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Uptake *in Vitro* of Nucleic Acid Precursors and Nucleic Acids by Zajdela Ascitic Hepatoma Cells

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Summary. Zajdela ascitic hepatoma cells are shown to take up pyrimidine bases at much lower rates than obtained in slices from normal rat liver. The rates of uptake of adenine and uridine by the Zajdela cells are, however, as high as in the slices. Like the slices, again, the Zajdela cells take up *E. coli* RNA and DNA at very low rates but, unlike the slices, these cells degrade rapidly the RNA taken up. The Zajdela cells resemble parenchymal cell suspensions derived from normal rat liver in regard to the uptake of pyrimidine bases and the ability to degrade heterologous RNA.

In the preceding paper (Kumar & Bhargava, 1975) we showed that in liver parenchymal cells, the rates of uptake of certain nucleic acid precursors and macromolecules depended on the state of organization of the cells. Since the normal pattern of intercellular organization is altered in malignant transformation, we have now compared the ability of the Zajdela ascitic hepatoma (a tumor of rat liver parenchymal cells) and of normal liver slices to take up orotic acid, uracil, adenine, uridine, RNA and DNA. We have found that the Zajdela cells behave more like liver parenchymal cells in suspension than like the organized tissue (the slices) with respect to the ability to take up orotic acid and uracil, but more like the organized tissue in regard to the uptake of adenine and nucleic acids.

Materials and Methods

Slices

Slices (500–750 μ thick) were cut free-hand with a razor blade from the livers of adult female albino Wistar rats of SPF strain originating from TNO, Netherlands.

Zajdela Ascitic Hepatoma

The Zajdela ascitic hepatoma (strain C) was originally derived from a spontaneous ascitic tumor of rat, obtained on chronic administration of dimethylaminoazobenzene (Zajdela, 1964); at the time these experiments were begun, it had gone through more than

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950 passages. Anatomopathological and cytological examination has shown that the tumor is derived from parenchymal cells.

The tumor cells used were obtained seven days after transplantation of 0.5 ml of a 7-day-old tumor without removal of the ascitic fluid. The tumor-bearing animal was killed by cervical dislocation, and the tumor cell suspension filtered through cloth and centrifuged at $150 \times g$ for 1–2 min. The ascitic fluid was decanted and the cells washed 2–3 times with 0.02 M Tris buffer (pH 7.4) containing 0.25 M sucrose, each time with a volume equal to the volume of the original ascitic fluid; this washing freed the tumor of contaminating blood cells. The washed tumor cells were suspended in the incubation medium to the desired cell concentration; cell count was made on a hemocytometer.

Usually, 30–50 ml of the tumor cell suspension containing approximately 20×10^6 cells/ml were obtained from one animal. The mitotic index of the cells used did not exceed 0.2%. The cells were usually found in clusters of 1 to 6 (average 2.4) cells. Viability tests (with eosine and negrosine) showed that the tumor contained less than 0.1% dead cells. Whenever tumor cells from one animal were insufficient, the cells from several animals were pooled.

The average dry weight of the cells was determined by suspending a known number of cells in a known volume of 0.9% NaCl, drying to a constant weight and subtracting the weight of the salt.

Homogenates

Homogenates (1:5) of both slices and Zajdela cells were prepared in a Potter-Elvehjem homogenizer. The proportion of the Zajdela cells disrupted varied and was determined by cell count.

Radioactive Materials

^{14}C-6-orotic acid, ^{14}C-2-uracil, ^{14}C-8-adenine, ^{3}H-5-orotic acid, ^{3}H-5-uracil, ^{3}H-2-adenine and ^{3}H-5-uridine were obtained from C.E.A., Saclay, France. The specific activities are given in the legends to the Tables and Figures.

Buffers

Krebs-Ringer's bicarbonate (KRB) buffer (Dawson, Elliott, Elliott & Jones, 1959) was used for the studies on the uptake of bases and nucleosides; it was aerated with water-saturated 95% air +5% CO_2 during incubation. Viability tests (trypan blue, eosine and negrosine) made on the Zajdela cells after 3 hr of incubation *(see below)* in this buffer, showed no loss of viability. For experiments on uracil catabolism, 0.02 M Tris–0.25 M sucrose buffer (pH 7.4), and for studies on the uptake of nucleic acids, KRB buffer containing 15 mM glucose were used.

Uptake of Bases and Nucleosides

Liver slices of a known wet weight, or a known number of Zajdela cells, were incubated in KRB buffer (3.0–5.3 ml) at 37 °C in either 25–50 ml Erlenmeyer flasks or in 20 ml flat-bottom plastic bottles, with shaking in a Warburg bath or in an air incubator. The material of which the transport was to be studied was added in 0.02–0.3 ml of 0.9% NaCl or KRB buffer, just before the commencement of incubation. A separate container was used for each time point.

