are related to dosage fed, pool size, and turnover of pool.

Discussion. Our results indicate that feeding a small dose (3.27 mg) of cholesterol-4-C^{14} without sodium taurocholate and oleic acid did not influence size or turnover rate of cholesterol pool of intestinal wall or produce an increase in lymph cholesterol level. Addition of sodium taurocholate and oleic acid to test meal produced an increase in transfer of fed cholesterol-4-C^{14} to the lymph. However, the amount transferred did not account for the observed increase in lymph cholesterol level. This increased amount of cholesterol in lymph may have been derived from increased synthesis of cholesterol in the intestine. It was previously postulated(2) that during fat absorption an increase in cholesterol synthesis in the intestine would occur to support chylomicron and lipoprotein formation.

The data on cholesterol levels and specific activity of the cholesterol in lymph and intestinal wall after feeding small doses of cholesterol-4-C^{14} and the relative dilution of large and small doses are in agreement with the concept that cholesterol entering from the lumen is mixed with a pool of free cholesterol in the mucosa prior to incorporation into the chylomicron.

Summary. Small doses of fed cholesterol-4-C^{14} lead to labeling of cholesterol fractions of mucosa and lymph without increase in level or turnover rate of free cholesterol pool of the mucosa or in amount of cholesterol in lymph. Feeding tracer dose with sodium taurocholate and oleic acid increases the turnover rate of pool which leads to an increased amount of labeled and unlabeled cholesterol in lymph.

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Propagation of Duck Hepatitis Virus in Tissue Culture.* (25003)

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Virus-induced hepatitis has been described in man(1,2), duck(3-5), dog(6), and mouse (7-9), and agents of the latter 2 have been propagated in tissue culture(10-14). Much interest in comparative viral hepatitis stems from failure to propagate the virus(es) of human hepatitis in the laboratory(15). Since identification of this agent now depends on hazardous exploitation of man, alternate procedures with other host-specific hepatitis agents have been employed as possible prototypes. In this respect, some biological properties of duck hepatitis virus (DHV) have been studied.

Materials and methods. Assay and identification of DHV.† DHV-infected chick embryos were emulsified (10% w/v) in Hank's balanced salt solution (BSS). The preparation was partially clarified by centrifugation at 3,000 rpm for 20 minutes, ampulated, and stored at dry-ice temperature. Assays for DHV were performed by inoculation of 0.1 ml amounts of 10-fold dilutions of test fluids in phosphate buffered saline (pH 7.2) into

