with respect to conversion of C^{14}-phytosterol to bile acids confirm and extend the observations by Werbin *et al.*(5) and Hanahan and Wakil(2) with H^3-β-sitosterol and C^{14}-ergosterol. Preliminary experiments were carried out in an attempt to identify the bile acids formed. They appeared to be different from those derived from cholesterol.

Summary. Data are presented on the comparative tissue distribution of C^{14}-free and esterified sterols following injection of cholesterol-4-C^{14} and C^{14}-phytosterols to normal and bile-fistula rats. At the end of 24 hours a large proportion of the injected C^{14}-phytosterols and cholesterol-4-C^{14} was recovered in the liver. All tissues examined (serum, liver, small intestine and adrenal) showed the presence of esterified C^{14}-phytosterols, but less was esterified than with cholesterol-4-C^{14}. A much larger proportion of the injected C^{14}-phytosterols was recovered in the feces and intestinal contents than in the cholesterol-4-C^{14} group. Conversion of the injected C^{14}-phytosterols to bile acid was observed; a larger proportion of the injected C^{14}-activity was recovered in the bile as C^{14}-sterol than with injected cholesterol-4-C^{14}. No conversion of C^{14}-phytosterols to cholesterol or related sterols could be demonstrated.

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Destruction of Chimpanzee Kidney Cells by Sera From Patients with Acute Infectious Hepatitis.*† (27077)

WILLIAM D. HILLIS (Introduced by S. S. Kalter)

Microbiology-Cellular Biology Branch, School of Aerospace Medicine, USAF Aerospace Medical Center (ATC), Brooks Air Force Base, Texas

Recent epidemiological evidence(1) has suggested that chimpanzees may act as transient carriers of human hepatitis virus. Since then, recurrent outbreaks of the disease among groups of chimpanzee handlers have further supported this hypothesis(2-4). The outbreaks were sufficiently well documented to lend support to a supposition that the virus may have been propagated in some unknown cellular system within the animals.

Accordingly, chimpanzee liver, omentum, spleen, bone marrow, testis, and kidney were removed from healthy adolescent animals, and standard tissue culture techniques were employed to obtain cellular outgrowths. The various cellular systems were exposed to acute sera from 3 to 12 cases of typical human infectious hepatitis, suspected of containing active virus. Some of the sera were from chimpanzee handlers at Holloman Air Force Base, New Mex.(1), whereas others were derived from outbreaks remote from Holloman and not associated with any known ape contacts.

Initial experiments indicated that some of the sera were capable of destroying outgrowths of chimpanzee macrophages from omental tis-

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† Since this paper was submitted for publication, it has not been possible to maintain the virus past the 4th chimpanzee kidney cell passage.

sue. The destruction of monolayers of chimpanzee kidney cells by 2 of 12 sera, however, was so striking and the latter cells were so much more satisfactorily grown, that attention was focused on these cells. The destructive agents have subsequently been followed in serial passage in these cultures and partially characterized.

Methods. Sera. Five sera were obtained from certain chimpanzee handlers at Holloman Air Force Base who had typical cases of infectious hepatitis, ranging from 1 to 6 days after onset of icterus. Seven additional sera from hepatitis patients, who had not handled apes, were similarly collected from various Air Force installations and from civilian hospitals in San Antonio, Texas. Control sera were derived from normal, healthy Air Force personnel. Sera had been stored at −20°C from 1 to 10 months.

Kidney Cells. Kidneys were removed surgically from chimpanzees weighing 45 to 50 lbs and immediately subjected to overnight trypsinization according to procedures described by Youngner(5) and Bodian(6). After trypsinization and washing, cells were suspended in 0.5% lactalbumin hydrolysate in Hanks' balanced salt solution(7) containing 5% calf serum (Microbiological Associates, Bethesda, Md.), 1% of a 10% $NaHCO_3$ solution, and penicillin and streptomycin (200 units/cc and 0.2 mg/cc, respectively), to a count of approximately 300,000 cells/cc. One cc volumes of the cell suspension were added to either screw-capped glass culture tubes or to Leighton tubes containing thin glass cover slips. Cultures were incubated, stationary, at 37°C, and medium was changed completely each 2 to 4 days, so that pH never fell below 7.0. When cellular growth was confluent, usually within 7 to 8 days, growth medium was removed, and the tubes were washed thoroughly with Medium 199(8) containing 0.1% $NaHCO_3$ and antibiotics as above (maintenance medium). One cc of fresh maintenance medium was then added to each tube. Following inoculation, tubes were maintained for 10 to 14 days at 37°C with the addition every 2 to 3 days of sufficient 10% $NaHCO_3$ to maintain pH above 7.0 (usually about 0.02 cc/tube).