At the desired time, the sample was chilled, 0.1–0.2 ml of a solution containing a high concentration of the unlabeled substrate in 0.9% NaCl was added, and the mixture was transferred quantitatively to a 10 ml centrifuge tube. The cells/slices were sedimented by centrifugation at low speed for 2–3 min, the incubation medium was decanted and the cells/slices washed rapidly 7 or 8 times in the cold, by centrifugation, each time with 5 ml of KRB buffer containing the unlabeled substrate. In several experiments, *all* the washings were counted; the sixth wash contained less than 0.1% of the radioactivity found in the cells/slices after this wash. Sometimes, the slices were washed in the cold by decantation which took only a few minutes, rather than by centrifugation which took longer; both methods were equally effective in freeing the slices of the radioactive incubation medium, and led to an identical content of radioactivity in the tissue.

For determination of the total uptake of nucleic acid bases and nucleosides, the washed slices/cells were treated with 5 ml of 5% trichloroacetic acid (TCA); the slices were disintegrated either with a glass rod or by homogenization. The mixture was then heated for 30 min at 98 °C, cooled, centrifuged and the supernatant collected. The residue was washed twice, each time with 3–5 ml of 5% TCA; the washings were combined with the original TCA extract. The radioactivity in the residue at this stage was less than 1% of that in the combined supernatants; the latter radioactivity was taken to be a measure of the total uptake (combined uptake in the acid-soluble and the acid-insoluble fractions).

To determine the distribution of the total radioactivity taken up in the acid-soluble and the acid-insoluble fractions, the initial precipitation with TCA was done in the cold. The mixture was centrifuged after 15–30 min in the cold, the supernatant collected and the sediment washed twice with 1–3 ml of 5% TCA. The washings were combined with the first supernatant to give the acid-soluble fraction. The residue was washed successively with ethanol, ethanol-ether (3:1) at 50 °C, and then finally with ether; it was dried and weighed, and is referred to as the "lipid-free TCA precipitate". Sometimes, the residual TCA was removed by ether from the 5% TCA-washed TCA precipitate, *without* prior washing with the lipid solvents; the precipitate was dried and weighed as above and is referred to as the "TCA precipitate". This precipitate (or the lipid-free TCA precipitate) constituted the acid-insoluble fraction.

To estimate the radioactivity in purified RNA, the cells/slices, after washing as above, were suspended at room temperature in 2.5–5.0 ml of acetate buffer (0.05 M, pH 5.1) containing 1 mM EDTA, 0.5% sodium dodecyl sulfate and 5 μg/ml of polyvinyl sulfate. While the Zajdela cells disintegrated immediately in this buffer, the slices had to be homogenized in a Potter-Elvehjem homogenizer to obtain complete disintegration. Total cellular RNA was then extracted and purified by dialysis as described by Raj and Rao (1969). RNA was estimated by measurement of absorbance at 260 nm (one optical density unit was taken to be equivalent to 40 μg of RNA).

Estimation of Protein

The lipid-free TCA precipitate was dissolved in 1–2 ml of 0.6 or 1.2 N NaOH and protein estimated by Folin's reagent (Fiszer, 1964).

Autoradiography

For autoradiography, the Zajdela cells, after incubation with the labeled base or nucleoside and removal of the incubation medium by centrifugation, were washed 3 times with KRB buffer and resuspended in this buffer. A drop of the cell suspension was put on a round microscope cover slip. Even spreading of the cells was obtained by centrifugation

following placement of the cover slip at the bottom of a specially designed centrifuge tube (F. Zajdela, *unpublished*). The cells were then fixed with methanol and rinsed with acetone. The cover slips were mounted onto the slides with "Permount" (Fisher Scientific Co., Pittsburgh, Pa.), coated with Illford K5 emulsion, and developed 48 or 120 hr later. The silver grains were counted over 20–50 separate microscopic fields. The background was negligible (less than 2 grains/400 μ^2). After development of the autoradiograph, the cells were stained with methyl green-pyronin in 0.1% phenol.

Uracil Catabolism

Oxidation of the 2-carbon of ^{14}C-2-uracil to $^{14}CO_2$ was studied essentially as described by Jacob and Bhargava (1964). The liver slices (known wet weight) or the Zajdela cells (known number) in 4 ml of the Tris-sucrose buffer, or 4 ml of a homogenate of the slices or of the Zajdela cells in the above buffer, was incubated at 37 °C with the labeled uracil in a Warburg flask, the center well of which contained 0.4 ml of freshly prepared 20% KOH. After the specified time, the reaction was stopped with 1 ml of 10% TCA, the alkali from the center well was removed quantitatively, made up to 10 ml with water and an appropriate aliquot counted. The radioactivity recovered in the alkali represented the extent of oxidation of the labeled uracil.

Uptake of Nucleic Acids

^{32}P-labeled total *E. coli* RNA and DNA were prepared as described earlier (Shanmugam, 1968; Shanmugam & Bhargava, 1969). The cells/slices were incubated in plastic bottles at 37 °C with the stated amount of labeled nucleic acid, in a total volume of 5 ml of the phosphate buffer. After the specified period, the cells/slices were washed as mentioned earlier, and treated with 10 ml of 5% TCA for 30 min at 95–98 °C. The mixture was centrifuged and the supernatant collected. Radioactivity and ribose or deoxyribose [depending on whether the uptake of RNA or DNA was studied; ribose by the method of Slater (1958), and deoxyribose by that of Burton (1956)] were estimated, and the total uptake and the RNA or DNA content calculated. The sediment (TCA precipitate) was washed once with 5 ml of 5% TCA and then twice with 20 ml of ether each time, dried in the oven and weighed.

