J. Membrane Biol. 26, 31–41 (1976)

A Comparison of the Ability of Normal Liver, a Premalignant Liver, a Solid Hepatoma and the Zajdela Ascitic Hepatoma, to Take up Amino Acids *in Vitro*

P.M. Bhargava*, D. Szafarz, C.A. Bornecque, and F. Zajdela

Fondation Curie–Institut du Radium, Unite 22 INSERM,
Faculte des Sciences, Batiment 110, 91405-Orsay, France

Received 7 December 1973; revised 15 August 1975

Summary. The net total uptake of several amino acids at low (0.8–3.1 μmoles/liter) as well as high (800–1200 μmoles/liter) extracellular concentrations, by normal rat liver, a premalignant liver, a solid hepatoma, and the Zajdela ascitic hepatoma cells, has been compared under conditions in which protein synthesis continues. At low amino acid concentrations, the initial (3 min) total uptake of the various amino acids in the Zajdela cells, was 3–10 (average 7) times more, and the intracellular concentration of the labeled amino acids taken up 14–45 (average 31) times more, than in normal liver. At the high amino acid concentrations, the total uptake in the Zajdela cells, at 60–120 min was 2–5 (average 3.5) times more, and the intracellular concentration of the amino acids taken up 8–18 (average 13) times more, than in normal liver; the corresponding values for the premalignant liver and the solid hepatoma were in between those for normal liver and the Zajdela cells. Further, the rate of the total uptake of amino acids, their intracellular concentration, the proportion of the amino acid taken up utilized for protein synthesis, the rate of incorporation of the amino acid taken up into protein, and the cellular growth rate, seemed to be correlated in the four cell/tissue preparations studied. In most cases, the rate of the net uptake fell drastically with time, the uptake virtually stopping after 90–180 min, probably due to lack of serum in the incubation medium.

In an accompanying communication (Bhargava, Allin & Montagnier, 1976), it is shown that when resting BHK cells are triggered into division, there is a several-fold increase in the rate of the net total uptake of amino acids by the time the cells divide. It seemed, therefore, to be of interest to compare the rates of the total uptake of amino acids in normal liver (comprised mostly of resting cells) with those in hepatomas (containing dividing cells). In this paper we show that the total uptake of amino acids in the Zajdela ascitic hepatoma cells *in vitro* is, on an average, threefold that in normal liver, irrespective of whether the amino acids are present in the incubation medium in trace (0.8–

* *Present address:* Regional Research Laboratory, Hyderabad 500009, India.

3.1 nmoles/ml) or high (0.8–1.2 μmoles/ml) concentrations. The uptake in a premalignant liver or a solid hepatoma was in between that in the Zajdela cells and in normal liver. We have also compared the intracellular concentration of the labeled amino acid taken up, and the distribution of the amino acid in the acid-soluble and the acid-insoluble fractions of the cell, in the above four preparations – one normal, one premalignant and two malignant – derived from rat liver.

Although it is generally known that the rates of uptake of various nutrients are higher in malignant than in normal cells (*inter alia*, Christensen & Henderson, 1952; Johnstone & Scholefield, 1965), there have been few studies in which a comparison has been made of these rates in normal cells with those in *homologous* tumor cells.

Materials and Methods

Animals and Tumors

The animals used were the same as in a previous paper (Bhargava, Szafarz, Bornecque & Zajdela, 1975).

Three types of tumors were used: (*a*) the Zajdela ascitic hepatoma described in the above-cited paper; (*b*) a solid hepatoma induced by dimethylaminoazobenzene in which, as revealed by histological examination, the number of cells per unit volume was only about 40% of that in normal liver and in which, therefore, the correction factor for hyperplasia was 2.5; (*c*) a premalignant rat liver (histopathologically, between normal and malignant liver), also obtained following administration of dimethylaminoazobenzene, in which the correction factor for hyperplasia was 1.25.

Slices were cut free-hand from normal liver, premalignant liver and solid hepatoma. The Zajdela cells had the highest mitotic activity and were followed, in decreasing order, by the solid hepatoma, the premalignant, and normal liver.