Inoculations. Each tube was initially inoculated with 0.2 cc of either acute hepatitis serum or "normal" serum. At least 4 tubes were inoculated with each serum in each of 2 initial experiments. In the case of sera producing destructive effects, serial passages were initiated by addition of 0.2 cc per tube of pooled fluid from frozen and thawed cultures which had shown extensive cellular destruction. Controls were derived from serial passage of fluids from cultures initially inoculated with "normal" sera.

Results. Chimpanzee kidney cells inoculated with 2 of the 12 hepatitis sera showed evidence of cellular damage in initial cultures on the sixth day following inoculation (Fig. 1-3). One destructive serum was derived from Capt. JEC, Veterinary Services Branch, Aeromedical Field Laboratory, Holloman Air Force Base, a close contact of numerous immature chimpanzees. The second was obtained from PEL, a patient at the Stead Air Force Base Hospital, Nevada, who as far as could be ascertained, had no contact with chimpanzees.

Cellular changes in fixed, stained preparations are illustrated in Fig. 4-6. By the seventh or eighth day after inoculation of the sera, destruction was complete. Original PEL serum, inactivated at 56°C for 30 minutes, similarly produced cellular destruction. Insufficient JEC serum was available for inactivation studies. Cells of the same age grown in serum from an individual with no known hepatitis showed no changes.

To characterize the destructive agents encountered in the 2 sera further, direct fluorescent antibody tests were performed on methanol-fixed cells 6 days after inoculation. Serum from Capt. JEC, 6 months convalescent from the acute episode of hepatitis, was labelled with fluorescein isothiocyanate according to methods outlined by Cherry et al.(9). Similarly, normal pooled serum from a group of 4 individuals at Holloman Air Force Base who had contracted no known hepatitis was labelled with fluorescein isothiocyanate for control purposes. Cells grown in JEC, PEL, and "normal" serum were fixed 6 days after inoculation, when destruction was still moderate. Each series was treated with labelled JEC serum and labelled normal serum pool. The fluorescent antibody test results are shown in Table I and Fig. 7-8.

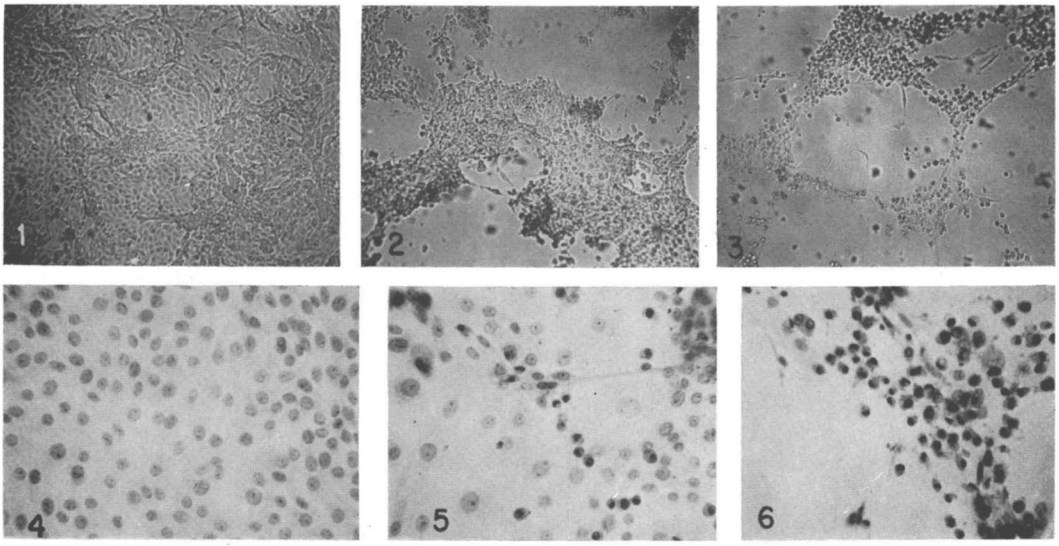

FIG. 1. Control chimpanzee kidney cells, grown in normal human serum for 6 days.

FIG. 2. Same as Fig. 1, except cells grown for 6 days in acute JEC serum.

FIG. 3. Same as Fig. 1, except cells grown in acute PEL serum.

FIG. 4. Chimpanzee kidney cells grown in normal human serum for 6 days, fixed and stained with hematoxylin and eosin.

FIG. 5. Same as Fig. 4, except cells grown in JEC serum.

FIG. 6. Same as Fig. 4, except cells grown in PEL serum.