Measurement of Radioactivity

Measurement of radioactivity was made either in a Tracerlab gas-flow solid G.-M. counter using planchets and having an efficiency of 20% for ^{14}C, or in a Packard liquid scintillation counter having an efficiency of 40% for ^{3}H, 80% for ^{14}C and 100% for ^{32}P. The insoluble materials (TCA precipitate or lipid-free TCA precipitate) were counted in solution in 0.6 N or 1.2 N NaOH in the liquid scintillation counter. Appropriate corrections were made for self absorption and quenching. The 0 hr values have not been subtracted, but given in each case.

Results

Uptake of Orotic Acid

The total uptake of orotic acid by normal liver slices was much higher than that by the Zajdela tumor cells (Table 1); on a cell-to-cell basis, the uptake in the slices was approximately 30 times more than in the Zajdela cells. The specific activity of the total cellular RNA as well, follow-

Table 1. Total uptake of orotic acid, uracil, adenine and uridine by normal rat liver slices and the Zajdela ascitic hepatoma cells[a]

Precursor	Cell preparation	Wet wt[b] (mg)	Cell no.[c] (millions)	Amount taken up per 10^6 cells (pmoles)[d]			
				0 hr	0.5 hr	1 hr	3 hr
^{14}C-orotic acid	Slices	262–283	21.2–22.9	4.7 (0.3)	44.2 (2.6)	56.5 (3.1)	104.0 (5.8)
^{14}C-orotic acid	Zajdela cells	—	148	0.5 (0.2)	1.3 (0.5)	2.0 (0.7)	3.1 (1.2)
^{14}C-uracil	Slices	257–274	20.8–22.2	0.8 (0.05)	—	1.6 (0.1)	1.7 (0.1)
^{14}C-uracil	Zajdela cells	—	148	0.3 (0.1)	2.0 (0.9)	—	3.6 (1.5)
^{14}C-adenine	Slices	231–275	18.7–22.3	15.9 (0.7)	376 (20.0)	479 (24.6)	176 (9.8)
^{14}C-adenine	Zajdela cells	—	148	2.8 (1.0)	151 (55.8)	208 (76.8)	134 (49.7)
^{3}H-uridine	Slices	249–279	20.2–22.6	1.0 (0.1)	17.2 (1.3)	—	15.0 (1.1)
^{3}H-uridine	Zajdela cells	—	148	0.4 (0.2)	61.1 (30.1)	62.3 (30.8)	47.2 (23.3)

[a] The slices or the Zajdela cells were incubated with ^{14}C-orotic acid (38.4 nmoles, 1.26×10^6 cpm), ^{14}C-uracil (34.2 nmoles, 1.20×10^6 cpm), ^{14}C-adenine (40.0 nmoles, 2.29×10^6 cpm), or ^{3}H-uridine (30.0 nmoles, 152×10^6 cpm), in 5 ml of KRB buffer for the specified period. The cells and the slices were then washed free of the incubation medium, treated with hot TCA, and the radioactivity estimated in the hot TCA supernatant as described in the text.

[b] Slightly different wet wt of slices was used for each time point.

[c] Calculated on the basis of the data of Iype, Bhargava and Tasker (1965) (nonparenchymal cells which constitute less than 10% of the dry weight of the parenchymal cells in liver, were not included).

[d] Values in parentheses are percentages of the total radioactivity initially put in the incubation medium that was taken up by the total quantity of the cells/slices incubated.

ing uptake of ^{14}C-orotic acid for 2 hr (beyond which no increase in the specific activity of RNA was obtained in the case of either cell preparation), was eightfold higher in the slices than in the Zajdela cells (Fig. 1). The acid-soluble radioactivity per mg of the TCA precipitate in the slices was, at 1 hr, 7 times greater than in the Zajdela cells (Table 2); this difference decreased with time but persisted. Autoradiography of the cells (Fig. 2 and Table 3) following uptake of the nucleic acid precursors, confirmed that the uptake of orotic acid by the Zajdela cells was very low, say

