The Uptake of Homologous Ribonucleic Acid by Rat-Liver Parenchymal Cells in Suspension

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1. Native or partially degraded RNA derived from intact rat liver, or from the parenchymal-cell or the non-parenchymatous fraction of liver, has been shown to be transported into rat parenchymal cells in suspension, without prior degradation to acid-soluble components, when the cell suspension is incubated with the RNA at 37°. The amount of RNA of exogenous origin present in the parenchymal cells in an acid-precipitable form increased rapidly up to 30–60 min., after which it gradually decreased, indicating intracellular degradation to acid-soluble components of the RNA taken up by the cells. 2. The RNA taken up by the parenchymal cells from the medium, and the acid-soluble products of its degradation within the cells, could be released back into the medium. 3. The RNA of exogenous origin present in acid-precipitable form in the parenchymal cells represented up to 5% of the RNA of the cells after 60 min. of incubation. 4. When the concentration of RNA in the medium was less than 200 µg./ml., over 10% of the RNA was transported in an acid-precipitable form in 60 min. into the parenchymal cells incubated at a concentration of 2.3×10^6 /ml. 5. Ribonuclease inhibited the uptake of exogenous RNA by the parenchymal cells, whereas 2,4-dinitrophenol, sodium azide, protamine sulphate and polyvinyl sulphate had no significant effect. 6. The uptake of exogenous RNA by liver slices proceeded at a rate which was 4–20% of that obtained in the parenchymal-cell suspensions; the RNA taken up did not appear to become degraded, unlike that taken up by the cell suspensions. 7. It is concluded that dispersion of liver tissue to a suspension of single cells increases the permeability of the parenchymal cells to macromolecular RNA and creates conditions that lead to a rapid degradation of the RNA taken up.

Several recent investigations have studied the uptake of RNA by mammalian cells. Most of these investigations, however, have been confined to a study of the effect of exogenous RNA on either the biological properties of the cells (Niu, 1960; Decarvalho & Rand, 1961; Aksenova, Bresler, Vorobyev & Olenov, 1962; Mannick & Egdahl, 1962; Hillman & Niu, 1963; Esposito, 1964) or of protein synthesis by them (Niu, Cordova & Niu, 1961; Amos & Kearns, 1962; Niu, Cordova, Niu & Radbill, 1962; Amos & Moore, 1963; Niu, 1963, 1965; Zimmerman, Zoller & Turba, 1963). In only a few studies was any attempt made to demonstrate the actual transport of RNA from the medium into the cells. Amos (1961) and Amos & Kearns (1963) observed an uptake of 2–6 µg. of *Escherichia coli* RNA by 10^7 chick-embryo fibroblasts. Niu *et al.* (1961) have reported the entry of ^{14}C -labelled liver RNA into ascites cells; by comparison of the distribution of radioactivity in the bases derived from ^{14}C RNA of liver with the distribution obtained with RNA of ascites cells after incubation

with the labelled RNA, they concluded that the ^{14}C RNA entered the tumour cells without degradation. More recently, Schwarz & Rike (1962) reported the uptake *in vivo* and *in vitro* of ^3H -labelled homologous RNA by normal and neoplastic mouse cells; they followed the uptake by radioautography and found most of the label in the cellular RNA. However, they found that DNA was also labelled and they attributed this to the partial degradation of RNA before or after incorporation in the cells. Only in one of these investigations (Niu, 1963) was the localization of the RNA taken up in the recipient cells studied, and in none was the fate of the RNA transported in the cells determined. Moreover, except in one report (Schwarz & Rike, 1962), either tumour cells or embryonic cells grown in tissue culture were used as the recipient cell system, and the exogenous RNA used was heterologous. The RNA that was used was not characterized in any of the above studies.

It seemed to us that, for adequate interpretation of the biological effects and the stimulation of

protein synthesis by exogenous RNA in mammalian systems, it was necessary to possess more definite information on the kinetics and extent of the uptake of well-characterized RNA by normal tissue cells, the nature of this uptake, and the localization and fate of the RNA taken up. The present paper describes the uptake by a suspension of rat parenchymal cells of [^{32}P]RNA isolated from whole rat liver or from the parenchymal-cell or the non-parenchymatous fraction of liver. Two different methods were used for the isolation of RNA, one of which gave undegraded RNA and the other RNA that was partially degraded. Considerable uptake of [^{32}P]RNA by the parenchymal cells was observed in every case; the RNA taken up was distributed in all the subcellular fractions. The uptake proceeded rapidly initially, reaching a peak at 30–60 min., after which the [^{32}P]RNA accumulating in the cells was degraded. The uptake of homologous [^{32}P]RNA by rat-liver slices was also studied and found to be 4–20% of that by the parenchymal cells in suspension; the RNA taken up by the slices did not appear to become degraded. A preliminary report of this work has been published (Bhargava, Jacob & Shanmugam, 1965).

EXPERIMENTAL

Materials

Animals. Adult albino rats (an inbred strain from the Nutrition Research Laboratory, Hyderabad) of both sexes were used.

Radioactive chemicals. [^{32}P]Phosphate was obtained from the Atomic Energy Establishment, Bombay, India, as orthophosphate in 0.9% NaCl buffered with phosphate buffer to give a concentration of 1 mg. of P/ml.

