STUDIES ON THE AGENT OF INFECTIOUS HEPATITIS

II. The Disease Produced in Human Volunteers by the Agent Cultivated in Tissue Culture or Embryonated Hen's Eggs*

By MILES E. DRAKE, M.D., ALBERT W. KITTS, M.D., MERCER C. BLANCHARD, M.D., JOHN D. FARQUHAR, M.D., JOSEPH STOKES, Jr., M.D., and WERNER HENLE, M.D.

With the Technical Assistance of Charles Ming and Mary Ming

(*From the Children's Hospital of Philadelphia, The Division of Virology, Department of Public Health and Preventive Medicine and The Department of Pediatrics, School of Medicine, University of Pennslyvania, Philadelphia*)

(Received for publication, June 16, 1950)

It has been shown in the preceding paper (1) that an agent has been transmitted from cases of infectious viral hepatitis to chick embryo tissue culture and to the embryonated hen's egg. It has been passed in series and proved capable of inducing hepatitis in volunteers. Its identity with the infectious hepatitis virus has not as yet been established beyond question, although the available information strongly suggests this. The inference is based largely on the similarity in the clinical pictures and the results of laboratory tests for hepatic dysfunction in volunteers challenged with natural virus and cultivated virus, and on their response, after convalescence, to skin test antigen consisting of infected amniotic fluid inactivated by ultraviolet irradiation (2).

Because of the fact that the clinical and laboratory data offer the main clues at present as to the identity of the agent propagated in tissue culture and in chick embryos, the present report describes in detail the illness induced by the "cultivated infectious hepatitis virus" and compares it with that resulting from exposure to "natural infectious hepatitis virus."

Methods and Materials

Virus.—The technic for the cultivation of infectious hepatitis virus has been discussed in the preceding paper. The materials used for infection of volunteers with "cultivated infectious hepatitis virus" were the 6th, 8th, and 10th tissue culture passages of the Akiba strain and the 3rd amniotic passage after 10 tissue culture transfers, designated as Akiba TC 6, 8, and 10, and Akiba Am 3, respectively, and the 10th passage tissue culture of the NL strain, NL TC 10. As source of "natural infectious hepatitis virus" pooled sera were used or filtrates of stools obtained in the early acute stage of infectious hepatitis during the outbreaks at Akiba and NL.

* This investigation was conducted in part under a contract from the Office of the Surgeon General, United States Army, and under the sponsorship of the Commission on Liver Disease, Armed Forces Epidemiological Board.

Volunteers.—The earlier tests with Akiba TC 6 were conducted in male volunteers at institution 1. All subsequent tests with cultivated and natural infectious hepatitis viruses were carried out in female volunteers of corrective institution 2.

The volunteers in institutions 1 and 2 were carefully screened before being selected for the project. For instance, of a total of 194 volunteers at institution 2, only 65 were judged suitable. At institution 1, 18 were admitted to the study. All volunteers had a complete physical examination, roentgenogram of the chest, hemogram, urine analysis, and serological tests for syphilis. Those who passed this initial screening were then studied for signs of liver dysfunction by appropriate tests of their serum and urine, in order to ascertain their fitness for the project. These preliminary tests included determinations of bromsulfalein excretion (Bsf.) (3), cephalin-cholesterol flocculation (C.C.F.) (4), thymol turbidity (T.T.) (5), thymol flocculation (T.F.) (6), colloidal red (C.R.) (7), and prompt and total serum bilirubin (8, 9). The

TABLE I

Interpretation of Laboratory Tests

Tests	Reference	Significant readings
Serum tests		
Serum bilirubin	(8, 9)	
Total		>1.2 mg. per 100 ml.
Direct—1 min.		>0.21 mg. per 100 ml.
Cephalin - cholesterol flocculation (C.C.F.)	(4)	> + in 24 hrs., > ++ in 48 hrs.
Thymol flocculation (T.F.)	(6)	> ++ in 18 hrs.
Collodial red (C.R.)	(7)	> ++ in 24 hrs.
Thymol turbidity (T.T.)	(5)	> 3 units
Bromsulfalein (Bsf.)	(3)	> 6 per cent in 30 min; 4–6 per cent in 45 min.
Urine tests		
Bilirubin, Harrison spot test	(10)	+ or stronger
Urobilinogen, Watson	(11)	> 1.9 Ehrlich units
Wallace and Diamond	(12)	1:30 or greater dilution

urine was examined for bilirubin by the Harrison spot method (10) and for urobilinogen by the methods of Watson (11) and Wallace and Diamond (12). No volunteer was acceptable who showed a positive urine bilirubin or urobilinogen, or who revealed a prompt serum bilirubin of over 0.21 mg. per 100 ml. Furthermore, a C.C.F. of 1+ at 24, or of 2+ at 48 hours, a T.T. of 3 units in 18 hours, a T.F. of 2+, a C.R. of 2+, or a Bsf. of 4 per cent or greater after 30 minutes was considered sufficient evidence of possible existing liver dysfunction to exclude the volunteer.

In the earlier experiments at institution 1, the virus (Akiba TC 6) was given orally (4 to 6 ml.) and subcutaneously (1 ml.). In the later experiments at institution 2 the infectious materials were given only by mouth (4 ml.). In both institutions the volunteers were isolated, after exposure, from the rest of the inmates, each volunteer having his or her designated unit and space. A shower, toilet, and wash basin were provided for each group of three. They had separate dining facilities; their diet, prepared by a separate cook, was high in calories, rich in proteins and vitamins with a daily multivitamin supplement. Every effort was made to maintain their morale and protect the remainder of the inmates from infection. That this

was accomplished is shown by the fact that no cases of hepatitis occurred in either of the institutions outside of the experimental units. Ample space was provided about the quarters for walking and sunning. The volunteers who were well and were deemed non-infectious were permitted to attend the institutional movies, but were seated in a segregated area. They were permitted to receive outside visitors during convalescence when the quarantine had been lifted.