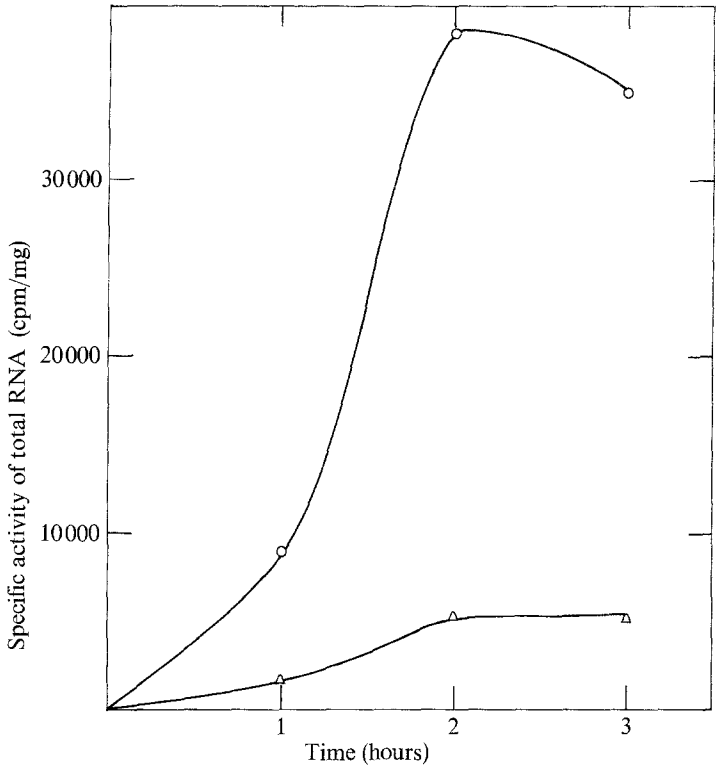

Fig. 1. Comparison of the incorporation of ^{14}C-orotic acid into RNA in normal rat liver slices and the Zajdela ascitic hepatoma cells. Normal liver slices (215–273 mg wet wt) or the Zajdela cells (19×10^6) were incubated in 5 ml of KRB buffer with 2.2×10^6 cpm (0.085 μmole) of ^{14}C-orotic acid for the specified period. The cells and the slices were then washed free of the incubation medium and RNA isolated by phenol extraction and purified as described in the text. ○, slices; △, Zajdela cells

in comparison to that of uridine which was about the same as in the slices *(see later)*. The reported inability of certain tumors to incorporate orotic acid into their RNA (Hurlbert & Potter, 1952) could be due to their inability to transport orotic acid.

Uptake of Uracil

Table 1 shows that the uptake of ^{14}C-2-uracil by the Zajdela cells was about the same as that of orotic acid. The accumulation of labeled uracil in the acid-soluble pool of the Zajdela cells also followed, both qualitatively and quantitatively, the same pattern as was obtained with

Table 2. Accumulation of adenine, uracil and orotic acid in the acid-soluble fractions of normal liver slices and the Zajdela ascitic hepatoma cells[a]

Time of incubation (hr)	Radioactivity in the acid-soluble fraction (cpm/mg lipid-free TCA ppt.)[b]					
	Adenine		Uracil		Orotic acid	
	Slices	Zajdela cells	Slices	Zajdela cells	Slices	Zajdela cells
0	1,009 (112)	1,644 (85)	11 (54)	120 (21)	40	10
1[c]	40,320 (5,502)	93,880 (15,330)	149 (111)	1,036 (965)	1,262	180
2	51,160	79,570	153	1,480	1,471	375
3	44,030	72,260	257	2,108	1,470	552

[a] The slices (258–270 mg wet wt, equivalent to 33.8–35.4 mg dry wt of lipid-free TCA ppt., in the case of adenine and uracil; 212–217 mg wet wt in the case of orotic acid) or the Zajdela cells (100×10^6 cells, equivalent to 83.7 mg dry wt of lipid-free TCA ppt., in the case of adenine and uracil; 19×10^6 cells in the case of orotic acid) were incubated in 5 ml of KRB buffer with 9.9×10^6 cpm (0.1 µmole) of ^{14}C-adenine, 9.9×10^6 cpm (0.085 µmole) of ^{14}C-uracil, or 2.2×10^6 cpm (0.085 µmole) of ^{14}C-orotic acid, for the specified period. The cells and the slices were then washed free of the incubation medium, treated with TCA, and the acid-soluble fraction and the acid-insoluble fraction (TCA ppt.) obtained as described in the text.

[b] The TCA ppt. was not freed of lipids in the case of orotic acid. Values in parentheses are specific activities (cpm/mg) of the lipid-free TCA ppt.

[c] For adenine, the acid-soluble and the acid-insoluble radioactivity taken together, represented 15 and 92% of the radioactivity put in the incubation medium in the case of the slices and the Zajdela cells, respectively.

orotic acid (Table 2). It would, therefore, appear that the uptake of uracil by the Zajdela cells was, like that of orotic acid, low. This view is supported by the results of autoradiography following uptake of ^{3}H-uracil (Table 2, Fig. 3).

The total radioactivity (or the radioactivity in the acid-soluble and the acid-insoluble fractions taken separately) in normal liver slices following uptake of labeled uracil for various periods, was lower than that in the Zajdela cells (Tables 1 and 2). The comparison is, however, misleading as fresh liver slices are known to oxidize the 2-carbon of uracil (Rutman, Cantarow & Paschkis, 1954; Canellakis, 1957) to carbon dioxide very rapidly. Therefore, for a valid comparison of the ability of the liver slices and the Zajdela cells to take up uracil, it would be necessary to have, concurrent with the data of Table 1, information on the relative ability of the two cell systems to oxidize uracil. Table 4 shows that on a per cell basis, the ability of the normal parenchymal cells in the slices to