Other chemicals. Yeast RNA and protamine sulphate were purchased from Nutritional Biochemical Corp. (Cleveland, Ohio, U.S.A.). Bentonite, ribonuclease, sodium dodecyl sulphate, 2,4-dinitrophenol and sodium azide were obtained from Fisher Scientific Co. (Fair Lawn, N.J., U.S.A.), L. Light and Co. Ltd. (Colnbrook, Bucks.), British Drug Houses Ltd. (Poole, Dorset), E. Merck A.-G. (Darmstadt, W. Germany) and Eastman Kodak (Rochester, N.Y., U.S.A.) respectively. Polyvinyl sulphate was a gift from Dr Melvin Cohn.

Buffer. Krebs original Ringer phosphate buffer (Dawson, Elliott, Elliott & Jones, 1959), to which glucose was added to give a final concentration of 15 mM, was used unless otherwise mentioned.

Methods

Preparation of liver parenchymal-cell suspensions. The parenchymal cells were obtained in suspension by the method of Jacob & Bhargava (1962).

Preparation of liver slices. The slices were cut freehand from liver freshly perfused as described by Jacob & Bhargava (1965a).

Preparation of the 'soluble portion of the non-parenchymatous fraction of liver'. The residue left on the 200-mesh

sieve during the preparation of the parenchymal-cell suspension was mixed with the supernatant obtained on centrifugation of the crude cell suspension at 200g to sediment the parenchymal cells; the mixture was homogenized at 4° in a Potter-Elvehjem homogenizer and centrifuged at 105 000g for 1 hr. The supernatant, which was predominantly derived from the cells of liver other than the parenchymal cells and from the non-cellular material in liver, is referred to as the 'soluble portion of the non-parenchymatous fraction of liver' (Iype & Bhargava, 1963, 1965).

Preparation and estimation of [^{32}P]RNA. [^{32}P]Phosphate (1 mc) was injected intraperitoneally and the animal killed after 24 hr. RNA was isolated from the whole liver, the parenchymal-cell suspension or the 'soluble portion of the non-parenchymatous fraction of liver' by one of the following two methods.

Method A. This was essentially the same as that described by Kirby (1956).

Method B. This was the same as that described by Scherrer & Darnell (1962), except that: (i) the concentration of polyvinyl sulphate added to the acetate buffer used for homogenization was 5 $\mu\text{g./ml.}$ instead of 2 $\mu\text{g./ml.}$; (ii) bentonite (0.5%) was also used with the polyvinyl sulphate; (iii) the extraction with phenol was done at 4° for 15 min. instead of 60° for 3 min.; (iv) the aqueous layer after phenol extraction was separated by centrifuging at 1500g for 30 min. instead of 20 000g for 1 min.

An extinction of 1.0 at 260 $\mu\text{m.}$ for an aqueous solution of RNA prepared as above was taken to correspond to 40 $\mu\text{g.}$ of RNA/ml. (Fleck & Munro, 1962).

Alkaline and ribonuclease-catalysed hydrolysis of [^{32}P]RNA. The former was carried out as described by Jacob & Bhargava (1965a); the alkaline hydrolysate was neutralized to pH 7 before further use. For the latter, the [^{32}P]RNA was treated at 37° for 1 hr. with ribonuclease (50 $\mu\text{g./ml.}$) in the buffer. The enzyme was removed by precipitation with trichloroacetic acid; a small amount of pure bovine serum albumin was added to the incubation mixture before addition of trichloroacetic acid, to facilitate removal of the precipitated enzyme. Trichloroacetic acid was removed by repeated extraction with ether.

Incubations. The tissue preparation (100–160 mg. wet wt. of liver slices, or 2×10^6 to 4×10^6 liver parenchymal cells in suspension, per ml. of the incubation medium) was incubated in the buffer with [^{32}P]RNA (or its alkaline or ribonuclease hydrolysate) in a series of 25 ml. conical flasks (one for each time-point) at 37° or at 4° (cold room), in a Gallenkamp constant-temperature water bath with shaking at 100 oscillations/min. Other additions, if any, to the incubation medium were as stated in the legends to the Tables.

In experiments in which the fate of [^{32}P]RNA taken up by the parenchymal cells in suspension was to be studied, the cell suspension after incubation for 30 min. at 37° as above was centrifuged and the sedimented cells were washed twice at 4°, each time with 3–4 ml. of the buffer containing 3–4 mg. of unlabelled yeast RNA. The cells were then washed once with the buffer, resuspended in the buffer, dispensed in a series of flasks (one for each time-point) and reincubated as above.

In the zero-time samples, the addition of [^{32}P]RNA to the tissue preparation was always done at 4°.

Preparation of subcellular fractions. The parenchymal-cell suspension, after incubation with [^{32}P]RNA, was

centrifuged and the sedimented cells were washed repeatedly as above with the buffer containing unlabelled yeast RNA until no radioactivity was detected in the last washing; the cells were then washed once with the buffer. Usually a total of three washings sufficed. The washed cells were suspended in 0.25 M-sucrose and the subcellular fractions (the nuclear-plus-membrane, mitochondrial, microsomal and supernatant fractions) prepared as described by Jacob & Bhargava (1965b).