A trained nurse or attendant was housed in the units and was available at all times. Adequate facilities were prearranged for intravenous fluids if they should be needed. Each volunteer was seen at least twice weekly by a physician, and daily when ill with hepatitis. All medical complaints of the volunteers were carefully checked and a complete physical examination was given once a week, or more often when indicated. The urines were examined daily or every other day during acute hepatitis. Liver function tests were run twice weekly during the acute phase, and once weekly otherwise. The tests employed, the references for the methods used, and the readings considered significant in this study are listed in Table I. Bromsulfalein retention tests were performed at infrequent intervals because of the reluctance of the volunteers to submit to the frequent venipunctures and of our concern regarding the possibility that one of them might be sensitive to the dye. These two factors were considered real hazards in the enlisting of future volunteers. In those tests which were done a retention of greater than 6 per cent in 30 minutes was considered significant. Hemograms were done on occasion when deemed necessary by the attending physician. In addition, heterophile antibody tests were conducted on each volunteer about midway in the convalescent period.

EXPERIMENTAL

Results of Exposure to Infectious Hepatitis Virus.—The results of exposure of volunteers to natural and cultivated infectious hepatitis virus are shown in Table II. It is apparent that of forty-two individuals receiving *natural* virus, seven failed to contract hepatitis and thirty-five developed clinical or laboratory signs of infection, or both. Of this group of thirty-five patients, twelve showed clinical signs compatible with hepatitis without confirmation by laboratory tests, twenty-three developed clinical signs of illness confirmed by laboratory tests, but only eleven became jaundiced. All those individuals who were still available at the time the skin test antigen became available (2) gave positive reactions with this material regardless of whether they had shown clinical signs and positive laboratory tests or illness without laboratory confirmation.

The results obtained with *cultivated* infectious hepatitis virus showed that of the thirty-six volunteers, nine remained well, four developed clinical signs of hepatitis without confirmation by laboratory tests, sixteen showed clinical and laboratory evidence of infection, and seven revealed abnormal liver function tests in the absence of demonstrable clinical signs or subjective complaints. There were no striking differences in response in the male and female volunteers of the two institutions. Again, all the volunteers available for skin tests who had previously shown signs of hepatitis with or without laboratory confirmation, gave positive reactions. Among those who failed to respond to exposure with illness only one gave a negative test, and this volunteer became jaundiced on subsequent challenge with natural infectious hepatitis virus 6 months later (1).

It appears then, on comparing the results of exposure to natural and to cultivated infectious hepatitis virus, that the total incidence of hepatitis was similar in the two groups. However, in the volunteers exposed to cultivated virus, the disease was generally milder and no jaundice was observed. In the group infected with natural virus, the occurrence of clinical signs of hepatitis, unconfirmed by laboratory tests, was more frequent than in volunteers exposed to the cultivated virus. In the latter group, on the other hand, abnormal liver function tests without clinical evidence of disease were observed in some volunteers, whereas such cases did not occur among the former.

Representative Cases of Induced Hepatitis.—For purposes of closer comparison between the diseases induced by natural and cultivated virus, representative case reports are summarized here. They concern patients with hepatitis without jaundice, with both clinical and laboratory evidence of illness, and with clinical signs only. No records are included of cases with abnormal laboratory tests in the absence of clinical evidence, since these occurred only in the experiments with tissue culture material of the 6th passage which had been stored for over 18 months at −20°C. prior to infection of the volunteers (1).

TABLE II

Response of Volunteers to Natural and Cultivated Virus

No. of volunteers	Inoculum	No hepatitis		Hepatitis									
				Clinical signs only		Clinical and laboratory signs				Laboratory signs only		Total	
						No jaundice		With jaundice					
		No.	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*
42	Natural IH virus	7	16.6	12	28.6	12	28.6	11	26.2	0	0	35	83.4
36	Cultivated IH virus	9	25.0	4	11.1	16	44.4	0	0	7	19.5	27	75.0

(*a*) *Patients with Clinical Signs and Symptoms of Hepatitis with Laboratory Confirmation*

1. Natural Virus.—Volunteer 41, age 21, female.

June 3, 1949. 4 ml. of NL strain of infectious hepatitis virus in serum pool 9, administered by the oral route.

June 1, 7, 12, 14, 19, 22, and 26. T.[1] between 98.4 to 98.6°F., P. 80 to 82, P.E. negative, no complaints. July 2. T. 100°F., P. 78, severe frontal headache, liver 2 cm. and tender,

[1] In order to conserve space the following abbreviations are employed in the individual case records: T. = temperature; P. = pulse rate; P.E. = physical examination; R.U.Q. = right upper quadrant; liver 2 cm. = liver palpable 2 cm. below costal margin.

anorexia, vomiting, R.U.Q. pain. July 6. T. 99°F., P. 80, felt better, still anorexia, nausea, and intermittent vomiting. Liver 2 cm. and tender. July 10. T. 98.6°F., P. 80, no anorexia or vomiting, felt better. Slight R.U.Q. pain, liver 1 cm. and mildly tender. July 13. T. 98.6°F., P. 80, liver 2 cm. and slightly tender. July 17. T. 98.6°F., P. 80, liver edge palpable, no tenderness, felt well. Lost 10 pounds.

July 20 to Aug. 12. Felt well, regaining weight, appetite good, and liver not palpable. Discharged.

The laboratory findings are presented in Fig. 1 and Table III.

2. Cultivated Virus, NL Tissue Culture.—Volunteer 33, age 29, female.

Oct. 14, 1949. 4 ml. of NL TC 10 administered by the oral route.

