pathogenicity for the mouse, and its capability of inducing DNA depolymerization and thymidine catabolism in the susceptible cell are also in keeping with the requirements of an effective oncolytic agent. This possibility is being explored.

Summary. The appearance of a transmissible cytopathic agent during the first blind passage of pooled human "liver" cultures previously infected with 4 acute infectious hepatitis bloods is described. The infectivity of this agent in the "liver" culture was cell-associated. The infective degenerated "liver" cells retained their infectivity for at least 16 weeks at 4°C and 8 weeks at 36°C. Heating for 1 hour at 56°C failed to destroy completely the infectivity of infected mouse fibroblasts. One cycle of freeze-thawing or homogenizing, however, abolished the infectivity with occasional exceptions. Characteristic cytopathology consisted of cytoplasmic granularity, cell rounding followed by diminution of cell volume leading to morphologic picture of shrunken cells. During an intermediate stage of degeneration, characteristic intranuclear basophilic globules were seen regularly; the globules were Feulgen positive. The established cell line was more susceptible to the destructive action of this agent than primary cells. Preliminary immunological study failed to establish the identity of this agent.

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Appearance of Marked DNA-Degrading and Thymine Catabolic Activities in a Human Cell Infected with a Transmissible Agent.* (26559)

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The preceding paper described the history and general properties of this unidentified transmissible cytopathic agent(1). The appearance of DNA-degrading and thymine catabolic activities in infected "liver" cultures is described in this report.

Materials and method. Cell cultures. Roller cultures were used exclusively. The "liver" cells(2) were grown directly on glass in monolayer. Nutrient medium consisted of 5% horse serum, preheated at 56°C for 30 minutes, in Eagle's basal medium fortified with double strength amino acids and the non-essential amino acids at 0.2 mM(3). Certain modifications of this basal medium were also made(4).

C^{14} labelled substrates. Thymidine-2-C^{14}, uridine-2-C^{14}, and uracil-2-C^{14} with specific activity of 6.29, 0.65 and 1.1 mC per mMole respectively, purchased from New England Nuclear Corp., were used at final concentrations of 0.5 μC per ml medium.

C^{14} labelled culture. "Liver" cultures grown in media containing either thymidine-2-C^{14} or uridine-2-C^{14} for 48 to 72 hours, were rinsed 3 times with 2 ml of regular medium, without C^{14} substrate, then allowed to grow for another 24 to 48 hours in the regular media.

Fractionation of C^{14} labelled cell culture. CO_2 was first absorbed with an alkaline trap suspended in the gaseous phase of the culture

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TABLE I. C^{14} Activity in Various Fractions of Uninfected Thymidine-2-C^{14} or Uridine-2-C^{14} Labelled "Liver" Cells.

Cells labelled with	Exp.*	C^{14} activity (cpm) in fractions: Acid soluble	Lipids	RNA	DNA	Protein	CO_2
Thymidine-2-C^{14}	1	3	0	21	885	225	0
	2	66	0	69	2104	738	0
Uridine-2-C^{14}	3	224	4	3537	1025	225	0
	4	1552	0	1134	540	108	2

* In Exp. 1, cells were grown in thymidine-2-C^{14} containing medium for 48 hr; cells were rinsed twice and further propagated in medium without thymidine for 3 more days.
A total of 765,000 cells was fractionated.
In Exp. 2, 2×10^6 cells were fractionated after 48 hr in thymidine-2-C^{14} containing medium.
Exp. 3 is similar to 1 except uridine-2-C^{14} was used and 1.6×10^6 cells fractionated.
Exp. 4 is similar to 2 except uridine-2-C^{14} was used.

(5). The whole culture, medium and cells, was then fractionated into fractions 1 (acid soluble), 2 (lipids), 3 (RNA), 4 (DNA), and 5 (proteins) by the method of Schmidt-Tannhauser-Schneider(6). Preliminary study showed that C^{14} activity of the labelled culture resided almost entirely in the nucleic acids and protein. Subsequently, the culture was merely fractionated into cold acid soluble and insoluble fractions, the latter containing the nucleic acids. In actual experiments, the cells (those adhered on glass) and the medium (including detached degenerating cells and fragment) were fractionated separately.