Isolation and estimation of the RNA fraction from the tissue preparations incubated with [32 P]RNA or from their subcellular fractions. The incubated tissue preparation, washed as above until the washings were free of radioactivity, or the subcellular fraction, was suspended in 3 ml. of the buffer and treated with an equal volume of 20% (w/v) trichloroacetic acid in the cold. The acid-soluble material and lipids were removed and the nucleic acids extracted with 10% (w/v) NaCl as described by Jacob & Bhargava (1965a). The NaCl extract was made 1% (w/v) with respect to sodium acetate and the total nucleic acids were precipitated with 2 vol. of ethanol in the usual way. RNA was obtained in the hydrolysed form and separated from DNA by treatment of the total nucleic acid fraction with alkali (Jacob & Bhargava, 1965a). An extinction of 1.0 at 260 m μ in the hydrolysed RNA fraction was taken to be equivalent to 33 μ g. of unhydrolysed RNA/ml. (Fleck & Munro, 1962).

In some experiments with parenchymal-cell suspensions, the precipitate obtained on addition of trichloroacetic acid was washed three times with ethanol saturated with sodium acetate, and then once with ether. The radioactivity in the residue was taken to be a measure of the radioactivity in the RNA of the cells.

Isolation of the RNA and the acid-soluble fractions from the parenchymal-cell suspensions first incubated with [32 P]RNA and then reincubated in the absence of [32 P]RNA. The reincubated cell suspension was centrifuged and the sedimented cells were washed once with the buffer. The combined supernatants are referred to as the 'medium' fraction. The cells were resuspended in 3 ml. of the buffer. A 1 ml. sample was used for estimation of the total radioactivity contained in the cells; the remaining cell suspension (2 ml.) was treated with an equal volume of 20% trichloroacetic acid in the cold and centrifuged. The sediment as well as the supernatant was freed of trichloroacetic acid by repeated washing with ether. The radioactivity in the sediment and the supernatant was taken to be a measure of the radioactivity in the RNA and the acid-soluble fractions of the parenchymal cells respectively.

Purified yeast RNA (1 mg.) was added as a carrier to the 'medium' fraction, which was then treated with an equal volume of 20% trichloroacetic acid in the cold and centrifuged. The sediment and the supernatant were freed of trichloroacetic acid as above; the radioactivity in the two fractions was taken to be a measure of the radioactivity in the RNA and the acid-soluble fractions of the medium respectively.

Separation and estimation of nucleotides. The ribonucleotides obtained by alkaline hydrolysis were separated electrophoretically and estimated as described by Davidson & Smellie (1952), by using 50 v/cm. for 90 min. in a high-voltage horizontal-paper-electrophoresis unit.

Measurement of radioactivity. The RNA (hydrolysed or unhydrolysed), nucleotides and the acid-soluble fraction were plated on aluminium planchets (2 cm.²) as described by

Jacob & Bhargava (1965b). The washed trichloroacetic acid precipitate of the parenchymal cells was plated on weighed planchets as a slurry in distilled water. When the specific activity of dried whole parenchymal cells was desired, the incubated cells were washed as described above and suspended in distilled water, and a sample was dried to constant weight on weighed planchets. When the total radioactivity in the cell suspension was to be determined, a known sample of the cell suspension in the buffer was plated directly. The radioactivity was measured in a Geiger-Müller counter with 10% efficiency for 32 P. All the radioactivity data were corrected for decay to an initial specific activity of 1 mc/mg. of P.

Ultracentrifugal studies. These were carried out in a Spinco model E analytical ultracentrifuge.

RESULTS AND DISCUSSION

Uptake by the liver parenchymal cells in suspension of radioactivity from [32 P]RNA prepared by two different methods. Fig. 1 (Expts. 1 and 2) and Table 1 (Expts. 1-4) show that, when the parenchymal cells in suspension were incubated with [32 P]RNA prepared by method A (Kirby, 1956) from whole liver, there was initially a rapid incorporation of radioactivity into the RNA of the parenchymal cells;

Fig. 1. Uptake by liver parenchymal cells in suspension and by liver slices of [32 P]RNA: 0, parenchymal-cell suspension, Expt. 1; ●, parenchymal-cell suspension, Expt. 2; □, liver slices, Expt. 3. The tissue preparation was incubated at 37° for various periods in 3 ml. of the buffer with [32 P]RNA [564 μ g. (37 800 counts/min.) in Expt. 1, 657 μ g. (24 900 counts/min.) in Expt. 2, and 581 μ g. (18 500 counts/min.) in Expt. 3] prepared by method A from the whole liver of an animal injected with [32 P]phosphate. Details of method A and of the isolation of RNA from the incubated parenchymal cells are given in the Experimental section; the specific activity of this RNA is plotted against the time of incubation.

Table 1. *Comparison of the uptake by liver parenchymal cells in suspension and by liver slices of [³²P]RNA isolated by two different methods from whole liver or its fractions*

The tissue preparation was incubated in 3 ml. of the buffer with the stated amount of [³²P]RNA at 37° for the specified period. Details of the preparation of [³²P]RNA by methods A and B, the preparation of the 'soluble portion of the non-parenchymatous fraction of liver' and of the isolation of RNA from the incubated parenchymal cells and the tissue slices are given in the Experimental section.