The data given for normal liver slices and the Zajdela cells are of typical reproducible experiments. Only one set each of animals was used as a source of the premalignant liver and the solid hepatoma.

Media

For suspension and incubation of the cells/slices, Krebs-Ringer's bicarbonate (KRB) buffer (Dawson, Elliott, Elliott & Jones, 1957) or a serum-free tissue culture medium (Bhargava *et al.*, 1976), both containing penicillin (100 μg/ml), was used. For washing, either KRB buffer or Dulbecco's phosphate-buffered saline containing azide (Bhargava *et al.*, 1976), was used.

Radioactive Compounds

^{3}H-labeled high specific activity leucine, phenylalanine, valine, methionine, lysine and arginine, were obtained from C.E.A., Saclay, France.

Incubation

The Zajdela cells ($69–120 \times 10^6$; one million of these cells gave, on an average, 0.96 mg dry wt of TCA precipitable material), or slices (210–270 mg wet wt, equivalent to 42–54 mg dry wt) derived from normal liver, the premalignant liver or the solid hepatoma, were incubated at 37 °C with the ^{3}H-labeled amino acid in 3 ml of the stated incubation medium in open plastic bottles with shaking in an atmosphere of water-saturated 95% O_2 + 5% CO_2. A separate bottle was used for each time point and for each amino acid.

Estimation of the Uptake

The cells/slices were transferred to weighed centrifuge tubes and washed with either KRB buffer or phosphate-buffered saline-azide containing unlabeled amino acids, as in Bhargava *et al.* (1975). After the last wash (which was counted and shown to be virtually free of radioactivity), 3 ml of ice-cold 5% TCA were added. The slices were disintegrated with a glass rod. The mixture was left in the cold for at least 30 min. It was then centrifuged and the supernatant removed by decantation; 0.2 ml aliquots of the supernatant were counted and the total acid-soluble radioactivity in the washed tissue preparation calculated.

The sediment (TCA precipitate) was washed thrice, each time with 5 ml of TCA; TCA was removed by washing with ether, and the residue dried and weighed. For estimation of radioactivity, the dried TCA precipitate was dissolved in 5 ml of 1.2 N NaOH by heating for 60 min at 95 °C, and the insoluble material (mostly lipids) removed by centrifugation. Suitable aliquots of the alkali solution were counted and the total radioactivity in the acid-insoluble fraction calculated after appropriate correction for quenching by alkali.

Unless otherwise mentioned, the term "uptake" refers to the total (acid-soluble + acid-insoluble) uptake of amino acids; as the back-flux was not estimated, the total uptake values represent the *net* total uptake.

Measurements of radioactivity were made as described in Bhargava *et al.* (1975).

Results

Uptake of Amino Acids at Low Extracellular Concentrations

Table 1 shows the total uptake of four ^{3}H-labeled amino acids by slices from normal liver and a premalignant liver and by the Zajdela ascitic cells; the concentration of the amino acids in the medium in these experiments ranged from 0.8 to 3.1 μM. The uptake (expressed as the total amount of the labeled amino acid taken up into the acid-soluble and the acid-insoluble fractions, per mg TCA ppt.) of all the amino acids was higher in the Zajdela cells than in either type of slices. The uptake in the premalignant liver was higher than in normal liver, except at 3 min for all the amino acids and at 180 min for two amino acids, in which cases it was about the same as in normal liver. The ratio of the uptake by the Zajdela cells to that by normal liver slices at 3 min varied between 3 and 10 (average 7). At 90 and at 180 min,

Table 1. Comparative uptake of amino acids present in trace amounts in the incubation medium, by normal liver slices, slices from premalignant liver, and the Zajdela ascitic hepatoma cells[a]