TABLE I. Fluorescence of Chimpanzee Kidney Cells Grown in Acute Hepatitis Sera and Normal Serum Treated with Fluorescent Antibody.

	Fluorescein-labelled JEC Serum	Fluorescein-labelled Normal Serum Pool
Cells grown in JEC serum	+++	−
" " " PEL "	+++	−
" " " normal "	−	−

The destructive agents from JEC serum and from PEL serum, tentatively named SAM-1 and SAM-2, respectively, have at this time been carried through 3 serial tissue culture passages. Fluids removed from primary cultures 3 days after inoculation, when no destruction was evident, produced no change on serial passage. After the initial passage, cellular destruction occurred within 48-72 hours.

Agents SAM-1 and SAM-2, after first passage, survived heat treatment at 56°C for 30 minutes. Following first passage, both agents were titrated in chimpanzee kidney cells. Titers were in the range of only 1×10^{-2}. Nevertheless, the destructive effects of both agents, undiluted, were neutralized by JEC convalescent serum and not by "normal" serum.

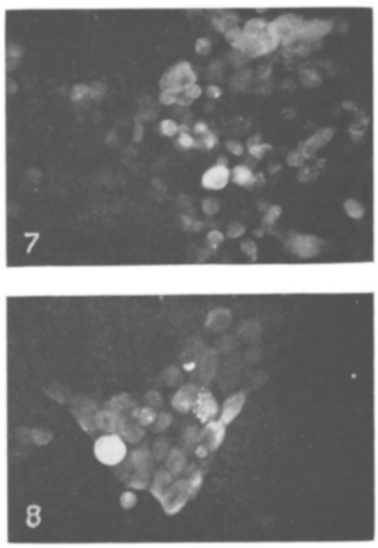

FIG. 7. JEC serum-infected cells (as in Fig. 2) treated with fluorescein-labelled hepatitis convalescent serum.

FIG. 8. PEL serum-infected cells (as in Fig. 3) treated with fluorescein-labelled hepatitis convalescent serum.

Discussion. The destructive effect of 2 acute hepatitis sera on chimpanzee kidney cells, capable of serial passage, suggests the action of a viral agent or agents. Fluorescent antibody and neutralization tests indicate that the agents are antigenetically reactive with convalescent serum from clinical infectious hepatitis. Heat inactivation studies further emphasize similarity to properties attributed to hepatitis virus. Studies to characterize the agents more clearly are presently in progress.

Summary. Destruction of chimpanzee kidney cells by 2 of 12 acute sera from patients with clinical infectious hepatitis was noted. The cellular effect has been seen in serial passage. The destructive agents, tentatively named SAM-1 and SAM-2, have been shown in fluorescent antibody and neutralization tests to be serologically cross-reactive with convalescent serum derived from clinical infectious hepatitis. The agents are resistant to heat treatment at 56°C for 30 minutes.

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Dose Dependent Effects of D-Sorbitol on Intestinal Absorption of Vit. B_{12}.* (27078)

KUNIO OKUDA
Department of Medicine, Yamaguchi Medical College, Ube, Japan

It has been shown that D-sorbitol enhances intestinal absorption of B_{12} under certain conditions in man(1) as well as in rats(2). Morgan and Yudkin(3) reported that growth of rats can be accelerated by supplement of sorbitol to a diet deficient in the B group vitamins. They postulated that sorbitol increased intestinal synthesis of these vitamins. Our study(4) also demonstrated that dietary sorbitol promoted growth of weanling rats placed on a B_6 deficient diet, with a concomitant increase in urinary output of B_6. No such effects were obtained with B_{12}, however. Inasmuch as increased synthesis of B_{12} in the intestine will not explain the enhancement of absorption of oral doses of B_{12}, a study was carried out in rats to elucidate the mechanism by which sorbitol improves B_{12} absorption. It was found that intestinal absorption of B_{12} could be either enhanced or reduced by coadministration of sorbitol, depending upon the dose. Such diverse, dose dependent effects of sorbitol will be reported herein.

Methods. Young adult rats of Wistar strain were used. Absorption of Co^{60} labeled B_{12} (Merck) was determined by one of the following methods. a) Absorption in intact rats: Animals were fasted overnight and then fed by a stomach tube a test dose of $Co^{60}B_{12}$, and absorption was estimated from the organ uptake of radioactivity. b) Absorption in ileostomized rats: In some animals, the ileum end was surgically severed and opened outside through the abdominal wall, the cecal end being closed. Thus, the cecum was excluded from the absorptive route, and absorption measured was that from the small intestine. The technique

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