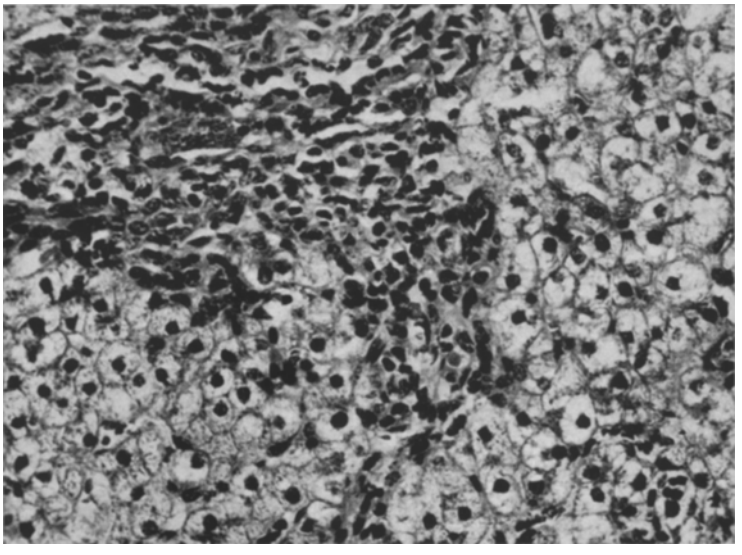

Fig. 7. Experimental viral hepatitis, patas monkey No. 517 (see Table 4). Biopsy of liver, 11 weeks after intravenous inoculation of 2 ml. Thrombin (Cutter). The hepatocytes present mild irregularities in size and shape. The stroma of the portal canals is rather densely infiltrated with monocytic cells, which in places extend into sinusoids. Hematoxylin and Eosin; × 230

observations from two experiments are presented in Table 4; Fig. 7 depicts representative histopathologic changes seen in a liver biopsy of animal 517.

The results of these experiments indicate that inoculation of chimpanzees and patas monkeys with serum or stool suspensions from human cases of infectious hepatitis can produce lesions compatible with hepatitis, provided that the animals have had no previous experience with this disease and that the material used for inoculation is potent. Similarly, materials capable of producing serum hepatitis in humans can cause laboratory and histopathologic alterations in patas monkeys compatible with viral hepatitis.

Unfortunately, these experimental studies with materials from cases of human serum hepatitis could not be repeated or extended to chimpanzees at this time.

Pilot Study for Testing the Effect of Re-Infection of Subhuman Primates Following an Attack of Viral Hepatitis

In order to test the effect of a previous attack of viral hepatitis on re-infection, chimpanzees and patas monkeys that had recovered from either spontaneous or induced viral hepatitis — proved by study of liver function tests and histologic examinations of liver tissue obtained by biopsy — were rechallenged with potent material from a human case of infectious viral hepatitis (P. M. stool suspension). Five ml each were given orally and intraperitoneally to each animal tested.

Experiments with Chimpanzees

a) Spontaneous Viral Hepatitis. Five chimpanzees that had overcome an attack of spontaneous hepatitis (Table 1) were challenged orally and intra-

Table 5. *Study for testing immunity. Challenge of chimpanzees after recovery from spontaneous presumably viral hepatitis with 5 ml P.M. Stool suspension each, orally and intraperitoneally*