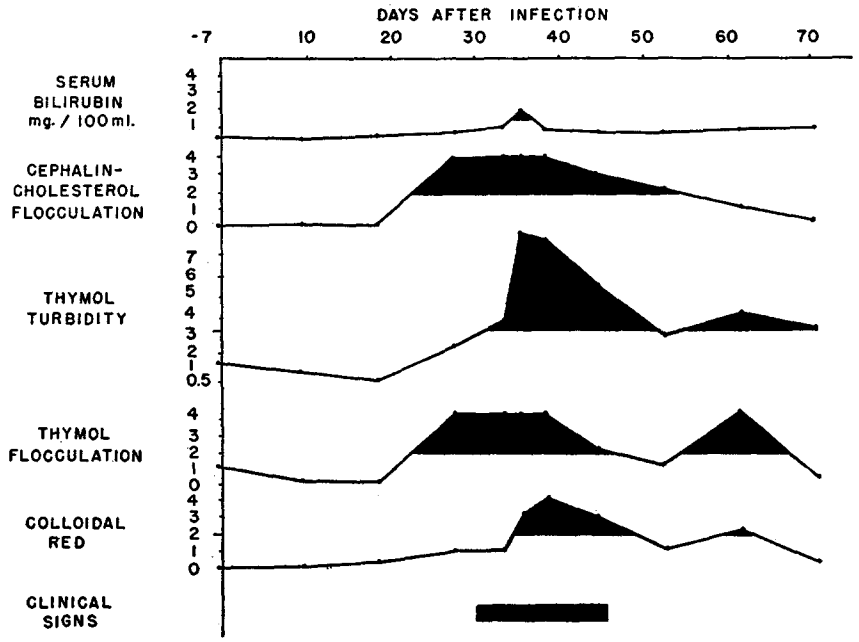

FIG. 1. Hepatitis without jaundice following exposure to natural virus NL strain, acute stage serum pool 9 (volunteer 41).

Oct. 20, 1949. T. 98.6°F., P. 84, menstruating, liver not palpable. Oct. 23, 1949. T. 99°F., P. 80, mild nausea, dysuria, R.U.Q. pain, liver 1 to 2 cm., tender. Oct. 24, 1949. T. 99°F., P. 80, nausea after breakfast. R.U.Q. pain and tenderness. Liver not palpable. Oct. 26, 1949. T. 99.6°F., P. 86, mild frontal headache, ocular soreness, epigastric pain. Vomited twice, liver 2 cm., tender. Nov. 1, 1949. T. 98.6°F., P. 82, appetite good, felt better, liver not tender and not palpable. Nov. 8, 1949. T. 99°F., P. 80, anorexia, liver edge palpable, tender. Nov. 15, 1949. T. 98.6°F., P. 80, felt well, liver 2 cm., tender. Nov. 22, 1949. T. 99.6°F., P. 78, epigastric pain and tenderness, feeling of epigastric fullness. Liver 3 cm., and tender. Nov. 28, 1949. Liver 4 cm., mildly tender, felt much better. Dec. 2, 1949. T. 98.6°F., P. 80, looked much better, liver 2 cm., slight tenderness, lost 10 pounds. Dec. 9, 1949. Liver edge palpable, no tenderness.

Dec. 21, 1949. T. 98.6°F., P. 78, felt well, liver not palpable, no tenderness. Regained 5 pounds. Discharged.

TABLE III

Laboratory Observations in Representative Cases Infected with Natural or Cultivated Infectious Hepatitis Virus

Volunteer No.	Inoculum	Day of onset of clinical signs	Laboratory studies									
			Blood							Urine		
			Day of study	T.T.	C.C.F.	T.F.	C.R.	Total serum bilirubin	Jaundice	Bilirubin Harrison spot	Watson 2 hr.	Watson and Diamond
								mg. per cent				
41	Natural virus	29	9	0.75	0	0	0	0.33	—	—	0.36	—
			18	0.7	0	0	0	0.45	—	—	0.48	—
			27	2.0	4+	4+	1+	0.50	—	—	0.68	—
			33	3.5	4+	4+	1+	0.90	—	—	0.90	—
			35	10.0	4+	4+	3+	1.70	—	3+	1.12	+1-50
			38	8.0	4+	4+	4+	0.5	—	2+		—
			44	5.5	3+	2+	3+	0.45	—	+—	0.88	—
			52	2.5	2+	1+	1+	0.42	—	Lavender	0.44	—
			61	3.7	1+	4+	2+	0.5	—	—		—
			70	3.1	0	0	0	0.5	—	—	0.48	—
33	NL	9	9	2.9	0	0	0	0.28	—	—	0.42	—
	Tissue culture		25	4.6	0	1+	1+	0.40	—	Lavender		—
	10th passage		32	5.2	0	4+	4+	1.40	—	1+	0.86	+ Undil.
			37	4.0	4+	4+	3+	0.50	—	Lavender		—
			42	5.8	1+	4+	4+	0.60	—	—	0.16	—
			48	4.2	0	3+	1+	0.20	—	Lavender		—
			55	4.8	0	4+	3+	0.20	—	—		—
			68	4.8	0	3+	2+	0.60	—	—		—
			74	4.6	0	3+	1+	0.40	—	Lavender	0.40	—
			90	2.8	0	0	0		—	—		—
23	Akiba	26	1	2.5	0	0	0	0.32	—	—	0.22	—
	Tissue culture		13		0	0	0	0.34	—	—		—
	10th passage		30	4.3	4+	4+	2+	0.50	—	—	0.52	—
			32	3.0	4+	3+	1+	0.90	—	—		+ Undil.
			41	4.8	4+	4+	2+	0.50	—	—	0.44	+1-20
			54	2.8	1+	1+	+—	0.40	—	—		—
28	Akiba	18	9	2.3	0	0	0	0.33	—	—	0.38	—
	3rd amniotic passage		19	2.5	0	+—	+—	0.25	—	—	0.40	—
			24	3.0	0	0	1+	0.40	—	—	0.44	—
			28	3.1	0	2+	1+	0.50	—	—	0.42	—
			32	4.1	+—	3+	1+	0.50	—	—	1.20	+1:50
			37	3.5	3+	4+	3+	0.40	—			
			45	3.5	4+	3+	1+	0.25	—	—	0.64	+ Undil.
			48	3.6	2+	2+	+—	0.15	—	Lavender		—
			55	4.1	0	3+	2+	0.6	—	—	0.40	—
			61	3.1	0	3+	3+	0.45	—	—		—
			74	2.3	0	2+	+—	0.5	—	—		—