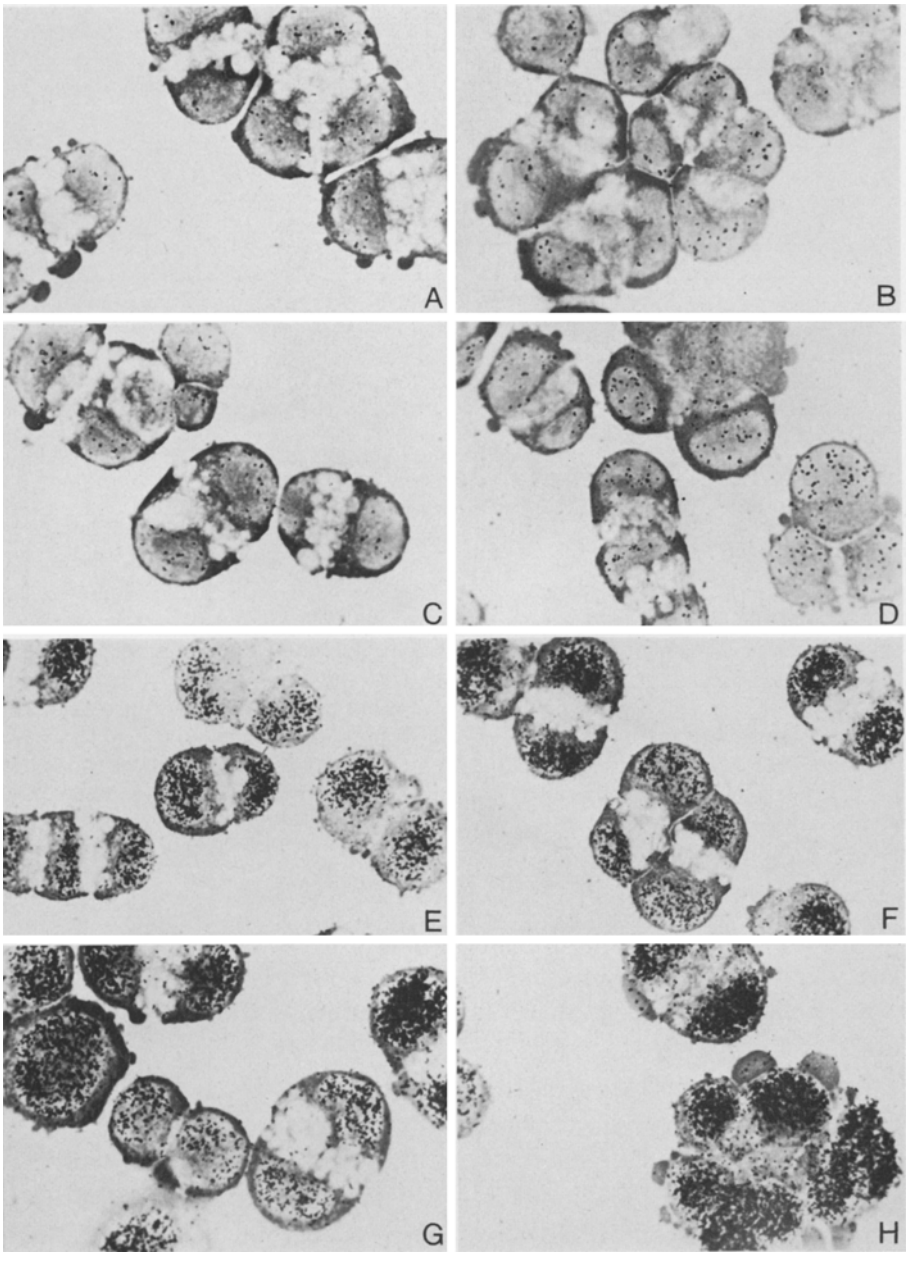

Fig. 2. Autoradiography of the Zajdela ascitic hepatoma cells following incubation with ^{3}H-labeled uracil, orotic acid, adenine or uridine for 30 min or 1 hr. For details, *see* legend to Table 3; the exposure time for the autoradiographs was 2 days (*E, F, G, H*) or 5 days (*A, B, C, D*). *A, B,* uracil; *C, D,* orotic acid; *E. F,* adenine; *G, H,* uridine. *A, C, E, G,* 30 min; *B, D, F, H,* 1 hr. *I,* phase-contrast micrograph of three clusters of unexposed cells in the incubation medium

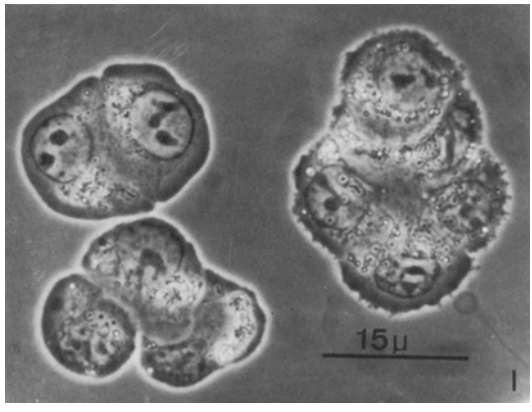

Table 3. Relative incorporation of tritiated orotic acid, uracil, adenine and uridine into acid-insoluble material in the Zajdela ascitic hepatoma cells as shown by autoradiography[a]