Amino acid	Tissue or cell type	Amino acid taken up (pmoles/mg TCA ppt)								
		Acid-soluble fraction			Acid-insoluble fraction			Total (acid-soluble + acid-insoluble)		
		3 min	90 min	180 min	3 min	90 min	180 min	3 min	90 min	180 min
Leucine	Normal liver	0.156	0.954	0.840	0.0022	0.055	0.121	0.158	1.01	0.961
	Premalignant liver	0.154	1.13	0.786	0.0022	0.089	0.201	0.156	1.22	0.987
	Zajdela cells	0.526	0.706	0.639	0.017	0.608	0.973	0.543	1.31	1.61
Phenyl-alanine	Normal liver	0.240	1.55	1.11	0.0024	0.189	0.334	0.242	1.74	1.44
	Premalignant liver	0.227	2.03	1.10	0.0018	0.197	0.410	0.229	2.23	1.51
	Zajdela cells	1.64	1.23	1.27	0.019	1.12	1.89	1.66	2.35	3.16
Valine	Normal liver	0.126	1.28	0.605	0.0005	0.095	0.140	0.127	1.37	0.745
	Premalignant liver	0.105	1.48	0.756	0.0003	0.126	0.255	0.105	1.60	1.01
	Zajdela cells	0.979	1.83	1.53	0.0007	1.21	1.68	0.980	3.04	3.21
Methio-nine	Normal liver	0.636	4.09	6.54	0.008	2.20	3.57	0.644	6.29	10.1
	Premalignant liver	0.665	7.25	8.36	0.008	2.62	3.79	0.673	9.87	12.2
	Zajdela cells	6.34	26.3	17.0	0.0106	17.6	20.7	6.35	43.9	37.7

[a] Normal or premalignant rat-liver slices (240–270 mg wet wt; 48–54 mg dry wt) or the Zajdela cells (112×10^6; 107.5 mg dry wt) were incubated in 3 ml of KRB buffer with 1.11×10^8 cpm (2.34 nmoles) of ^{3}H-leucine, or 1.08×10^8 cpm (4 nmoles) of ^{3}H-phenylalanine, or 0.85×10^8 cpm (2.99 nmoles) of ^{3}H-valine, or 0.69×10^8 cpm (9.26 nmoles) of ^{3}H-methionine, for the stated period. The slices or the cells were then washed with KRB buffer, treated with TCA, and the radioactivity in the acid-soluble and the acid-insoluble fractions estimated as in the text.

the uptake in the Zajdela cells was, on an average, threefold (range 1.3- to sevenfold) that in normal liver. The uptake of the four amino acids in the premalignant liver was, on an average, 30% higher than in normal liver at 90 min, and 16% higher at 180 min, although no significant and consistent differences were observed between the two tissue preparations at 3 min. In the Zajdela cells, at 180 min, the amount of labeled amino acid found in the cell accounted for 8, 9, 12 and 44% of the labeled leucine, phenylalanine, valine and methionine put initially in the medium.

In all the tissue/cell preparations, the rate of the total uptake during the first 3 min far exceeded that obtained subsequently; the ratio of the uptake at 3 min to that at 90 min was, however, much lower for the two types of slices than for the Zajdela cells. The drastic reduction observed in the rates of total uptake in the Zajdela cells after the first few minutes, in comparison to slices from normal or premalignant liver, is unlikely to be due to a lowering of metabolic activity, as the incorporation of the labeled amino acids into protein in these cells continued between 90 and 180 min at about 50% of the rate observed between 3 and 90 min (Table 1).

The calculated average intracellular concentration (I)[1] of the labeled amino acid, that is, the amount present per unit total cellular volume, was also the highest in Zajdela cells at all the time points and for all the amino acids (Table 2). At 3 min, I in the Zajdela cells was 14–45 times (average, 31 times) that in normal liver; at the later time points, I in the former was 3–30 times (average, 9 times) that in the latter. In the premalignant liver at 3 min, I was about the same as that in normal liver, but at subsequent time points, I in the premalignant liver was 25%–200% more than in the normal tissue cells.