The laboratory findings are shown in Fig. 2 and Table III.

Akiba Tissue Culture.—Volunteer 23, age 22, female.

June 3, 1949. 4.0 ml. Akiba TC 10, administered by the oral route.

June 10, 13, 15, 22, 28, 1949. T. 98.4 to 98.8°F., P. 78 to 88, P.E. negative. No complaints. June 29, 1949. T. 100.6°F., P. 74, anorexia, nausea, frontal headache, ocular pain, severe lumbar pain, and R.U.Q. pain. Liver 3 cm. and tender. June 30, 1949. T. 99°F., P. 80, nausea, vomiting, anorexia, R.U.Q. pain, liver 2 cm., slightly tender. July 2, 1949. T. 99.8°F., P. 78, nausea, intermittent vomiting, anorexia, R.U.Q. pain, liver 3 cm. and tender, no jaundice. July 5 to 11, 1949. T. 98.6°F., P. 80. Anorexia, mild R.U.Q. pain. Liver 3 cm. and tender. July 13, 1949. T. 98.6°F., P. 80. Appetite good, lost 8 pounds. Liver 2 cm., not tender. July

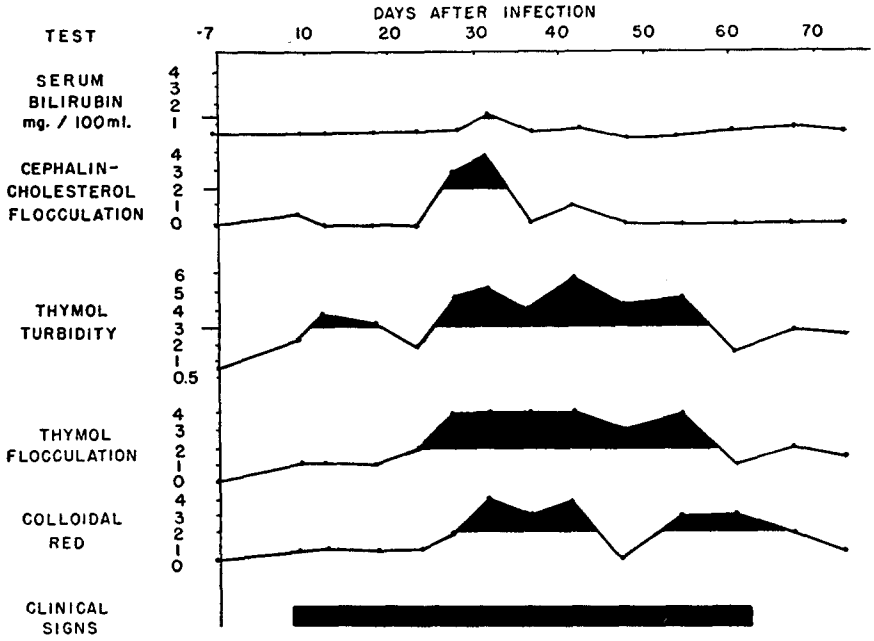

FIG. 2. Hepatitis without jaundice following exposure to the 10th tissue culture passage of the NL strain (volunteer 33).

17 to 23, 1949. T. 98.6°F., P. 80 to 84. Appetite good. Feeling well. Gained 1 pound. Liver 3 cm., not tender. July 30, 1949. Regained 5 pounds. Appetite good. Felt well. Liver 1 cm., not tender. Aug. 2, 1949. Regained 6 pounds. No complaints. Liver edge palpable, not tender.

Aug. 12, 1949. Regained 7 pounds. No complaints. Liver not palpable. All liver function tests were normal. Discharged.

The laboratory findings are shown in Fig. 3 and Table III.

Akiba Amniotic Passage.—Volunteer 28, age 29, female.

Oct. 14, 1949. 4.0 ml. of Akiba Am 3, administered by the oral route.

Oct. 20, 23, 28, 1949. T. 98.6 to 99.8°F., P. 84 to 88, P.E. negative. No complaints. Nov. 1, 1949. T. 100°F., P. 76. Chills, anorexia, nausea, R.U.Q. pain, headache, liver 2 cm. and tender. Nov. 3, 1949. T. 99°F., P. 78. Nausea, anorexia, intermittent vomiting, headache, liver 3 cm. and tender. Nov. 5 to 18, 1949. T. 98.6°F., P. 80. Feeling better. Appetite improving. Lost 11