Expt. no.	Method of preparation	Data on [³² P]RNA			Tissue preparation used for uptake of [³² P]RNA	Sp. activity of RNA of the tissue preparation (counts/min./mg.)			
		Source	Sp. activity (counts/min./mg.)	Concn. (μg./ml.)		0 min.	60 min.	120 min.	180 min.
1	A	Whole liver	38 000	254	Cell suspension	935	1190 (1600*, 940†)	865	306‡
2	A	Whole liver	71 600	180	Cell suspension	175	1010	—	548
3	A	Whole liver	56 000	221	Cell suspension	1260	2940	—	2100
4	A	Whole liver	81 500	207	Cell suspension	1160	1970	1420	1050
5	B	Whole liver	16 200	227	Slices	97	386	540	690
6	B	Whole liver	100 000	465	Cell suspension	—	1340	545	404
7	B	Whole liver	18 900	275	Cell suspension	3500	5300	3840	700
8	B	Parenchymal-cell suspension	63 700	273	Slices	140	625	690	—
		'Soluble portion of the non-parenchymatous fraction of liver'	114 000	273	Slices	75	200	205	270
					Cell suspension	935	1210	1010	965
					Cell suspension	303	536	510	404

* 30 min.

† 90 min.

‡ 150 min.

this incorporation reached a maximum at 30–60 min., after which the incorporated radioactivity was progressively lost from the cellular RNA. However, ultracentrifugal analysis of the [^{32}P]RNA used in these experiments showed the presence of two components with sedimentation coefficients ($S_{20,w}$) of only 4–5s and 8–10s, suggesting that the ribosomal RNA is partially degraded during isolation by Kirby's (1956) method, presumably by the action of tissue ribonuclease. Therefore, in another series of experiments, [^{32}P]RNA was isolated from whole liver as well as from the parenchymal-cell suspensions and the 'soluble portion of the non-parenchymatous fraction of liver' by method B, a modification of the procedure of Scherrer & Darnell (1962), in which bentonite and polyvinyl sulphate were used to inhibit ribonuclease action. In sedimentation runs, the RNA prepared by method B from the first two sources was found to contain three major components (4s, 15–16s and 23–28s), and from the last source only one component (5–6s). The uptake by the parenchymal cells in suspension of radioactivity from [^{32}P]RNA prepared by method B from all the three sources was of the same magnitude and followed the same pattern as was obtained with partially degraded [^{32}P]RNA prepared from whole liver by method A (Table 1: Expts. 5, 6 and 8).

Nature of the radioactivity incorporated from [^{32}P]RNA into the RNA of the parenchymal cells in suspension. The observed incorporation in the parenchymal-cell RNA of radioactivity from [^{32}P]RNA prepared either from whole liver or fractions of liver by various methods (Fig. 1 and Table 1) could be due to: (a) transport and association with the cell RNA of non-nucleic acid ^{32}P -containing phosphorus compounds that might be present as a contaminant in [^{32}P]RNA (Davidson, Frazer &

Hutchison, 1951; Jacob & Bhargava, 1965a); (b) incorporation into the cell RNA of ^{32}P -containing precursors of RNA that might be formed after extracellular or intracellular breakdown of such contaminants in [^{32}P]RNA; (c) association with the cell RNA of non-nucleic acid ^{32}P -containing phosphorus compounds newly formed in the cell during incubation; (d) degradation of [^{32}P]RNA outside the cell and the uptake of low-molecular-weight acid-soluble ^{32}P -labelled precursors of nucleic acids; (e) adsorption of [^{32}P]RNA on the cell membrane; (f) transport of [^{32}P]RNA or its acid-insoluble degradation products from the outside to inside the cell. Possibilities (a) and (c) are ruled out because the [^{32}P]RNA used in the uptake studies was dialysed before use, and the electrophoretic separation (Table 2) of nucleotides obtained on alkaline hydrolysis of [^{32}P]RNA, or of RNA of the parenchymal cells after incubation with [^{32}P]RNA, showed that virtually all the radioactivity in RNA was due to ^{32}P in internucleotide linkage. Possibility (b) is ruled out since the incorporation of ^{32}P from the non-nucleic acid ^{32}P -containing contaminants of [^{32}P]RNA into the cellular RNA is likely to proceed only through their degradation to [^{32}P]phosphate, which would be diluted to such a large extent by the [^{31}P]phosphate of the medium or the cells (depending on whether the degradation occurs outside or inside the cells) that it could not account for the high level of radioactivity observed in the RNA of the cells.