The proportion of the labeled amino acid taken up incorporated into the acid-insoluble fraction, increased with time in the case of all the tissue/cell preparations and for all the amino acids: from 0.4–1.3% at 3 min to 13–35% (average, 22%) at 180 min in normal liver; from 0.3–1.3% to 20–31% (average, 26%) in the premalignant liver, and from 0.1–3.1% to 52–60% (average, 57%) in the Zajdela cells. This proportion was the highest for the Zajdela cells and generally the lowest for normal liver.

In the Zajdela cells, except for methionine, the total uptake continued to increase till 180 min; in the other two tissue preparations, the total amount of radioactivity found in the tissue at 180 min was significantly less than at 90 min (except in the case of methionine), suggesting a

[1] The intracellular concentration values given in Table 2 must be considered only a first approximation as they are based on the *total* volume of the cells and not on the intracellular fluid space. The general conclusions are unlikely to be in error as the intracellular fluid space in mammalian cells appears to be about 75% of the total cell volume (e.g. Foster & Pardee, 1969). It must also be noted that the values given refer only to the concentration of the *labeled* amino acid taken up and do not take into account the unlabeled amino acid already present in the cell. The values therefore do *not* represent the true intracellular concentration of the amino acid, which would be a sum of the concentration of the labeled amino acid taken up in the free pool and that of the preexisting unlabeled amino acid in the pool.

Table 2. Intracellular concentration of labeled amino acids following their uptake in vitro by normal liver, a premalignant liver, a solid hepatoma, and the Zajdela ascitic hepatoma[a]

Amino acid	Tissue or cell type	External concentration of the labeled amino acid (μmoles/liter incubation medium)	Intracellular concentration of the labeled amino acid (nmoles/ml cellular volume)				
			3 min	60 min	90 min	120 min	180 min
Leucine	Normal liver	0.78	0.04	–	0.22	–	0.19
	Premalignant liver	0.78	0.04	–	0.33	–	0.23
	Zajdela ascitic hepatoma	0.78	0.56	–	0.75	–	0.68
Leucine	Normal liver	800	–	409	–	436	522
	Solid hepatoma	800	–	884	–	1,680	1,650
	Zajdela ascitic hepatoma	800	–	3,370	–	7,580	6,730
Valine	Normal liver	1.00	0.03	–	0.30	–	0.14
	Premalignant liver	1.00	0.03	–	0.43	–	0.22
	Zajdela ascitic hepatoma	1.00	1.04	–	1.95	–	1.63
Valine	Normal liver	800	–	375	–	478	785
	Solid hepatoma	800	–	1,290	–	2,120	2,170
	Zajdela ascitic hepatoma	800	–	5,780	–	8,480	11,300
Phenyl-alanine	Normal liver	1.33	0.06	–	0.36	–	0.26
	Premalignant liver	1.33	0.07	–	0.59	–	0.32
	Zajdela ascitic hepatoma	1.33	1.75	–	1.31	–	1.35
Methionine	Normal liver	3.09	0.15	–	0.95	–	1.52
	Premalignant liver	3.09	0.19	–	2.11	–	2.43
	Zajdela ascitic hepatoma	3.09	6.76	–	28.0	–	18.13
Lysine	Normal liver	800	–	787	–	1,130	1,220
	Solid hepatoma	800	–	1,400	–	3,810	3,920
	Zajdela ascitic hepatoma	800	–	8,040	–	8,520	8,360
Arginine	Normal liver	1,200	–	313	–	438	636
	Solid hepatoma	1,200	–	660	–	1,190	2,020
	Zajdela ascitic hepatoma	1,200	–	4,750	–	4,740	4,420

[a] The values given above have been calculated from the data for the acid-soluble fraction given in Tables 1 and 3, the average volume of parenchymal cells from normal liver, the premalignant liver and the solid hepatoma (taken to be 10,800 μ^3 in each case; Iype,

loss of labeled material from the cells between 90 and 180 min. This loss may be caused by leakage of labeled protein (such as serum albumin) synthesized during incubation. No loss was observed in the case of Zajdela cells which apparently synthesize but do not secrete serum albumin (C. Bornecque & D. Szafarz, *unpublished*).