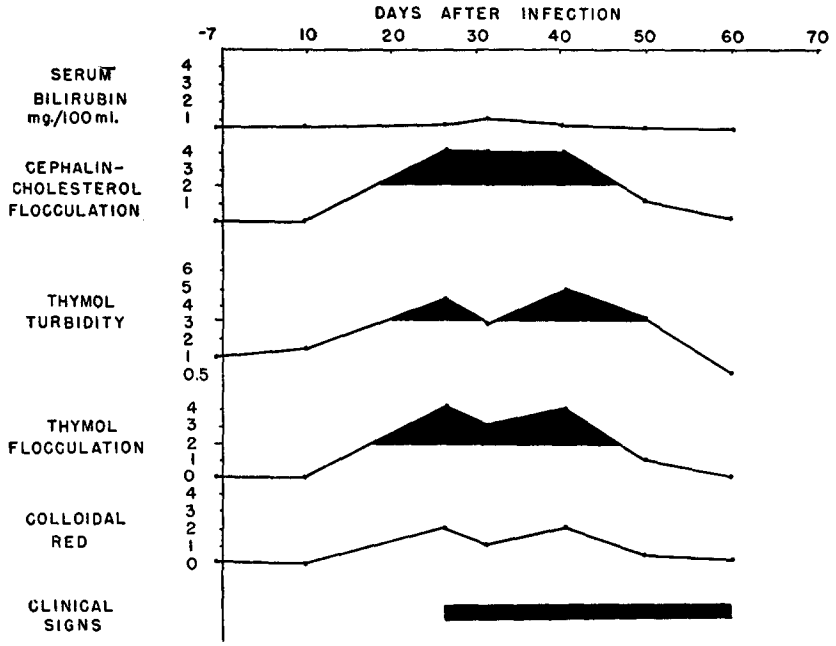

FIG. 3. Hepatitis without jaundice following exposure to the 10th tissue culture passage of the Akiba strain (volunteer 23).

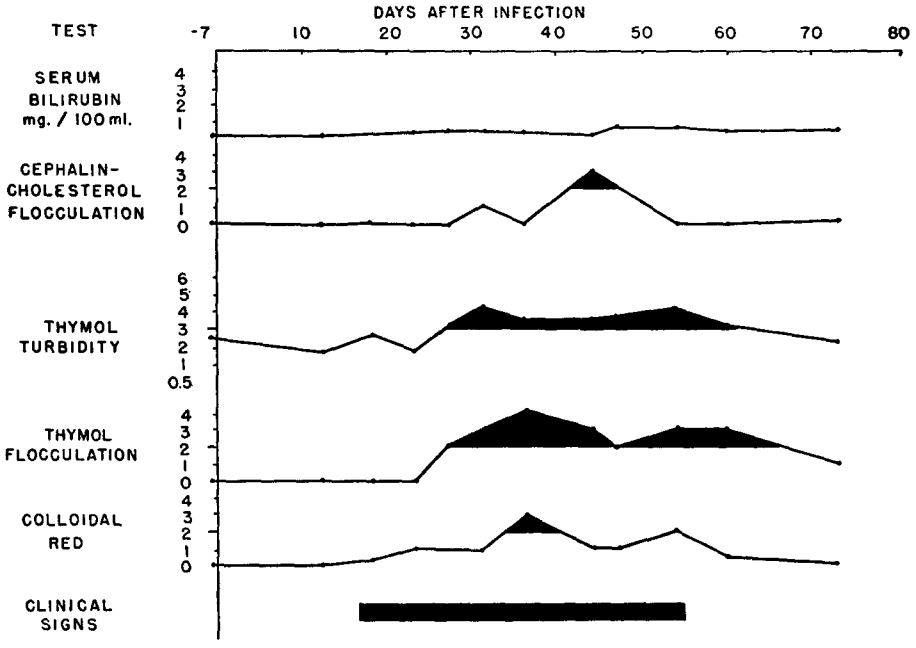

FIG. 4. Hepatitis without jaundice following exposure to the 3rd amniotic passage of the Akiba strain (volunteer, 28).

pounds in weight. Liver 3 to 4 cm., and intermittently tender. Nov. 25, 1949. T. 99°F., P. 82. Feeling well, no complaints. No gain in weight. Liver 4 cm., and not tender. Dec. 2, 1949. T. 98.6° F., P. 88, Felt well. No complaint. Liver 3 cm., not tender. Dec. 8, 1949. T. 98.4°F., P. 84. Felt well. Regained 6 pounds. Liver edge palpable, not tender.

Dec. 13, 18, 23, 27, 1949. No complaints. Liver not palpable. Discharged.

The laboratory findings are shown in Fig. 4 and Table III.

(b) Patients with Clinical Signs Unconfirmed by Laboratory Tests

1. Natural Virus.—Volunteer 38, age 20, female.

Mar. 21, 1949. 4.0 ml. NL strain of infectious hepatitis virus in serum, pool 7, administered by the oral route.

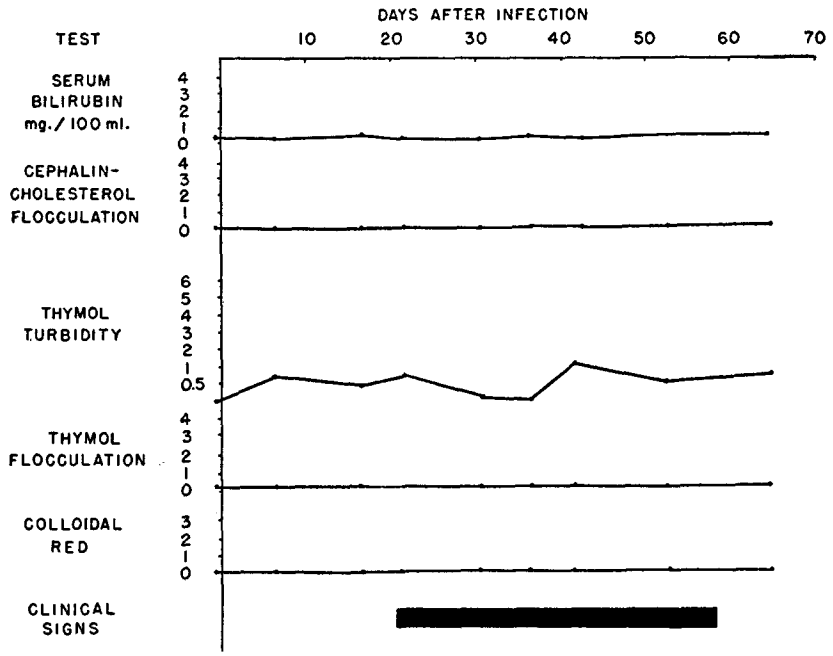

FIG. 5. Hepatitis without jaundice unconfirmed by laboratory tests following exposure to natural virus NL strain, acute stage pool 7 (volunteer 38).