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7-day-old chick embryos *via* the chorio-allantoic cavity. Stock virus preparations usually killed the embryo in 2-4 days when diluted 10^6-fold. Infected embryos showed hemorrhagic lesions in skin and viscera. The lethal effect of DHV was neutralized by immune serum prepared in chickens and rabbits. Two intramuscular inoculations of virus in adjuvant (virus : mineral oil : arlacel; 0.5 ml : 1 ml : 0.1 ml, respectively) were given 2 weeks apart. Animals were exsanguinated 2 weeks later and sera were stored at –20°C. One ml aliquots of DHV pool were sealed in ampules and incubated at 37°C. At 5-day intervals, samples were assayed for virus content. Portions of virus pool were mixed with ⅓ volume of di-ethyl ether (Squibb) and stored overnight at 4°C. Mixtures were centrifuged at 2,000 rpm for 10 minutes, and aqueous fractions were assayed for virus content. Aliquots of virus pool were mixed twice with ½ volume of fluorocarbon (CCl_2F-$CClF_2$) (Genetron: General Chemicals, Inc., N. Y.) in a Virtis homogenizer as described by Gessler *et al.* (16). Aqueous portions were assayed for virus content. Seven-day-old chick embryos were washed in BSS and their bodies minced into approximately 1 mm pieces. Four to 6 washed explants were imbedded in chicken plasma onto the wall of each screw-cap tube. Nutrient fluid (NF) for tissue cultures contained BSS (90%), heat-inactivated chicken serum (10%), penicillin (100 units/ml), and streptomycin (0.1 mg/ml). Occasionally Eagle's medium or solution 199 was substituted for BSS. The NF was decanted from proliferating explants after 48 hours incubation at 37°C in a roller drum apparatus. After addition of 0.1 ml of undiluted DHV pool and 0.9 ml of NF to each tube, they were reincubated. Cultures were examined daily, by low-power magnification, for evidence of CPE. At intervals, contents of several tubes were quick-frozen and thawed twice, pooled, and assayed for virus content. Subsequent virus passages involved addition of 0.1 ml of DHV tissue culture pool plus 0.9 ml of NF to tubes containing new chick embryo explants. At different passage levels, DHV was checked for identity by neutralization tests with immune serum. Some aspects of DHV replication in tissue explant cultures were studied by 1) assay of cultures at daily intervals following addition of DHV, 2) assay of NF from cultures renewed at 6-day intervals, 3) assay of cultures inoculated with 10-fold dilutions of DHV to determine minimal infective dose of virus, 4) assay of infected explants inoculated at various ages, and 5) assay of aging cultures in which the NF was not changed after the addition of DHV. Seven-day-old chick embryos were minced in BSS and treated repeatedly for 5 minutes with 0.25% Difco Trypsin 1:250 at 37°C. All resulting cells, except those from first treatment, were pooled, and washed at low-speed centrifugation. Approximately 100,000 cells in NF were transferred to each screw-cap tube. Cultures were incubated in stationary racks at 37°C for 3-6 days, then inoculated with DHV according to schedule described above with tissue explants. After 6 days at 37°C, cultures were quick-frozen and thawed twice, pooled, and assayed for virus. Pooled culture fluids were passaged (0.1 ml) through 5 or 6 series of trypsinized-cell cultures. Standard cell lines (HeLa, McCoy human fibroblast, and mouse "L" cell) and monkey kidney were propagated in stationary tubes at 37°C. Cells were loosened by agitation and/or by scraping from the tube-wall and transferred to tubes (approximately 100,000 cells/ml). Cultures were incubated in stationary racks for 5 days when confluent cell-sheets had formed and were inoculated with 10^6 LD_{50} of DHV. Subsequent examinations for CPE and for virus were similar to that described above with chick embryo explants.

Results. Properties of DHV. DHV is a relatively stable virus. It resisted treatment with ether and with fluorocarbon and survived for at least 3 weeks at 37°C (Fig. 1).

Propagation of DHV in tissue cultures. DHV has been propagated in chick embryo explants through 25 consecutive passages at 6-day intervals and production of virus particles exceeded amount in original inoculum (Table I). The cumulative time of DHV in tissue culture with sustained titer was 150 days which exceeded decay time of virus. Definite CPE was not observed; in fact, less virus was recovered from tissues which ap-

TABLE I. Propagation of DHV on Explanted and Trypsinized Chick Embryo Cultures. Passaged at 6-day intervals.

Tissue cultures	Passage No. and virus titer								
	Base	1	2	3	4	5	15	20	25
Trypsinized	10^{-6}	10^{-4}	10^{-3}	10^{-1}	Neg.	Neg.			
Explanted	10^{-7}	NT*	NT	NT	10^{-4}	10^{-5}	10^{-3}	$10^{-4.5}$	$10^{-3.5}$

* Not tested.

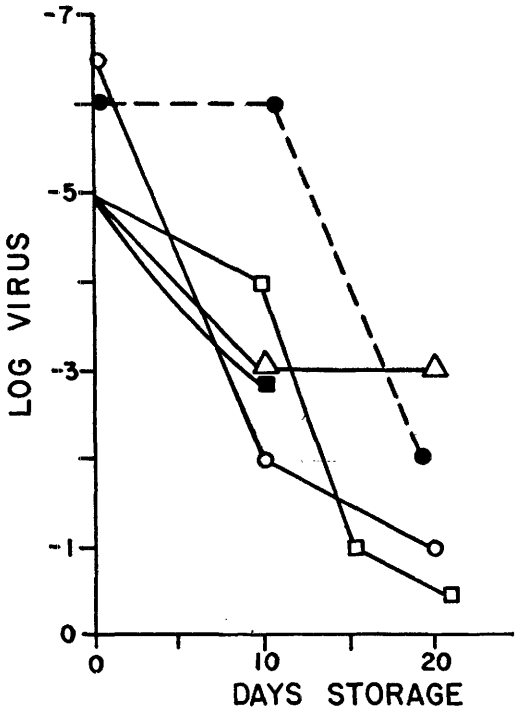

FIG. 1. Survival of duck hepatitis virus stored without tissue at 37°C. Each line represents a separate test series.