Chimpanzee No.		Weeks after challenge 0	3	6	8	12	Changes in liver[a]	Weeks after spontaneous hepatitis[b]
		LB 1	LB 2	LB 5	LB 7	LB 9		
1858	SGOT	29	24	24	18	18	none	22
Juvenile	SGPT	30	30	30	21	34		
male	ICD	456	306	420	330	461		
	T. Bi	0.4	0.3	0.2	0.3	0.8		
1857	SGOT	21	34	39	34	29	none	21
Juvenile	SGPT	34	34	42	38	30		
female	ICD	396	276	366	240	390		
	T. Bi	0.4	0.3	0.2	0.1	0.3		
1854	SGOT	44	29	39	44	29	none	19
Juvenile	SGPT	50	30	46	42	30		
male	ICD	618	234	420	405	420		
	T. Bi	0.5	0.2	0.2	0.2	0.2		
1895	SGOT	34	21	18	29	34	none	14
Juvenile	SGPT	46	24	24	21	18		
female	ICD	705	252	366	207	396		
	T. Bi	0.4	0.3	0.3	0.2	0.2		
1894	SGOT	78	29	16	29	34	none	12
Juvenile	SGPT	83	26	26	24	24		
male	ICD	1,260	390	225	165	420		
	T. Bi	0.5	0.4	0.4	0.4	0.2		

[a] Histologie changes in sections of serial liver biopsies.

[b] See Table 1. SGOT, SGPT, ICD activities expressed in units/ml. T.Bi = Total serum bilirubin mg-%.

peritoneally with 5 ml each of a stool suspension from a human case of infectious viral hepatitis (animal caretaker P. M.) 12 to 22 weeks afterwards. Serial liver function tests and liver needle biopsies performed during 12 weeks following this challenge showed no evidence of recurrence of the hepatitis (Table 5).

b) Induced Viral Hepatitis. Three chimpanzees (1528, 1530, 1526) of the group of 5 animals (Table 3) that had developed viral hepatitis following inoculation of infectious material from a human case of infectious viral hepatitis (P. M. stool suspension) were re-inoculated with infectious material from the same source 37 to 39 weeks after the first infection. Neither liver function tests nor histologic studies of sections of liver needle biopsies showed any significant changes up to 12 weeks following the re-inoculation.

Experiments on Erythrocebus Patas

a) Induced Infectious Viral Hepatitis. Animal 67, which had been successfully infected with plasma from a human case of infectious hepatitis (Smetana, 1965) was rechallenged with 5 ml each orally and intraperitoneally of material from a human case of infectious viral hepatitis (P. M. stool suspension) 52 months after the first infection. Neither liver function tests nor study of sections from

serial needle biopsies of the liver showed any evidence of reaction during 12 weeks of observation following the rechallenge.

b) Induced Homologous Serum Hepatitis. 1. Thrombin (Cutter). Two patas monkeys which had shown signs of the effect of transfer of homologous serum hepatitis (517, 808, Table 4) were inoculated both orally and intraperitoneally with 5 ml each of material from a human case of infectious viral hepatitis (P. M. stool suspension) 26 months afterwards. No significant consequences could be observed in results of serial liver function tests or in sections of liver needle biopsies up to 12 weeks following this challenge.

2. Hyland Laboratory Plasma. One patas monkey (262) which had shown mild signs of viral hepatitis 14 to 16 weeks following the subcutaneous inoculation with Hyland Laboratory Plasma was challenged with 5 ml of material from a human case of infectious viral hepatitis (P. M. stool suspension) intraperitoneally as well as orally, 24 months after this previous episode. No significant biochemical or histologic changes developed during the observation period of 12 weeks following this rechallenge.

The results of this pilot study indicate that previous infection with the virus of viral hepatitis, be it spontaneous or induced, of the infectious or homologous serum type, can produce immunity in chimpanzees or patas to re-infection with materials capable of inducing infectious viral hepatitis in chimpanzees or patas monkeys. These experiments will have to be repeated on a large scale with infectious materials from different sources and performed in several susceptible species of experimental animals before these results can be fully accepted.

Discussion

"Spontaneous" viral hepatitis is a rather common disease in chimpanzess but can apparently also occur on patas monkeys. It can be transferred to other animals of the colony as well as to humans by contact. The means of propagation from animal to animal as well as to humans is most probably by the oral route with materials contaminated by fecal matter.

The two most important prerequisites for the transmission of this disease from animal to animal and probably also to man is the lack of previous experience with the virus of viral hepatitis and the potency of the infectious material.