Mar. 28 to Apr. 2, 1949. T. 98.6°F., P. 84 to 86. No complaints. P.E. negative. Apr. 10, 1949. T. 99.6°F., P. 76. Chills, anorexia, R.U.Q. pain. Liver tender but not palpable. Apr. 12, 1949. T. 100.1°F., P. 74. Malaise, anorexia, epigastric pain, nausea, and chills. Liver 4 cm. and tender. No jaundice. Apr. 15, 1949. T. 99°F., P. 80. Anorexia and frontal headache. Liver 2 cm. and tender. No jaundice. Apr. 17 to 19, 1949. Anorexia, headache, liver 3 cm. and tender. Apr. 22, 1949. Malaise, vomiting, epigastric pain. Liver 3 cm. and tender. Apr. 24, 1949. Felt better. Liver 2 cm. and tender. Apr. 27, 1949. Felt bad in general. Anorexia. Liver 1 cm. and tender. Apr. 29, 1949. T. 98.6°F., P. 82. Anorexia, R.U.Q. pain, losing weight. Liver 3 cm. and tender. May 1, 1949. Anorexia. Slight abdominal pain. Liver 4 cm. and tender. May 3, 1949. Frontal headache. Otherwise felt better. Liver 3 cm. and mildly tender. Lost 12

pounds. May 10, 1949. T. 98.6°F., P. 82. Mild anorexia. Liver 3 cm. and tender. May 13, 1949. T. 99°F., P. 84. Vomited once. Felt well otherwise. Liver 3 cm. and mildly tender. May 18, 1949. T. 98.4°F., P. 80. Feeling well. Liver 2 cm. and not tender.

May 24, 1949. Felt well. Liver not palpable. No complaints. Discharged.

The laboratory data are shown in Fig. 5.

2. Cultivated Virus, Akiba Amniotic Passage.—Volunteer 31, age 20, female.

Oct. 14, 1949. 4.0 ml. Akiba Am 3, administered by the oral route.

Oct. 20, 23, 24, Nov. 1, 8, 1949. T. 98.2 to 98.6°F., P. 86 to 80. No complaints. Felt well. P.E. negative. Nov. 15, 1949. T. 100°F., P. 70. Malaise, anorexia, frontal headache, nausea, R.U.Q. pain. Liver 2 cm. and tender. No jaundice. Nov. 18, 1949. T. 98.2°F., P. 80. Menstruating. Nauseated. R.U.Q. pain, anorexia, frontal headache. Liver 2 cm. and tender. Nov.

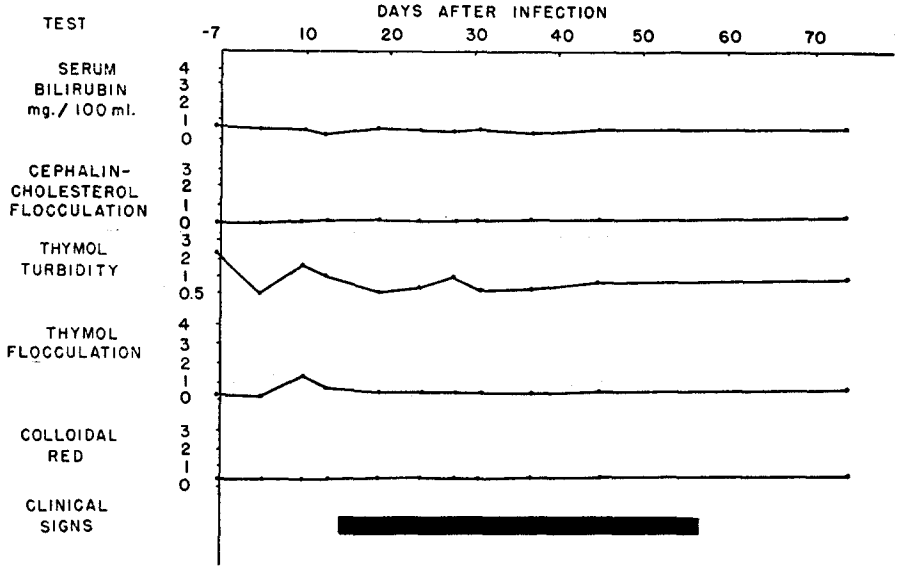

FIG. 6. Hepatitis without jaundice unconfirmed by laboratory tests following exposure to the 3rd amniotic passage of the Akiba strain (volunteer 31).

21, 1949. T. 98.2°F., P. 80. Feeling better but still nauseated. Liver 2 cm. and tender. Nov. 23, 1949. T. 99°F., P. 82. Felt well. Liver edge palpable and tender. Nov. 28, 1949. T. 98°F., P. 80. No complaints, but attendant stated that she was not eating well and tired easily. Liver 2 cm. and very tender. Dec. 2, 1949. T. 98°F., P. 82. No complaints. P.E. negative. Lost 10 pounds. Dec. 9, 1949. Felt well. Liver area tender. Liver not palpable. Much guarding over liver area.