Uptake of Amino Acids at High Extracellular Concentrations

The amount of an amino acid taken up by mammalian cells increases with an increase in its extracellular concentration within a wide range (*inter alia,* Clark & Schmidt, 1967; Taylor & Stanners, 1967); therefore, for any comparison of the ability of different cell systems to take up amino acids to be valid, it would be advisable to study the uptake at at least two (preferably more, to allow kinetic analysis) concentrations, one low (as in Table 1) and the other high. For this reason, we also studied the total uptake of four amino acids (leucine, valine, lysine and arginine) in normal liver, in a solid hepatoma and in the Zajdela ascitic hepatoma cells, in a serum-free tissue culture medium in which the above amino acids were present at concentrations varying from 0.8 to 1.2 mM, that is, about a 1 000-fold higher than in Table 1. The total uptake at various time points, and its distribution in the acid-soluble and the acid-insoluble fractions, are given in Table 2.

The uptake of the amino acids expressed as the total amount taken up per mg TCA precipitate was, again, at all time points, higher in the Zajdela cells than in the other two tissue preparations; at 60–120 min it was 2–5 (average, 3.5) times higher than in normal liver (Table 3). The uptake by the solid hepatoma at 60 min was about the same as by normal liver, or in between that for normal liver and the Zajdela cells, depending on the amino acid; at 120 and 180 min, however, the uptake by the hepatoma was 20–100% (average 50%) higher than that by normal liver.

Whereas in the case of the Zajdela cells, the uptake stopped at 60 or 120 min, or proceeded only very slowly beyond 120 min, in slices

---

Bhargava & Tasker, 1965) and of the Zajdela cells (900 μ^3), and the information given in the legends to Tables 1 and 3 on the amount of cells/slices used. The number of parenchymal cells in normal liver, the premalignant liver and the solid hepatoma has been taken to be 81×10^6, 64.8×10^6 and 32.4×10^6 cells per gram wet wt of the tissue, and it is assumed that all the uptake occurred in the parenchymal cells (*see* text).

Table 3. A comparison of the uptake by normal liver slices, slices from a solid hepatoma, and the Zajdela ascitic hepatoma cells, of amino acids present in the incubation medium in physiological concentrations[a]

Amino acid	Tissue or cell type	Amino acid taken up (nmoles/mg TCA ppt)								
		Acid-soluble fraction			Acid-insoluble fraction			Total (acid-soluble + acid-insoluble)		
		60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Arginine	Normal liver	1.34	1.88	2.73	0.02	0.13	0.13	1.36	2.01	2.86
	Solid hepatoma	1.13	2.04	3.48	0.08	0.32	0.33	1.21	2.36	3.81
	Zajdela cells	4.53	4.53	4.22	0.34	0.90	0.87	4.87	5.43	5.09
Leucine	Normal liver	1.76	1.87	2.24	0.04	0.22	0.25	1.80	2.09	2.49
	Solid hepatoma	1.52	2.88	2.83	0.25	0.77	0.78	1.77	3.65	3.61
	Zajdela cells	3.21	7.24	6.42	1.20	3.22	3.84	4.41	10.5	10.3
Lysine	Normal liver	3.38	4.85	5.25	0.06	0.15	0.32	3.44	5.00	5.57
	Solid hepatoma	2.41	6.55	6.74	0.70	1.31	1.12	3.11	7.86	7.86
	Zajdela cells	7.68	8.13	7.98	0.84	3.04	3.07	8.52	11.2	11.1
Valine	Normal liver	1.61	2.05	3.37	0.03	0.09	0.33	1.64	2.14	3.70
	Solid hepatoma	2.21	3.65	3.73	0.41	0.68	0.69	2.62	4.33	4.42
	Zajdela cells	5.51	8.09	10.7	0.57	2.82	2.00	6.08	10.9	12.7