^{3}H-precursor	Time incubation (hr)	Grains/cell	
		At 2 days	At 5 days
Orotic acid	0.5	0–9	22
	3	0–36	52
Uracil	0.5	0–7	22
	3	0–22	41
Adenine	0.5	112	>200
	3	>200	>200
Uridine	0.5	123[b]	>200[b]
	3	138[b]	>200[b]

[a] The Zajdela cells (148×10^6) were incubated in 5 ml of KRB buffer with ^{3}H-orotic acid (120 μCi; sp. act. 20 Ci/mmole), ^{3}H-uracil (600 μCi; sp. act. 25 Ci/mmole), ^{3}H-adenine (600 μCi; sp. act. 19 Ci/mmole), or ^{3}H-uridine (300 μCi; sp. act. 10 Ci/mmole), as in Table1. The cells were then washed, spread on round cover slips, and autoradiographed as described in the text. Two sets of slides were prepared for each time point of each precursor for counting of grains after 2 days and 5 days. The data given are the average (range in the case of orotic acid and uracil) for 20–50 cells. More than 200 grains/cell could not be counted.
[b] For purposes of comparison with other precursors, the values given for uridine should be multiplied by two as the specific activity of ^{3}H-uridine used was about half of that of the other precursors.

oxidize labeled uracil was several thousand times greater than that of the Zajdela cells. Further, the amount of uracil oxidized by the slices (Table 4) was 20–100 times more than the uracil taken up by the Zajdela cells (Table 1). These results, taken together, show that the uptake of

Table 4. Comparison of uracil oxidation by the Zajdela ascitic hepatoma cells and normal liver slices[a]

Expt. no.	Time of incubation (hr)	^{14}C-uracil oxidized (nmoles/10^8 cells)[b]			
		Zajdela cells[c]	Slices[d]	Zajdela cells homogenate[c]	Slices homogenate[d]
1	4.0	0.009 (0.072)	35.7 (43.9)	0.139 (1.16)	6.91 (8.46)
2	2.0	—	—	—	4.30 (5.48)
	4.0	0.002 (0.016)	22.4 (28.5)	—	7.38 (9.53)
3	0	0.010 (0.030)	0.011 (0.033)	—	—
	1.5	0.009 (0.026)	2.67 (7.84)	—	—
	3.0	0.007 (0.020)	6.74 (19.8)	—	—
4	3.0	0	—	0.026 (0.075)	—

[a] The cells or the slices (or their homogenates) were incubated in 4 ml of the Tris-sucrose buffer with ^{14}C-2-uracil [$2{,}245 \times 10^3$ cpm (17 nmoles) in Expts. 1 and 2, and $4{,}490 \times 10^3$ cpm (34 nmoles) in Expts. 3 and 4] in a Warburg flask containing 0.4 ml of 20% KOH in the center-well. At the desired time, the alkali in the center-well was removed quantitatively by a Pasteur pipette, made up to 10 ml and a 0.5–1.0 ml aliquot counted as described in the text.

[b] Values in parentheses represent the percentage of ^{14}C-uracil oxidized by the total tissue/cell preparation.

[c] 142, 120, 45 and 105×10^6 cells were used, as such or after homogenization, for each time point in Expts. 1, 2, 3 and 4, respectively; 100×10^6 cells yielded approximately 96 mg dry wt of the TCA ppt. or 72 mg of protein. The homogenate contained 20% broken cells in Expt. 1 and 95% in Expt. 2.

[d] 203–271 mg wet wt of the slices was used, as such or after homogenization, for each time point (100 mg wet wt was equivalent to approximately 20 mg dry wt of the TCA ppt. or 17 mg of protein). One gram wet wt of liver is taken to contain 81×10^6 parenchymal cells (Iype *et al.*, 1965); it is assumed that the oxidation of uracil to CO_2 in liver is carried out only by the parenchymal cells.

uracil by the Zajdela cells is very small when compared to that by the parenchymal cells in normal liver.

Homogenization of Zajdela cells resulted in a significant increase in their ability to oxidize uracil, even though the level of oxidation achieved was much lower than that obtained with the homogenate of normal liver (Table 4). It would, therefore, seem that the Zajdela cells contain the

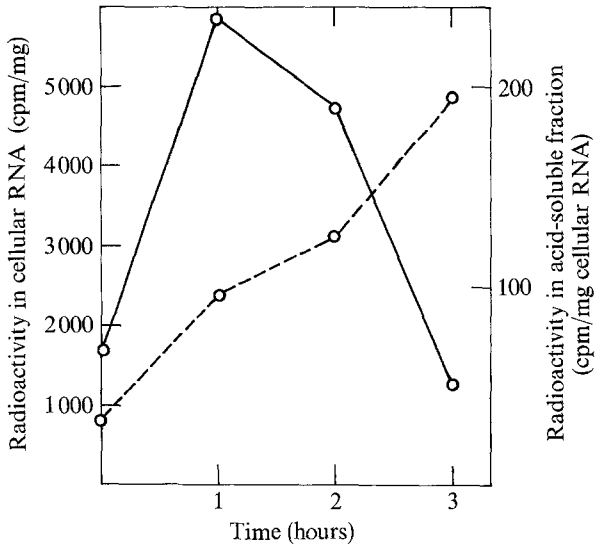

Fig. 3. Uptake of highly polymerized *E. coli* RNA by the Zajdela ascitic hepatoma cells. The Zajdela cells (135×10^6, yielding 3.54 mg RNA) were incubated with ^{32}P-labeled *E. coli* RNA (2.12 mg, 28.4×10^6 cpm) in 5 ml of KRB buffer for the specified period. The cells were then washed free of the incubation medium, and the cellular RNA and the acid-soluble fraction isolated as described in the text. o——o, cellular RNA; o-----o, cellular acid-soluble fraction