Dec. 21, 1949. T. 98.6°F., P. 80. Felt well. P.E. negative. Discharged.

The laboratory data are shown in Fig. 6.

Comparison of Hepatitis Induced by Natural and Cultivated Infectious Hepatitis Virus.—In the preceding section, there have been presented individual cases of hepatitis without jaundice, induced by natural or cultivated virus, which were considered representative. It is apparent that the two types of viral prepa-

rations may produce illnesses indistinguishable from each other. This similarity is maintained if the two groups of patients are considered as a whole, except for the complete absence of jaundice in the volunteers exposed to cultivated virus. For reasons stated above mainly the patients of institution 2 will be considered in the further analysis.

As shown in Table I, the incidence of hepatitis following infection with natural and cultivated infectious hepatitis virus was not strikingly different; *i.e.*, 83 and 75 per cent, respectively, developed some signs of infection. The average incubation period for the natural virus disease was 24.4 days; for the cultivated virus, 23.4 days.

The disease induced by both natural and cultivated virus can be divided into a primary and a secondary stage. The earlier stage, lasting 2 to 5 days,

TABLE IV

Average Duration of Incubation Periods, Primary and Secondary Phases of Hepatitis, and Laboratory Changes Following Infection with Natural or Cultivated Infectious Hepatitis Virus

Inoculum	No. of volunteers	Incubation period	Duration of						
			Primary stage	Interval	Secondary stage		Total time of illness	Laboratory changes	
					Icteric cases	Non-icteric cases		Icteric cases	Non-icteric cases
		days	*days*	*days*	*days*	*days*	*days*	*days*	*days*
Natural virus	35	24.4	5	2.3	29.8	21.0	28.3	25.6	24.3
Cultivated virus	13	23.4	3.2	1.9	—	22.4	27.6	—	24.8

is characterized by chills, malaise, frontal headache, ocular pain, anorexia, nausea and vomiting, epigastric pain, lumbar pain, and often dysuria and occasional diarrhea. The temperature may be normal or elevated. This is followed by a short period of 1 to 3 days, during which the patient feels well and the appetite returns. After this interval the second stage begins, all the above symptoms recur and the liver becomes enlarged and tender. Jaundice occurred in only one-third of the cases of hepatitis induced by natural infectious hepatitis virus and not at all in those infected with cultivated virus. The average duration of the two stages and of the interval between them in the two groups of patients is given in Table IV. No significant differences may be discerned. The same conclusion holds for a comparison of the incidence of the various clinical signs and symptoms in the two groups, as shown in Table V. Furthermore, the average temperatures and pulse rates recorded during the acute illnesses failed to reveal marked differences between the two groups, as summarized in Table VI. Finally, the weight loss resulting from the infection was, if anything, more

marked in the groups exposed to the cultivated virus than in those infected with the natural agent (Table VII).

TABLE V

Clinical Signs and Symptoms of the Primary and Secondary Phases in Cases Induced by Natural Virus or Cultivated Infectious Hepatitis Virus

Inoculum	No. of volunteers	Elevated temperature		Anorexia		Headache		Nausea		Vomiting		Enlargement of liver		R.U.Q. pain		Lumbar pain		Dysuria	
		No.	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*
Natural virus	35	20	57	35	100	35	100	35	100	30	86.0	35	100	35	100	21	60	10	28.6
Cultivated virus	13	10	77	13	100	13	100	13	100	10	77.0	13	100	12	93	8	69	3	23.0

TABLE VI

Incidence of Elevated Temperatures and Pulse Rates at the Height of Primary and Secondary Phases of Hepatitis Induced by Natural or Cultivated Infectious Hepatitis Virus

Inoculum	Phase	No. of volunteers	Temperature (oral)				Pulse					
			100°–104°F.		<100°F.		>90		70–90		<70	
			No.	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*	*No.*	*per cent*
Natural virus	Primary	35	20	57	15	43	8	23	19	54	8	23
	Secondary		18	51	17	49	5	14	15	43	15	43
Cultivated virus	Primary	13	7	54	6	46	2	17	8	60	3	23
	Secondary		10	77	3	23	1	8	6	46	6	46

TABLE VII

Comparison of Average Weight Loss in Pounds as the Result of Infection Induced by Natural or Cultivated Infectious Hepatitis Virus

Inoculum	No. of volunteers	Average admission weight	Average terminal weight	Average weight loss
		lb.	*lb.*	*lb.*
Natural virus	35	127	120	7
Akiba TC 8	3	127	118	9
Akiba TC 10	2	138	124	14
NL TC 10	3	141	131	10
Akiba Am. 3	5	133	120	13

Of the thirty-five cases of hepatitis induced by natural virus, twelve or 34.3 per cent failed to show confirmatory laboratory evidence of disease, whereas of the thirteen patients infected with cultivated virus, three or 23 per cent fell

into that category. Confirmation of hepatitis by laboratory tests was obtained in twenty-three of the thirty-five cases of the natural virus disease, or in 65.7 per cent, and in the cultivated virus group, in ten of thirteen, or 77 per cent. In these cases, all the serum tests for hepatic dysfunction used were significantly positive at some time during the course of the studies, except for the determinations of serum bilirubin. In keeping with the absence of jaundice in all the cases induced by cultivated virus, total serum bilirubin was slightly elevated in only three patients (1.3 mg. to 1.4 mg. per cent). The cases of hepatitis without jaundice in the natural virus group fell in line with this observation, with values not exceeding 1.7 mg. per cent.

The bromsulfalein retention was determined only at infrequent intervals as previously stated. However, enough evidence was obtained to demonstrate that in the cases infected with cultivated infectious hepatitis virus there was retention of 8 to 22 per cent in some of the volunteers. These values are comparable to those found in patients having hepatitis without jaundice following response to natural virus, who showed retention of 8 to 30 per cent.