[a] Slices (210–270 mg wet wt; 42–54 mg dry wt) from normal liver or a malignant hepatoma, or the Zajdela cells (69×10^6; 66 mg dry wt) were incubated in 3 ml of the tissue culture medium with 22.4×10^6 cpm (concentration in the tissue culture medium, 2.4 μmoles) of ^{3}H-valine, or 31.8×10^6 cpm (2.4 μmoles) of ^{3}H-lysine, or 47.3×10^6 cpm (3.6 μmoles) of ^{3}H-arginine, or 28.8×10^6 cpm (2.4 μmoles) of ^{3}H-leucine, for the stated period; all the ^{3}H-amino acids were of high specific activity ($11–22 \times 10^{12}$ cpm/nmole). The slices or the cells were then washed with Dulbecco's phosphate-buffered saline, treated with TCA, and the radioactivity in the acid-soluble and the acid-insoluble fractions estimated as in the text.

from normal liver the total uptake of all the four amino acids increased significantly between 120 and 180 min (Table 3). In the solid hepatoma, the uptake stopped after 120 min in the case of three out of the four

amino acids. It is noteworthy that at the time of cessation of the uptake in the Zajdela cells, each of the amino acids taken up was present in the free pool in an amount equivalent to at least 0.1% of the weight of the TCA precipitate of the cells.

The *I* values for the Zajdela cells, for the various amino acids at different time points, were 7–18 times greater than for normal liver (Table 2); the value for the solid hepatoma was, in every case, in between those for normal liver and the Zajdela cells.

The proportion of the labeled amino acid taken up found in the acid-insoluble fraction was, in the case of all the amino acids and at all time points, the highest for the Zajdela cells and the lowest for normal liver, with the solid hepatoma again falling in between (Table 3); the actual values for the acid-insoluble radioactivity at 180 min were: 16–37% (average, 25%) in the Zajdela cells, 9–22% (average, 15%) in the solid hepatoma, and 5–10% (average, 7%) in normal liver.

Discussion

In this study we have compared the net total uptake of amino acids by thin slices from normal liver, a premalignant liver, and a solid hepatoma, and the Zajdela ascitic hepatoma cells. We have discussed in a previous communication (Kumar & Bhargava, 1975) the problems arising from the use of slices in such studies. We must also point out that the comparisons we have made here between the Zajdela cells and the slices would be valid only if the diffusion of amino acids into the slices were not a limiting factor during the 3-hr period of incubation. Although it is unlikely that diffusion was limiting (Kumar & Bhargava, 1975), we have no direct proof that this was not so. Further, although we have shown (M.A. Siddiqui, G.K. Kumar and P.M. Bhargava, *unpublished work*) that in liver slices and liver cell suspensions, the rate of catabolism of the amino acids used here is low, so that most of the acid-soluble radioactivity found in the cells following uptake of labeled amino acids for short periods is recovered in amino acids chromatographically purified from the acid-soluble fraction, we have not shown that degradation of amino acids by the four cell/tissue preparations used here, under the experimental condition employed in this study, could not vitiate the results. Subject to the above limitations, our results strongly suggest that the total uptake of amino acids measured for a period of up to 180 min under conditions in which incorporation of

the amino acid taken up into protein may continue, is significantly higher in the Zajdela ascitic hepatoma cells than in normal liver, both at low ($\sim$1 μM) and high ($\sim$1 mM) extracellular amino acid concentrations. Although the ratio of the total uptake in the Zajdela cells to that in normal liver varied considerably from amino acid to amino acid and from time point to time point, in no case and in no experiment was the total uptake in the Zajdela ascitic cells lower than in normal liver. It is possible that the above variation from amino acid to amino acid may be due to a variation in the relative requirement for the various amino acids in the case of the two preparations, one (the Zajdela ascitic hepatoma) consisting predominantly of *dividing* cells and the other (normal liver) containing predominantly *resting* cells. Our observations also suggest that in the above four cell/tissue preparations representing different states of liver parenchymal cells, the rate of the total uptake of an amino acid, its intracellular concentration in the free pool, the proportion of the amino acid taken up incorporated into protein, the rate of incorporation of the amino acid into protein, and the cellular growth rate, are correlated.