Infectious inocula. Completely degenerated cultures of "liver" cells infected with the agent were centrifuged at 2000 rpm for 10 minutes in a horizontal centrifuge. The sediment, which contained the infectivity, was washed 3 times with 0.25 M sucrose containing 0.003 M $CaCl_2$, and was resuspended in sufficient sucrose solution so that 0.2 ml would contain sufficient infectivity to cause complete degeneration of a liver culture in 2 or 3 days. Other viral agents used for comparative study were vaccinia, adeno 3, herpes simplex, polio 1 and Coxsackie B1. The vaccinia virus was prepared from the chorio-allantoic membrane of the chick embryo, the other viruses from cultures of HeLa cells. The dosage of these viruses was also adjusted so that 0.2 ml contained sufficient virus to cause complete degeneration of a "liver" culture within 2 or 3 days.

DNA or RNA degradation study. Two of a replicate set of C^{14} labelled cultures were first fractionated (considered zero hour). The remainder were then infected by the various agents. A culture from each set was fractionated on the 24th, 48th, and 72nd hour of experiment. Regular media without C^{14} substrate were used.

Measurement of C^{14} activity. Thin specimens containing 0.5 of each fraction were prepared. Their C^{14} activity was monitored with a gas flow Geiger-Mueller tube of about 15% efficiency. Total time required to register 1000 counts was determined for each sample. The results were expressed as counts per minute above background per culture. Net values less than 20% of the background count (5 or less) were arbitrarily considered insignificant. No attempt was made to remove the trichloracetic acid in the acid soluble fraction. This amount of trichloracetic acid reduced the C^{14} activity of a sample by approximately one-half.

Results. Distribution of radioactivity in various fractions of "liver" cells grown in thymidine-2-C^{14} or uridine-2-C^{14} containing medium. Several experiments were performed to determine if the "liver" cell, like most other cells, utilizes thymidine solely for DNA synthesis and uridine for RNA and DNA syntheses. Results are presented in Table I; C^{14} activity does reside mostly in the RNA and DNA fractions. Of interest is the failure to detect radioactivity in the CO_2 evolved by this cell, suggesting that the mechanism for reductive catabolism of uracil or thymine (7) is inhibited or absent. The C^{14} activity of the protein fraction is believed due to incomplete separation of DNA from protein; that in the DNA fraction of uridine-2-C^{14}

TABLE II. Radioactivity in CO_2, Acid Soluble and Insoluble Fractions of Thymidine-2-C^{14} Labelled "Liver" Cells Treated with Various Viral and Physical Agents.

		Radioactivity* in fractions						
Agent	Day		CO_2	Acid soluble	Acid insoluble		CO_2	Acid soluble	Acid insoluble
None	0	(0) †	0	5	1528	(0) †	2	10	4694
	1	(0)	0	4	1542	(0)	0	14	4756
	2	(0)	0	10	1602				
	3	(0)	0	4	1376	(0)	2	60	3928
The agent	1	(+)	20	23	1398	(++)	516	368	1878
	2	(++)	108	120	698				
	3	(+++)	274	232	210	(+++)	822	434	216
Adeno 3‡	1	(+)	0	8	1658	(+)	2	12	4088
	2	(++)	0	14	1468				
	3	(+++)	1	62	1540	(++)	2	34	3012
Freeze-thawing	1	(+++)	0	6	1328	(+++)	0	22	3490
	2	(+++)	0	4	1262				
	3	(+++)	0	14	1538	(+++)	4	44	2774

* cpm above background per culture.
† 0, +, ++ and +++ signified respectively no, early, advanced and complete degeneration.
‡ Vaccinia, herpes simplex, polio 1 and Coxsackie B1 gave similar results.

labelled cells may be readily explained by the well known conversion of uridylate to thymidylate; while the low activity in the RNA fraction of thymidine-2-C^{14} labelled cells is probably due to a partial hydrolysis of DNA during separation of these 2 nucleic acids(8). It seems reasonable to conclude that, for our purpose, most if not all thymidine is utilized in DNA synthesis, and most or all uridine, in RNA, DNA and acid soluble nucleotides syntheses.

DNA degradation and thymine catabolism. Following infection of thymidine-2-C^{14} labelled cells with the agent, C^{14} activity of the CO_2 and acid soluble fraction increases while that of the acid insoluble fraction decreases in thymidine-2-C^{14} labelled cultures. No such changes have been observed for uninfected healthy cultures, cultures killed by freeze-thawing, cultures nourished in a deficient medium consisting of balanced salt solution minus glucose, and cultures infected by vaccinia, adeno 3, herpes simplex, Coxsackie B1 or polio virus. Results of 2 such experiments are shown in Table II. The apparent loss of total radioactivity of cultures infected by the agent may be due to our inability to collect all the CO_2 evolved and the interference of C^{14} measurement by trichloracetic acid present in the acid soluble fractions.