The urinary changes indicating liver dysfunction likewise were similar in both groups of patients. In the cases caused by cultivated virus there was a tendency for the urinary changes to be somewhat less marked and more intermittent than in the patients infected with the natural agent. However, on account of the geographic separation of the institutions from the laboratory, it is felt that the results do not represent true values because of the time elapsing between voiding the urine and the laboratory test.

There were too few hemograms done to permit a comparison between the two groups of patients. However, the total and differential counts done in some of the patients during the acute stage revealed a slight leucopenia in both the cases infected with natural and with cultivated virus, and both showed slight increases of monocytes.

None of the patients of either group revealed positive heterophile antibody tests during the course of the illness.

DISCUSSION

The data presented show that the virus propagated in tissue culture and in the embryonated hen's egg is capable of inducing in volunteers hepatitis without jaundice, and that the clinical picture obtained is indistinguishable from non-icteric hepatitis following infection with natural infectious hepatitis virus. Exposure to natural or cultivated infectious hepatitis virus resulted in a similar incidence of illness, with or without confirmatory laboratory tests. The incubation periods, average duration of the diseases produced, and the division into primary and secondary stages were closely similar. The changes in laboratory tests were comparable if the frankly jaundiced cases in the natural virus group are excluded. Leucopenia occurred in both types of patients and none developed positive heterophile agglutination reactions. Both groups lost weight to about

the same extent as a result of the infections. In addition, marked upsets in the menstrual cycles were recorded in the female volunteers infected with the two types of viral preparations. This latter finding will be elaborated upon in a separate communication (13).

In the spontaneous disease it is undetermined how often hepatitis occurs without jaundice. That it happens more frequently than is usually suspected is suggested by the occurrence of characteristic symptoms and signs of hepatic dysfunction without jaundice in many patients during an epidemic when the diagnosis is suggested by the epidemiologic situation and corroborated by tests of hepatic function (14). In the present studies with natural virus only eleven out of thirty-five patients, or 31.4 per cent, became jaundiced. This would indicate that as many as 70 per cent of infections in an epidemic might be nonicteric. This is a figure which is certainly higher than that usually given. However, it is realized that the number of volunteers in the studies reported above is too small to permit any definite conclusions on this point.

In view of the absence of jaundice in patients infected with the cultivated virus it is interesting to speculate on the possibility that propagation of the infectious hepatitis virus in chick embryo tissues may have led to an attenuation of the agent. In order to verify this suggestion it would be necessary to attempt transfer of serum or feces obtained during the acute stage from patients infected with the tissue culture virus to new volunteers in order to ascertain whether a similarly mild illness would result, or whether a more severe hepatitis accompanied possibly by jaundice might be obtained. Such experiments have not been feasible as yet.

The degree of attenuation, if this is the explanation, is insufficient as yet to permit any thoughts of immunization of susceptible individuals with the cultivated virus. Furthermore, the question whether the cultivated agent produces immunity against the natural virus cannot be considered answered at present. Although preliminary evidence points toward this possibility (1), such immunity tests will have to be conducted on a larger scale.

SUMMARY

The successful cultivation of the virus of infectious hepatitis in chick embryo tissue culture and in the amniotic cavity of the embryonated hen's egg is supported by a comparison of the disease induced in volunteers by the cultivated virus with hepatitis without jaundice resulting from experimental infection with natural infectious hepatitis virus. Both types of viral preparations produced illnesses in comparable percentages of volunteers (83 and 75 per cent, respectively) after similar average periods of incubation (24.4 and 23.4 days, respectively) and of similar average duration (28.3 and 27.6 days, respectively). The disease could be divided in both groups of patients into a primary stage, followed after a short interval of relative well being by the secondary stage. The ill-

nesses in both instances were characterized by anorexia, nausea, vomiting, enlarged, tender livers and abnormal liver function tests, and frequently temperature elevations. They differed in that jaundice was observed in 31 per cent of the cases resulting from infection with natural virus but not in any patients infected with the cultivated virus.

BIBLIOGRAPHY

1. Henle, W., Harris, S., Henle, G., Harris, T. N., Drake, M. E., and Stokes, J., Jr., *J. Exp. Med.*, 1950, **92,** 271.
2. Henle, G., Drake, M. E., Henle, W., and Stokes, J., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1950, **73,** 603.
3. Cantarow, A., and Wirts, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47,** 252.
4. Hanger, F. M., *Tr. Am. Assn. Physn.*, 1938, **53,** 148; *J. Clin. Inv.*, 1939, **18,** 261.
5. MacLagan, N. R., *Brit. J. Exp. Path.*, 1944, **25,** 234.
6. Neefe, J. R., *Gastroenterology*, 1946, **1,** 393.
7. Ducci, H., *Rev. med. y aliment. Chile*, 1946, **74,** 773.
8. Ducci, H., and Watson, C. J., *J. Lab. and Clin. Med.*, 1945, **30,** 293.
9. Malloy, H. T., and Evelyn, K. A., *J. Biol. Chem.*, 1937, **119,** 481.
10. Godfried, E. G., *Biochem. J.*, 1934, **28,** 2056.
11. Watson, C. J., Schwartz, S., Sborov, V., and Bertie, E., *Am. J. Clin. Path.*, 1944, **14,** 605.
12. Wallace, G. B., and Diamond, J. S., *Arch. Int. Med.*, 1925, **35,** 698.
13. Drake, M. E., Mangold, F., Stokes, J., Jr., and Henle, W., unpublished data.
14. Barker, M. H., Capps, R. B., and Allen, F. W., *J. Am. Med. Assn.*, 1945, **128,** 997.