With regard to possibility (c), there is no evidence for the secretion by the parenchymal cells of nucleases that would degrade the [^{32}P]RNA of the medium. Even if nucleases were secreted, the products of their action on this RNA would be nucleoside monophosphates, which are known to be unable to enter mammalian whole-cell systems

Table 2. *Distribution of radioactivity in nucleotides derived from [^{32}P]RNA used in the RNA-uptake studies and from the RNA of liver parenchymal-cell suspensions incubated with the [^{32}P]RNA*

[^{32}P]RNA was prepared by method A from the whole liver of an animal injected with [^{32}P]phosphate, as described in the Experimental section. A 3 ml. portion of the parenchymal-cell suspension was incubated at 37° for the specified period with 656 μg . (28700 counts/min.) of the [^{32}P]RNA. Details of the isolation of RNA from the incubated cell suspension, of the hydrolysis of both the RNA preparations and of the separation of the resulting nucleotides are also given in the Experimental section. The total radioactivity recovered in the nucleotides accounted for 90–100% of the radioactivity contained in the RNA fraction before electrophoresis.

Source of nucleotides	Sp. activity of nucleotides (counts/min./mg.)			
	CMP	AMP	GMP	UMP
[^{32}P]RNA	25400	39400	28300	43500
RNA from the parenchymal cells in suspension incubated with the [^{32}P]RNA for 0 hr.	525	410	670	600
RNA from the parenchymal cells in suspension incubated with the [^{32}P]RNA for 20 min.	1950	1210	1150	1670

without undergoing dephosphorylation; such a cleavage would generate free [^{32}P]phosphate that, again, would be diluted so extensively (10–30-fold if all the [^{32}P]RNA was broken down, and more if only a part was degraded) by the phosphate contained in the incubation medium that it could not account for the high (up to 10% of ^{32}P present in the medium as RNA) incorporation observed. No radioactivity was found in the RNA of the parenchymal cells when they were incubated with a ribonuclease or alkaline digest of [^{32}P]RNA (Table 3). Further, the presence of ribonuclease along with [^{32}P]RNA in the incubation medium, in the presence or absence of Ca^{2+} , had an inhibitory effect on the maximal incorporation of radioactivity into the RNA of the parenchymal cells (Table 4); it has been shown that, though high-molecular-weight DNA can be taken up by ascites cells, deoxyribonuclease-treated DNA is not (Sung & Quastel, 1963). Moreover, since ribonuclease appears to be the most

widely distributed of nucleolytic enzymes present in mammalian tissues, the products of the degradation (if any) of [^{32}P]RNA of the medium are likely to be nucleoside 3'-phosphates, which even if transported into the cell could not serve as immediate precursors of RNA.

Although the present experiments do not rule out some adsorption of [^{32}P]RNA on the parenchymal cell surface (possibility *e*), they show that such adsorption alone could not account for all the radioactivity found in the RNA of the parenchymal cells after removal of the incubation medium. The radioactivity due to [^{32}P]RNA adsorbed as above would be expected to be confined to the 700g sediment (nuclear-plus-membrane fraction) obtained on subcellular fractionation of the parenchymal cells. The specific activity of RNA from the cell supernatant was more than twice, and that of RNA from the mitochondrial and microsomal fractions 50–60%, of the specific activity of RNA of the nuclear-plus-

Table 3. *Uptake of alkaline and ribonuclease digests of [^{32}P]RNA by liver parenchymal cells in suspension*

A 3 ml. portion of a solution (in the buffer) of [^{32}P]RNA (6.63 mg.; 264 000 counts/min.) prepared by method A from the whole liver of an animal injected with [^{32}P]phosphate was distributed equally in three tubes. The [^{32}P]RNA in one of the tubes was hydrolysed with alkali and in another with ribonuclease; the hydrolysates were evaporated to dryness and the residue was dissolved in each case in 1 ml. of the buffer. Samples (0.3 ml.) of the unhydrolysed [^{32}P]RNA and of the two hydrolysed [^{32}P]RNA preparations were incubated in separate tubes with 2.7 ml. of the parenchymal-cell suspension at 37° for the specified period. Details are given in the Experimental section.

RNA preparation in the incubation mixture	Sp. activity of RNA of the parenchymal cells in suspension (counts/min./mg.)		
	0 min.	60 min.	180 min.
[^{32}P]RNA	1260	2940	2100
Alkaline hydrolysate of [^{32}P]RNA	0	15	0
Ribonuclease hydrolysate of [^{32}P]RNA	0	0	0

Table 4. *Effect of ribonuclease on the uptake of [^{32}P]RNA by liver parenchymal cells in suspension*

A 3 ml. portion of the parenchymal-cell suspension in the medium stated was incubated at 37° for the specified period with 540 μg . (39 000 counts/min.) of [^{32}P]RNA prepared by method A from the whole liver of an animal injected with [^{32}P]phosphate. The concentration of ribonuclease when added to the incubation medium was 16.7 μg /ml. Details of method A and of the isolation of RNA from the incubated parenchymal cells are given in the Experimental section.

Medium	Addition	Sp. activity of RNA of the parenchymal cells in suspension (counts/min./mg.)	
		0 min.	60 min.
Krebs-Ringer original phosphate buffer containing glucose	None	175	1010
The above buffer	Ribonuclease	55	342
The above buffer made without CaCl_2	Ribonuclease	56	302

Table 5. *Intracellular distribution of [32 P]RNA taken up by liver parenchymal cells in suspension*

A 3 ml. portion of the parenchymal-cell suspension was incubated at 37° for the period stated with the specified concentration of [32 P]RNA prepared by method A from the whole liver of an animal injected with [32 P]phosphate. The specific activity of [32 P]RNA was between 40000 and 80000 counts/min./mg. Details of method A, of the preparation of the subcellular fractions from the incubated parenchymal cells and of the isolation of RNA from these fractions and from the unfractionated whole parenchymal cells are given in the Experimental section.