To determine if the absence of thymine-2-C^{14} to $C^{14}O_2$ conversion in cultures other than those infected by this DNA-degrading agent may be due to an insufficient degradation of DNA, the experiments were repeated using unlabelled culture in thymidine-2-C^{14}, uridine-2-C^{14} or uracil-2-C^{14} containing medium. Again only the CO_2 evolved from cultures infected with the agent shows significant radioactivity.

RNA degradation. When these experiments were repeated using uridine-2-C^{14} labelled cells, vastly different results were obtained. Increases of C^{14} activity in the acid soluble fractions were found under all experimental conditions leading to cell death. The liberation of significant quantity of $C^{14}O_2$ was, however, restricted to cultures infected with the agent. Table III shows the results of such an experiment. Since RNAse has been demonstrated in horse serum, it is possible that the serum RNAse may be responsible for the RNA degradation following cell death. To rule out this possibility, the experiment was repeated using the "liver" cell adapted to grow in a chemically defined medium by Holmes(9). Again, results similar to those presented in Table III were obtained.

Discussion. The appearance of DNA degradative activities in the human "liver" cells following infection with this unidentifiable transmissible cytopathic agent seems well established. These degradative activities consist of conversion of acid insoluble polynu-

TABLE III. Radioactivity in CO_2, Acid Soluble and Insoluble Fractions of Uridine-2-C^{14} Labelled "Liver" Cells Treated with Various Agents.

Agent	Day	Degeneration*	C^{14} activity, cpm/culture, in CO_2	Acid soluble	Acid insoluble
None	0	0	0	60	3522
	1	0	0	72	2654
	3	0	2	256	1800
The agent	1	++	78	138	2094
	3	+++	270	282	512
Adeno 3	1	+	2	74	2868
	3	++	0	172	1825
Freeze-thawing	1	+++	2	322	2214
	3	+++	0	540	1524
Vaccinia†	3	+++	4	742	1232

* 0, +, ++ and +++ represented no, early, advanced and complete degeneration respectively.
† Polio 1, Coxsackie B1, and herpes simplex gave similar results.

cleotides to acid soluble components and the cleavage of thymine with CO_2 formation. It is not known whether such active degradative activities are secondary to cell death although they must be associated with cell death. The real significance is that such degradative activities are seen only in cells killed by this transmissible agent and not in those killed by other agents (*e.g.*, 3 DNA viruses, 2 RNA viruses, freeze-thawing and 1 form of multiple nutrient depletion). The possible presence of low DNAse activity in cultures killed by these various other agents is suggested by the small but fairly consistent increase in radioactivity of the acid soluble fractions.

Study on induced RNA degradation was obscured by the finding of significant conversion of acid insoluble to acid soluble radioactive compounds in uridine-2-C^{14} labelled cultures which had degenerated under a variety of experimental conditions. The RNA degradation in uninfected healthy culture may be best explained by the well known observation that, in most monolayer culture, a varying portion of the total cell population is constantly undergoing degenerative changes.

To our knowledge, such extensive degradation of host DNA into acid soluble nucleotides following infection by any transmissible agents has been reported for a few bacterial viruses(10). Induction of reductive catabolism of thymine and uracil in a host cell by a transmissible agent has hitherto not been described. The exact mechanism of these induced changes and the nature of this transmissible agent are currently being investigated.

Summary. Following infection by a transmissible cytopathic agent and with the appearance of degeneration, the following were regularly observed for thymidine-2-C^{14} labelled human "liver" cultures: 1. progressive increase in quantity of $C^{14}O_2$ liberated, 2. progressive increase in radioactivity of the acid soluble fractions, and 3. progressive decrease in radioactivity of the acid insoluble fractions. Similar changes had not been demonstrated in uninfected culture, culture killed by freezing or by nutrient depleting, and cultures infected with vaccinia, adeno 3, herpes simplex, polio 1, or Coxsackie B1 viruses. Significant conversion of acid insoluble to acid soluble radioactive compounds was repeatedly demonstrated in uridine-2-C^{14} labelled "liver" cultures under all the described experimental conditions leading to cell degeneration. Formation of $C^{14}O_2$ from uracil-2-C^{14} or uridine-2-C^{14} was, however, restricted to cultures treated with this DNA-degrading agent.

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