peared degenerated than from actively growing tissues. LD_{50} titers of DHV from individual tissue culture passages ranged 10^{-3} to 10^{-5}. Tissue explants were infected by as little as 10 LD_{50} of DHV. Substitution of Eagle's medium or solution 199 for BSS did not influence virus production significantly. DHV multiplied in tissues which had been explanted 4-21 days previously. When NF of infected cultures was replaced 4 times at 6-day intervals, DHV reappeared in the NF with LD_{50} titers between 10^{-2} and 10^{-3}. When NF of infected cultures was not changed for 32 days, DHV was recovered in dilutions of 10^{-4}. Initial titer of virus in NF (10^{-5}) declined to LD_{50} 10^{-1} on first day after inoculation and climbed gradually to sustained titer of LD_{50} 10^{-4} after 4 days.

Duck hepatitis virus was recovered from first 3-4 passages in trypsinized chick embryo and disappeared thereafter (Table I). Virus assays on consecutive trypsinized-cultures simulated the normal decay curve (Fig. 1) and in some instances this decay was accelerated. DHV disappeared more rapidly in cultures of established cell lines than in cell-free medium alone. CPE was not noted in inoculated cell lines.

Discussion. Failure to detect propagation of DHV in mammalian cells is not surprising in view of its restricted spectrum of infectivity. However, failure of DHV to propagate in trypsin-treated chick embryo cells is more difficult to explain: trypsinization may have destroyed either a substance essential to virus replication or individual cells in which DHV reproduced selectively. As a check on technic, influenza A virus was passaged successfully at 72 hour intervals through 6 series of similarly prepared trypsinized chick embryo cells. This emphasizes the unusual relationship of DHV to trypsin-treated cells.

DHV has been propagated in chick embryo explant tissues without CPE. Absence of definite CPE in tissue explants infected with mouse hepatitis virus(12-14) and in tissue cells presumably infected with human hepatitis virus(17) indicates the need for other procedures of virus detection. Addition of an essential nutrient to the culture fluid may elicit a CPE, as was done with measles virus (18).

Summary. Duck hepatitis virus (DHV) survived storage for at least 21 days at 37°C. It survived treatment with di-ethyl ether and with fluorocarbon. DHV has been propagated through 25 consecutive passages in tissue cultures prepared with chick embryo explants. It was not propagated in cultures of trypsi-

nized chick embryo cells or in cell lines of mammalian origin.

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Electroencephalographic Evidence of Osmosensitive Elements in Olfactory Bulb of Dog Brain.* (25004)

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Verney and his associates(1,2) presented physiological evidence of the presence, within the bed of internal carotid artery of the dog brain, of elements sensitive to changes in osmotic pressure of blood. Activation of these "osmoreceptors" by hypertonic solutions stimulated release of neurohypophysial antidiuretic hormone (ADH). The precise location of the receptors was unknown, though it was suggested that they might lie within the supraoptic nucleus of the hypothalamus. Studies which employed electroencephalographic (EEG) recording technics in rabbits and in cats(3,4,5,6) have indicated that osmosensitive elements may lie rostral to the hypothalamus in the olfactory system. Characteristic EEG changes were observed in olfactory projection areas following intracarotid injection of hypertonic saline which activated release of oxytocin(4) as well as ADH(5). In a related investigation(6) hypertonic solutions were shown to act independently on hind brain to induce consistent changes in arterial pressure. The vast amount of information assembled by Verney *et al.* in the dog made it desirable to investigate osmoreceptor function in this species with electrophysiological methods.

Methods. A total of 24 mongrel dogs of both sexes were prepared under ether or sodium pentothal (20 mg/kg) anesthesia. Subsequently only a local anesthetic (4% procaine) was employed while animals were immobilized with gallamine triethiodide (Flaxedil) administered intravenously, and maintained with respirator pump. Blood pressure was recorded from femoral artery with a Statham transducer and a Varian inkwriter. Intracarotid injections (1-5 ml) of the following solutions were given through a polyethylene cannula: 0.15 M NaCl (isotonic), 1-1.5 M NaCl (hypertonic), 2 M sucrose and glucose. The scalp was incised and a skull flap removed. Using a stereotaxic instrument

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