enzyme system for oxidizing the 2-carbon of uracil to CO_2 but are unable to do so because of a block in the transport of uracil; this may be true also of the other tumors which have been shown to be incapable of oxidizing uracil (Canellakis, 1957; Heidelberger, Leibman, Harbers & Bhargava, 1957). Since the ability to oxidize the 2-carbon of uracil to CO_2 is, as far as is known, a characteristic of liver, this investigation further substantiates the view that the Zajdela ascitic tumor is a tumor of liver cells.

Uptake of Adenine

The total uptake of adenine by the Zajdela cells was of the same order as that by liver slices (Table 2), and much higher than that of uracil or orotic acid. Both cell systems showed a remarkable capacity to concentrate adenine. In one experiment (Table 2), 10^8 Zajdela cells (equivalent to 83.7 mg dry weight of TCA precipitate) were able to remove 92% of the labeled adenine (0.1 μmole) from the incubation medium in 1 hr. This ability accounts for the fact that the total uptake of adenine as well as the amount present in the acid-soluble and the acid-insoluble fractions taken separately, reached a peak at 1 hr and then fell, both in normal liver and in the Zajdela cells (Tables 1 and 2).

Table 5. Comparison of the incorporation of ^{3}H-uridine into RNA in normal rat liver slices and the Zajdela ascitic hepatoma cells[a]

Time of incubation (hr)	Sp. activity of RNA (cpm/mg)	
	Slices	Zajdela cells
0	191	269
1	923	21,060
2	1,221	24,040
3	1,331	22,930

[a] Normal liver slices (223–232 mg wet wt) or the Zajdela cells (30×10^6) were incubated in 5 ml of KRB buffer with 88×10^6 cpm (0.01 μmole) of ^{3}H-uridine for the specified period. The cells and the slices were then washed free of the incubation medium, and RNA isolated by phenol extraction and purified, as described in the text.

Autoradiography of the Zajdela cells following uptake of labeled adenine also showed that these cells were capable of incorporating adenine into nucleic acid at considerably higher rates than those obtained with uracil or orotic acid (Table 3 and Fig. 2).

Uptake of Uridine

Table 1 shows that uridine is taken up efficiently by liver slices, the rate of its uptake, however, being less than that of orotic acid. The ability of Zajdela cells to take up uridine (Table 1), particularly to incorporate it into RNA (Table 5), was much higher in comparison to the slices. That uridine was an excellent precursor of RNA in Zajdela cells—in fact better than adenine—was also shown by autoradiography (Table 3 and Fig. 2). Ehrlich ascites tumor cells have also been shown to take up ^{3}H-uridine very efficiently, 25–52% of the exogenous precursor being removed from the medium by the cells in 1 hr (Harwood & Itzhaki, 1973).

Comparison of the Rates of Uptake of the Four Nucleic Acid Precursors

In the case of liver slices, the total uptake was the highest for adenine, followed, in decreasing order, by uracil, orotic acid and uridine (Table 1). Adenine was also the best precursor for labeling of RNA in the slices and was followed, in decreasing order, by orotic acid, uridine and uracil (the incorporation of uracil into RNA being very small in normal liver due to its rapid oxidation) (Table 3 and Fig. 2). In the case of Zajdela cells too, adenine was taken up at the highest rate, and was followed,

in decreasing order, by uridine, orotic acid and uracil, the uptake of the last two precursors being small (Table 1). Uridine was, however, incorporated into RNA by the Zajdela cells better than adenine; the incorporation of orotic acid and uracil into RNA in these cells was small (Table 3 and Fig. 2).

Uptake of Nucleic Acids

Figs. 3 and 4 show that Zajdela cells can take up both RNA and DNA and accumulate them in macromolecular form in the cells. As was observed in liver cells in suspension (Shanmugam & Bhargava, 1966, 1969; Bhargava & Shanmugam, 1971), the initial rate of uptake (the "zero hour" uptake) of both types of nucleic acids was high in comparison to the subsequent uptake.