In all the cell/tissue preparations in both the media used (Tables 1 and 3), excepting perhaps in normal liver in the tissue culture medium, the total uptake generally slowed down with time, continuing only at a very low rate (in comparison to the initial rate), if at all, after 90–120 min. This could be due to the absence of serum in the incubation medium. Although the initial uptake of amino acids by mammalian cells in a tissue culture medium appears to be the same in the absence of serum as in its presence, serum has been shown to be required for continuance of the net total uptake of the amino acids beyond about one hour (Bhargava & Vigier, 1976).

In both normal liver and the Zajdela cells, the amount of labeled amino acid taken up as well as the I values obtained with the high extracellular concentration (0.8–1.2 mM) of amino acids, were 10^3–10^4 times greater than the corresponding values obtained with the low concentration (0.8–3.1 μM) of the amino acids (*cf.* Clark & Schmidt, 1967; Taylor & Stanners, 1967). In both preparations, a greater proportion of the amino acid taken up was present in the acid-soluble fraction when the higher external concentration of the amino acids was used. In the Zajdela cells, the cessation of increase in radioactivity in the acid-soluble pool at 60 – 120 min was accompanied by a cessation of incorporation of the labeled amino acid into protein when the extracellular concentration of the amino acids was high, in contrast to the con-

tinuance of incorporation of the amino acid into protein beyond 120 min when the extracellular concentration of the amino acids was low. A more sophisticated kinetic analysis of the uptake and of the variation in the pool size will be required to understand the significance of these observations.

One of the authors (P.M.B.) is grateful to the International Union Against Cancer for the award of an Eleanor Roosevelt International Cancer Research Fellowship, during the tenure of which this work was carried out; to the Director, Regional Research Laboratory, Hyderabad, India, for leave of absence; and to several of his colleagues at Hyderabad, for assistance in the preparation of the manuscript.

References

Bhargava, P.M., Allin, E.P., Montagnier, L. 1976. Uptake of amino acids and thymidine during the first cell cycle of synchronized hamster cells. *J. Membrane Biol.* **26**:1

Bhargava, P.M., Szafarz, D., Bornecque, C.A., Zajdela, F. 1975. Uptake *in vitro* of nucleic acid precursors and nucleic acids by Zajdela ascitic hepatoma cells. *J. Membrane Biol.* **22**:341

Bhargava, P.M., Vigier, P. 1976. Control of the uptake of amino acids by serum in chick embryo cells, untransformed or transformed with Rous sarcoma virus. *J. Membrane Biol.* **26**:19

Christensen, H.N., Henderson, M.E. 1952. Comparative uptake of free aminoacids by mouse ascites carcinoma cells and normal tissues. *Cancer Res.* **12**:229

Clark, C., Schmidt, E.L. 1967. Uptake and utilization of aminoacids by resting cells of *Nitrosomonas europea*. *J. Bacteriol.* **93**:1309

Dawson, R.M.C., Elliott, D.C., Elliott, W.H., Jones, K.M. 1957. Data for Biochemical Research. p. 208. Oxford University Press, London

Foster, D.O., Pardee, A.B. 1969. Transport of aminoacids by confluent and non-confluent 3T3 and polyma virus-transformed 3T3 cells growing on glass cover slips. *J. Biol. Chem.* **244**:2675

Iype, P.T., Bhargava, P.M., Tasker, A.D. 1965. Some aspects of the chemical and cellular composition of adult rat liver. *Exp. Cell Res.* **40**:235

Johnstone, R.M., Scholefield, P.G. 1965. Aminoacid transport in tumor cells. *Adv. Cancer Res.* **9**:144

Kumar, G.K., Bhargava, P.M. 1975. Comparison of some permeability properties of rat liver slices, liver cells in suspension, and *in vivo*-produced aggregates of dispersed liver cells. *J. Membrane Biol.* **22**:329

Taylor, J.M., Stanners, C.P. 1967. A note on the incorporation of aminoacids by hamster embryo cells in monolayer culture. *Biochim. Biophys. Acta* **138**:627