Expt. no.	Concn. of [32 P]RNA (μ g./ml.)	Time of incubation (min.)	Whole parenchymal cells	Sp. activity of RNA (counts/min./mg.)			
				Nuclear + membrane fraction	Mitochondrial fraction	Microsomal fraction	Supernatant fraction
1	197	30	4700	3960	2940	—	10400
2	450	30	3540	3120	2090	1880	—
3	217	0	124*	102*	260*	108*	780*

* Sp. activity (counts/min./mg.) of the washed trichloroacetic acid precipitate of the parenchymal cells.

membrane fraction (Table 5; Expt. 1 and 2). Since the mitochondrial, microsomal and supernatant fractions of the parenchymal cells, put together, contain more RNA than the nuclear-plus-membrane fraction, it is clear from the results in Table 5 that little, if any, of the radioactivity found in the RNA of the parenchymal cells could be due to adsorption of [32 P]RNA on the cell surface. The intracellular distribution of [32 P]RNA in the recipient parenchymal cells, observed by us, is similar to the intracellular distribution of [14 C]RNA (from liver) taken up by ascites-tumour cells, reported by Niu (1963).

On the basis of the above considerations, it can be concluded that one or more components of native homologous RNA or its partial degradation products ($S_{20,w} > 4s$) can be transported into the parenchymal cells in suspension, at least as macromolecular acid-precipitable RNA if not in the native form (possibility *f*). This investigation does not shed any light on the preferential uptake, if any, by the parenchymal cells, of various RNA types (messenger RNA, ribosomal RNA, soluble RNA). However, it suggests that these cells in suspension may be permeable to messenger RNA, since RNA prepared by method B from all the three tissue preparations used, namely whole liver, the parenchymal-cell suspensions and the 'soluble portion of the non-parenchymatous fraction of liver', stimulated amino acid incorporation into protein in a ribosomal system, the largest stimulation being obtained by RNA from the last-mentioned source which gave a single peak ($S_{20,w}$ 5–6s) in the ultracentrifuge and appeared relatively free of soluble RNA and ribosomal RNA (R. R. Reddy & P. M. Bhargava, unpublished work).

A significant incorporation of radioactivity from exogenous [32 P]RNA into the RNA of the paren-

chymal cells was observed in the zero-time samples (Fig. 1 and Table 1); this radioactivity was shown to be due to 32 P in internucleotide linkage (Table 2) and distributed in all the subcellular fractions, most of it being in the mitochondrial, microsomal and supernatant fractions (Table 5; Expt. 3). In these samples, though the addition of [32 P]RNA to the cell suspension and the subsequent centrifugations to separate the cells from the [32 P]RNA-containing medium were done at 4°, 10–15 min. was needed for the centrifugations. It therefore seems that there is initially a rapid temperature-independent (between 4° and 37°) uptake of [32 P]RNA by liver parenchymal cells in suspension. A comparison of the time-course of the uptake at 4° and 37° is given in Fig. 2. Fig. 2 shows that, after the initial rapid uptake of [32 P]RNA at 4°, further uptake by the parenchymal cells in suspension is temperature-dependent; the increase in the first 30 min. (at which period the cells were found to contain the maximum amount of [32 P]RNA) in the amount of [32 P]RNA found in the cells, over the zero-time value, was, at 37°, four times that at 4°.

Effect of concentration of [32 P]RNA on its uptake by the parenchymal cells. When increasing amounts of [32 P]RNA were added to a parenchymal-cell suspension containing 2.3×10^6 cells/ml., the quantity of [32 P]RNA (undegraded to acid-soluble components) found inside the cells at 60 min. increased linearly up to a [32 P]RNA concentration of about 100 μ g./ml., after which it fell (Fig. 3). From the results in Fig. 3 and the assumptions that the average dry weight of parenchymal cells is 1.5 mg./ 10^6 cells (Iype & Bhargava, 1963, 1965) and that the cells contain 10% of RNA on dry-weight basis, it can be calculated that, at an extracellular [32 P]RNA concentration of 39, 79, 197, 1276 and

Fig. 2. Comparison of the uptake by liver parenchymal cells in suspension of $[^{32}\text{P}]\text{RNA}$ at 37° and 4° : O, 37° ; ●, 4° . The cell suspension was incubated at the specified temperature for various periods in 3 ml. of the buffer with $[^{32}\text{P}]\text{RNA}$ ($717 \mu\text{g.}$, 19100 counts/min.) prepared by method B from the whole liver of an animal injected with $[^{32}\text{P}]\text{phosphate}$. Details of incubation, of method B and of the isolation of RNA from the incubated parenchymal cells are given in the Experimental section; the specific activity of this RNA is plotted against the time of incubation.