The amount of *E. coli* RNA found within the cells in macromolecular form increased up to 1 hr, beyond which it decreased rapidly, suggesting that after 1 hr the rate of degradation of the RNA taken up exceeded the rate of its uptake. It would also be clear from the data of Fig. 3 that only a part of the degradation products were retained within the

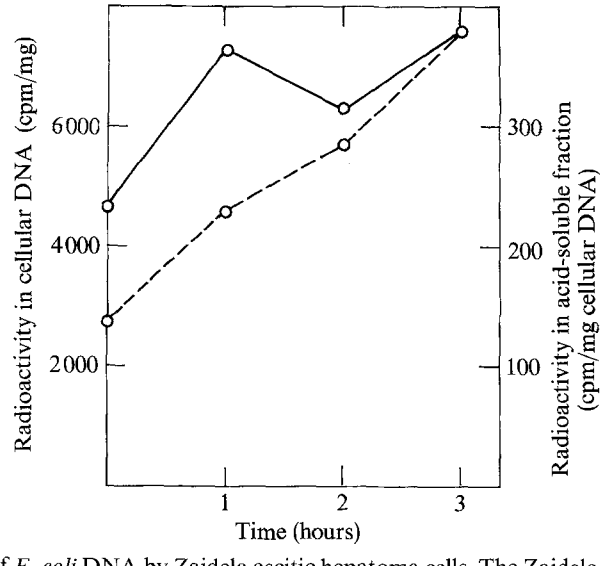

Fig. 4. Uptake of *E. coli* DNA by Zajdela ascitic hepatoma cells. The Zajdela cells (135×10^6, yielding 1.52 mg DNA) were incubated with ^{32}P-labeled *E. coli* DNA (1.20 mg, 15.3×10^6 cpm) in 5 ml of KRB buffer for the specified period. The cells were then washed free of the incubation medium, and the cellular DNA and the acid-soluble fraction isolated as described in the text. o——o, cellular DNA; o-----o, cellular acid-soluble fraction

Table 6. Comparison of the rates of uptake of *E. coli* DNA and RNA by normal liver cells in suspension and the Zajdela ascitic hepatoma cells

	RNA		DNA	
	Liver cells	Zajdela cells	Liver cells	Zajdela cells
Concentration of nucleic acid in the medium (μg/ml)	167–333	425	150–450	250
Cell concentration ($\times 10^6$/ml)	2–4	27	2.3	27
Duration of uptake (min)[a]	2.5	60	180	180
Nucleic acid taken up:[b]				
(*a*) % of input nucleic acid	5.5–16.2	0.08	1.2	0.081
(*b*) μg/10^6 cells	4.9–13.5	0.012	1.2	0.0072
(*c*) % of host cell nucleic acid	5.8–16.8	0.05	6.7	0.064

[a] Period of maximum uptake in the case of RNA.
[b] The data for liver cells in suspension is from Shanmugam and Bhargava (1968), Bhargava and Shanmugam (1971), and Kumar and Bhargava *(unpublished observations)*.

cells. In these respects, the Zajdela cells resembled primary liver cell suspensions (Shanmugam & Bhargava, 1966, 1969).

The amount of *E. coli* DNA found within the cells in macromolecular form reached a plateau at about 1 hr (Fig. 4); the amount of acid-soluble radioactivity in the cells, derived from the donor DNA taken up, represented less than 10% of the radioactivity in macromolecular form. In the case of liver cells in suspension, no degradation of *E. coli* DNA taken up was detected (Bhargava & Shanmugam, 1971, and *unpublished*).

A comparison of the capacity of the Zajdela cells to take up heterologous nucleic acids with that of normal liver parenchymal cells in suspension and of normal liver slices, showed that macromolecular nucleic acids were taken up poorly by the Zajdela cells in comparison to the cells in suspension (Table 6). It is also of interest to note that the rate of transport of macromolecular nucleic acids into the Zajdela cells is probably the lowest reported for any ascitic tumor cell [compare the data of Table 6 with that compiled in the review by Bhargava and Shanmugam (1971); *also see* Crooke, Okada and Busch (1971), and Okada and Busch (1972)].

Discussion

For reasons given in the preceding paper, the comparison made in the present study between liver slices and a homologous tumor, the Zajdela ascitic hepatoma, should be of value in spite of the limitations inherent

in the use of slices, lack of kinetic analysis of the uptake in these studies, and other considerations discussed earlier (Kumar & Bhargava, 1975).

This investigation shows that the Zajdela ascitic hepatoma cells have largely lost the ability to take up orotic acid and uracil and, in this respect, resemble primary cell suspensions from normal rat liver. This investigation, however, does not indicate whether the mechanisms underlying the loss of the ability to transport these two nucleic acid precursors are similar in the case of the two cell types.

On the other hand, the Zajdela cells resemble liver slices in their ability to take up adenine and uridine. The extraordinary ability of both normal liver and the Zajdela cells to concentrate adenine and to incorporate it into RNA could explain the reported repression of purine biosynthesis by adenine in mammalian hepatoma cells (Martin & Owen, 1972). The Zajdela cells show greater resemblance to the liver slices also in regard to the quantitative aspect of transport of macromolecular RNA and DNA; only trace amounts of the nucleic acids were transported. On the other hand, they resemble liver cells in suspension in regard to the ability to rapidly degrade the RNA taken up.

The observations suggest that some—not all—of the permeability changes accompanying malignant transformation may be similar to those obtained on dispersion of a tissue to a single cell suspension. As the loss of the ability to transport pyrimidine bases on dispersion of liver tissue to a single cell suspension and on malignant transformation of liver cells, may have the same underlying cause, further studies on primary cell suspensions may provide useful information on malignant transformation.

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