There are obvious similarities as well as differences between the manifestations of hepatitis in human beings and subhuman primates. The ease of transmission from chimpanzees to man and the possibility of transfer of infectious and serum hepatitis from man to subhuman primates suggests at least a similarity — if not identity — of the infective agent albeit in a different host.

Spontaneous viral hepatitis in chimpanzees and patas monkeys is difficult to recognize because of the absence of significant clinical symptoms. Thus, the disease may sweep through a colony before any control measures are taken. By contrast, viral hepatitis in humans acquired by persons in contact with infected subhuman primates is frequently a severe and even dangerous disease. The infection is probably transferred by physical contact with a carrier of the virus

by the oral route, through carelessness or lack of personal hygiene following handling of a chimpanzee[3].

Regularly performed laboratory studies, particularly liver function tests and serial needle biopsies of the liver are indeed desirable, even imperative, for experimental studies of viral hepatitis in subhuman primates. However, only strict isolation of the primates and sanitary measures of the handling can prevent spreading of the infection to other animals and to human contacts.

Although oral transfer of the disease from humans to human volunteers had been claimed by Voegt (1942), using duodenal juice from a case of hepatitis, the data presented are fragmentary. Attempts to transfer hepatitis from humans to subhuman primates in the past have yielded disappointing results (Evans, 1954, Hepatitis Surv. No. 26, 1966). More recently, however, apparently successful transfer of viral hepatitis has been reported: to chimpanzees (Deinhardt *et al.*, 1962; Atchley and Kimbrough, 1966; Smetana, 1969); to patas monkeys (Bearcroft, 1963, 1964; Smetana, 1965, 1969) and to marmosets (Deinhardt *et al.*, 1967a and b; Holmes *et al.*, 1967).

The methods used for diagnosis of hepatitis in human medicine can also be applied to the recognition of hepatitis in subhuman primates. Unfortunately, there are no clinical symptoms in most instances of hepatitis in chimpanzees and patas monkeys; the outcome of laboratory tests, particularly for the activities of SGOT, SGPT and ICD depends partly on the age of the animal: the younger, the less significant is the response. This fact greatly diminishes the reliability of laboratory tests alone for the diagnosis of hepatitis.

While older animals may have had spontaneous viral hepatitis, making them ineffectual for experimental studies of this disease, infantile and young juvenile primates do not fully respond physio-chemically to the infection, thereby increasing the difficulties of recognition of successful transfer. The only constant feature characteristic of infectious hepatitis in animals and man is the damage of liver cells, often resulting in necrosis of individual hepatocytes, accompanied by a distinctive reactive inflammatory response. The diagnosis of viral hepatitis in subhuman primates depends, therefore, largely on the recognition of the histopathologic alterations in the liver, strengthened by judicious interpretation of laboratory findings, correlated with the length of the incubation period — when it is known — and by comparison with control animals.

In view of the common occurrence of spontaneous viral hepatitis, especially in chimpanzees, the many failures of transfer of the disease from humans to subhuman primates suggest immunity acquired from prior infection. The results of the pilot study reported above tend to confirm this assumption.

There are good indications that histopathologic changes seen in biopsies of the liver in human beings, even when taken at the earliest stage at which hepatitis can be recognized, represent repair and healing processes following injury of the hepatocytes weeks previously. The elevated titers of the serum enzymes and of other liver function tests, including serum bilirubin are also

3 In a number of vivaria, particularly handling chimpanzees, gamma globulin is being used for the protection of the caretakers of the animals; the actual value of this preventative measure is now under critical investigation. (Personal communication, Col. M. E. Conrad, M. C., U.S. Army, 1969).