Fig. 3. Effect on the uptake of $[^{32}\text{P}]\text{RNA}$ by liver parenchymal cells in suspension, of the concentration of the RNA in the medium. A 3 ml. portion of the parenchymal-cell suspension (7×10^6 cells) was incubated at 37° for 60 min. with the stated amount of $[^{32}\text{P}]\text{RNA}$ (specific activity 40000 counts/min./mg.) prepared by method A from the whole liver of an animal injected with $[^{32}\text{P}]\text{phosphate}$. Details of method A and of the isolation of RNA from the incubated parenchymal cells are given in the Experimental section; the specific activity of this RNA is plotted against the initial concentration of $[^{32}\text{P}]\text{RNA}$ in the incubation medium.

Table 6. Fate of radioactivity taken up by liver parenchymal cells in suspension incubated with $[^{32}\text{P}]\text{RNA}$

A 9 ml. portion of the parenchymal-cell suspension was incubated at 37° for 30 min. with 3.6 mg. of $[^{32}\text{P}]\text{RNA}$ (specific activity 40000 counts/min./mg.) prepared by method B from the whole liver of an animal injected with $[^{32}\text{P}]\text{phosphate}$. The cells were then washed and resuspended in 9 ml. of the buffer, and 3 ml. portions incubated at 37° , one for each time point; details are given in the Experimental section. From each sample, the RNA and the acid-soluble fractions of the parenchymal cells and of the medium were isolated and their radioactivity was determined as described in the Experimental section. All the operations were performed quantitatively. The radioactivity data given are for 9 ml. of the original preincubated washed parenchymal-cell suspension. The values in parentheses are percentages on the total radioactivity (5300 counts/min.) in 9 ml. of the above cell suspension just before reincubation.

Incubation period (min.)	Radioactivity (counts/min.)									
	Cells					Medium				
	RNA fraction B	Acid-soluble fraction C	Total		RNA fraction E	Acid-soluble fraction F	Total, calculated E + F	Total (cells + medium)		
			Calculated B + C	Observed D				Calculated B + C + E + F	D + E + F	Theoretical G
0	3420 (64.6%)	1330 (25.0%)	4750 (89.6%)	4920 (92.8%)	97 (1.8%)	312 (5.9%)	409 (7.7%)	5160 (97.3%)	5330 (100.5%)	5300
60	650 (12.3%)	700 (13.2%)	1350 (25.5%)	1150 (21.7%)	840 (15.9%)	2640 (49.7%)	3480 (65.6%)	4830 (91.1%)	5630 (87.3%)	5300
120	415 (7.8%)	790 (14.9%)	1205 (22.7%)	1000 (18.9%)	1250 (23.6%)	2760 (52.0%)	4010 (75.6%)	5220 (98.3%)	5010 (94.5%)	5300

355 µg./ml., at 60 min. 10.3, 8.8, 5.7, 4.7 and 4.3% respectively of the extracellular RNA was found in the acid-precipitable form inside the cells, and that this RNA represented 1.2, 2.1, 3.4, 3.9 and 4.6% respectively of the RNA initially contained in the cells.

Fate of [³²P]RNA taken up by the parenchymal cells. The fall in the specific activity of intracellular RNA 30–60 min. after incubation of parenchymal cells with [³²P]RNA (Fig. 1 and Table 1), suggested that beyond this period the rate of the uptake of [³²P]RNA by the cells was exceeded by the rate of degradation of the RNA taken up. Rapid degradation of [³²P]RNA transported into the cells was confirmed by an experiment in which the uptake of the RNA was allowed to proceed for 30 min., and then the cells were washed, suspended in a medium containing no RNA (labelled or unlabelled) and reincubated for 1 or 2 hr. (Table 6). At the beginning of reincubation, almost all (92.8%) of the radioactivity in the cell suspension was located intracellularly, about three-quarters being in the acid-insoluble fraction of the cells. During reincubation, the radioactivity in the medium, expressed as a percentage of the total radioactivity in the cell suspension, increased from 7.7% at 0 hr. to 75.6% at 2 hr.; at the latter time-point about two-thirds of the radioactivity was in the acid-soluble fraction and one-third in the acid-insoluble fraction. These observations show that bulk of [³²P]RNA taken up by the parenchymal cells in 30 min. is degraded intracellularly to acid-soluble components within 2 hr., and that most of the degradation products leak into the medium from the cells; some of the [³²P]RNA taken up by the cells can also be transported back to the medium. The observed presence of [³²P]RNA in the medium after reincubation could not be due to breakage of cells since such a breakage should result in a concomitant increase in the protein and DNA content of the medium, and it has been shown (Layek Hussain & P. M. Bhargava, unpublished work) that little protein or DNA is released from the parenchymal cells in suspension on incubation for up to 3 hr. The possibility that the degradation of [³²P]RNA taken up by the cells occurs after the return of the RNA to the medium is unlikely since there was no evidence of the loss of intracellular nucleases to the medium. An uptake and loss of DNA, similar to that observed by us for RNA, has been reported for ascites cells by Baldwin (1961). Most of the transforming DNA taken up by *Haemophilus influenzae* has also been reported to be depolymerized and released into the medium soon after the uptake (Stuy, 1965).

The intracellular degradation of [³²P]RNA taken up by the parenchymal cells in suspension, was, like part of the uptake, temperature-dependent (Fig. 2).