indications of established hepatic injury and appear at about the same time as morphologic changes of liver cells become obvious. The recognition that clinical disease following infection with the virus of viral hepatitis is a late result of accomplished liver injury by the virus, is supported by the work of Blumberg *et al.* (1967), Prince (1968), and London *et al.* (1969), who demonstrated the presence of a specific viral antigen (the Australia antigen) in the blood of human volunteers during the incubation period of homologous serum hepatitis, several weeks prior to the appearance of clinical symptoms and laboratory signs of this disease. Most recently Hirschman *et al.* (1969) described the presence of the Australia antigen in the serum of human patients with infectious and serum hepatitis, as well as in the serum of subhuman primates. Associated with this antigen were virus-like particles which were identical in both infectious and serum hepatitis of man and subhuman primates. Sera taken from normal human beings, chimpanzees or gibbons did not contain these virus-like particles. These findings indeed suggest the identity of the virus either causing infectious or serum hepatitis in man or in subhuman primates. Refinement of the methods for detection of the antigen and antibody following infectious hepatitis may also lead to the possible recognition of virus carriers with the elimination of prospective infective blood donors. In the present study Australia antigen was demonstrated in the serum of the animal caretaker P. M. 6 days after the appearance of jaundice due to acute viral hepatitis. In the serum of chimpanzee 397 this antigen was present 8 weeks after the intraperitoneal inoculation of P. M. stool suspension; the serum of chimpanzee 1530 showed continuous presence of Australia antigen from 4 to 22 weeks following the inoculation of P. M. stool suspension, but this antigen could not be demonstrated after the rechallenge with this material (see Table 3).

The finding of the Australia antigen is reminiscent of the demonstration of a specific antigen in the reticuloendothelial system 18 to 72 hours after experimental infection of rhesus monkeys with yellow fever virus (Tigertt *et al.*, 1960; Smetana, 1962). The antigen appeared during the incubation period prior to the development of clinical symptoms, laboratory findings and histopathologic alterations of liver cells.

This phenomenon was interpreted as evidence of multiplication of the virus within the cells of the reticuloendothelial system before the invasion and destruction of the liver cells by the agent.

Conclusions

Spontaneous viral hepatitis occurs rather frequently in chimpanzees and is not uncommon in patas monkeys. Because of lack of overt symptoms the disease is difficult to recognize clinically, and may, therefore, spread silently through a colony of animals and also affect humans by contact. While viral hepatitis in subhuman primates is usually a mild condition, the disease in human beings following its transmission from animals is much more serious.

Infectious viral hepatitis can be transmitted from humans to chimpanzees and patas monkeys, provided that the material used for inoculation is potent and that the animals have had no previous experience with this disease. Similarly, inocula-

tion of materials capable of producing homologous serum hepatitis in humans, can induce viral hepatitis in patas monkeys.

A pilot study with chimpanzees and patas monkeys indicates that spontaneous or induced infectious viral hepatitis renders the animals immune to re-infection with materials capable of causing viral hepatitis in previously unaffected animals. Induced homologous serum hepatitis protected patas monkeys from the effect of inoculation with materials from a human case of infectious viral hepatitis, that has been shown capable of causing hepatitis in subhuman primates.

In the absence of clinical symptoms the diagnosis of viral hepatitis in juvenile and mature subhuman primates is based on laboratory findings and on histopathologic alterations of the liver; in infantile and young juvenile animals the laboratory findings may be minimal and therefore, the diagnosis of hepatitis is based mainly on histopathologic changes in the liver.

It has not been possible to determine whether the virus of spontaneous viral hepatitis in subhuman primates is of human or animal origin, but results of recent investigations (Hirschman *et al.*, 1969) suggest that the virus of spontaneous hepatitis in subhuman primates is similar, if not identical, to the agent causing the human disease. The disease caused by the transmission of the virus from animals to humans is indistinguishable from primary human viral hepatitis acquired by the oral route or by transfusion of blood or blood products independent of contact with subhuman primates.

The many reported failures of experimental transfer of viral hepatitis from humans to subhuman primates are probably due to previous infection of the animals employed or to the use of an impotent inoculum.

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