Uptake of [³²P]RNA by liver slices and comparison with the uptake by the parenchymal-cell suspensions. Fig. 1 (Expt. 3) and Table 1 (Expts. 4, 6 and 7) give the results of a study of the uptake of [³²P]RNA (derived from whole liver) by rat-liver slices. The incorporation of radioactivity into the RNA of the slices proceeded at a much lower rate than that obtained with the parenchymal-cell suspensions; at 0 hr. the incorporation in the slices was 4–10% and at 30–60 min. (the period of peak incorporation in the cell suspensions) 10–20% of that obtained in the cells in suspension. By analogy with the cell suspensions, the [³²P]RNA found inside the slices in these experiments is likely to be derived from [³²P]RNA of the medium without degradation of the latter to acid-soluble components. The uptake of [³²P]RNA by the slices increased with time up to 3 hr., unlike in the cell suspensions (Fig. 1), suggesting that in the slices the rate of degradation, if any, of the [³²P]RNA taken up was at no time higher than the rate of its uptake. The uptake of [³²P]RNA by the slices, as by the cell suspensions, was qualitatively and quantitatively the same whether the RNA was isolated by method A (in which the tissue RNA was partially degraded) or by method B (in which most of the tissue RNA was not degraded).

The above observations suggest that the parenchymal cells in suspension are more permeable to homologous RNA than are the cells in tissue slices, and that dispersion of the tissue to a suspension of single cells results in the activation of an intracellular enzyme system that enhances the degradation of the RNA taken up by the cells. This view is supported by the observation that up to 25% of the cellular RNA is degraded on incubation of liver parenchymal-cell suspensions for 3 hr. under conditions similar to those used in the present study; the degradation in the tissue slices is much less, if any (Layek Hussain & P. M. Bhargava, unpublished work).

Effect of 2,4-dinitrophenol and sodium azide on the uptake of [³²P]RNA by the parenchymal-cell suspensions. The metabolic inhibitors, 2,4-dinitrophenol and sodium azide, have been shown to inhibit the uptake of transforming DNA by *H. influenzae* (Barnhart & Herriot, 1963). However, no significant inhibition by these substances of the uptake of [³²P]RNA by the parenchymal cells in suspension was observed (Table 7). This suggests either that the transport of RNA does not require energy, or that the energy requirements for the transport process are met by the normal complement of energy-rich compounds present in the fresh parenchymal-cell suspensions.

Effect of protamine sulphate and polyvinyl sulphate on the uptake of [³²P]RNA by the parenchymal-cell suspensions. Protamine sulphate has been shown to enhance the uptake of RNA and to prevent the

Table 7. *Effect of 2,4-dinitrophenol and sodium azide on the uptake of [³²P]RNA by liver parenchymal cells in suspension*

A 3 ml. portion of the parenchymal-cell suspension was incubated at 37° for the specified period with the stated amounts of the inhibitor and [³²P]RNA prepared by method A from the whole liver of an animal injected with [³²P]phosphate; the concentration of [³²P]RNA was 410 and 220 µg./ml. in Expts. 1 and 2 respectively, and the specific activity was approx. 50000 counts/min./mg. Details of method A and of the isolation of RNA from the incubated parenchymal cells are given in the Experimental section.

Expt. no.	Inhibitor		Period of incubation (min.)	Sp. activity of RNA of the parenchymal cells in suspension (counts/min./mg.)
	Name	Concn. (mm)		
1	2,4-Dinitrophenol	0.0	30	945
		2.5	30	905
	Sodium azide	0.0	30	810
		1.0	30	757
		5.0	30	800
2	2,4-Dinitrophenol	0.0	60	266*
		5.0	60	230*

* Sp. activity (counts/min./mg.) of dry cells.

Table 8. *Effect of protamine sulphate and polyvinyl sulphate on the uptake of [³²P]RNA by liver parenchymal cells in suspension*

A 3 ml. portion of the parenchymal-cell suspension was incubated at 37° for the specified period with [³²P]RNA isolated by method B from the whole liver of an animal injected with [³²P]phosphate; the concentration of [³²P]RNA was 341 and 227 µg./ml. in Expts. 1 and 2 respectively, and the specific activity was approx. 20000 counts/min./mg. The concentrations of protamine sulphate and polyvinyl sulphate were 50 and 33 µg./ml. respectively. Details of method B and of the isolation of RNA from the incubated parenchymal cells are given in the Experimental section.

Expt. no.	Addition to incubation medium	Sp. activity of RNA of the parenchymal cells in suspension (counts/min./mg.)			
		0 min.	60 min.	120 min.	180 min.
1	None	630	1420	—	—
	Protamine sulphate	790	1660	—	—
2	None	—	1340	545	404
	Protamine sulphate	—	865	435	338
	Polyvinyl sulphate	—	1210	490	274

degradation of the RNA taken up in chick-embryo fibroblasts (Amos, 1961; Amos & Kearns, 1963). In the present study, protamine sulphate or polyvinyl sulphate did not appear to have a significant effect on either the uptake of [³²P]RNA by the parenchymal cells in suspension, or the degradation of the RNA taken up (Table 8); this may be partly due to the inability of these substances to be transported in the parenchymal cells.

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