Metabolism of Tissue Cells in Suspension

SYNTHESIS OF RIBONUCLEIC ACID BY LIVER CELLS IN SUSPENSION

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1. Liver cells in suspension are shown to incorporate several RNA precursors into their RNA. 2. The incorporation of [^{32}P]phosphate and [^{14}C]adenine into the RNA of the cell suspension is usually of the same order as that in the perfused (or unperfused) liver slices. However, the initial lag in the incorporation of adenine into the RNA of the cell suspensions is much longer than that obtained for the tissue slices, and the optimum incorporation of adenine in the former, unlike that in the latter, needs exogenous glucose and probably a high concentration of phosphate. 3. The cell suspensions also differ from the tissue slices in being unable to incorporate [^{14}C]orotic acid into their RNA, and resemble tumour tissues in incorporating uracil into their RNA at a rate significantly higher than that obtained with the tissue slices. 4. The above differences in the metabolic behaviour of liver-cell suspensions and tissue slices are considered to be due to the different levels of organization of the liver cells in the two tissue preparations.

Though a considerable amount of work has been done on the metabolic behaviour of tissue slices, homogenates and subcellular fractions, very little is known about the metabolic activities of tissue cells in suspension, when the cells are not organized in tissue-specific patterns. In the present paper we describe some studies on the incorporation of nucleic acid precursors into the total RNA of hepatic cells obtained in suspension by a method described by Jacob & Bhargava (1962). Several significant differences have been found between the cell suspensions and the tissue slices with regard to the synthesis of RNA; though liver-cell suspensions can incorporate [^{32}P]phosphate and [^{14}C]adenine into their RNA at overall rates comparable with those obtained in the perfused tissue slices and [^{14}C]uracil at rates that are usually higher than that obtained in the tissue slices, the cell suspensions, unlike the tissue slices, are unable to incorporate [^{14}C]orotic acid into their RNA.

EXPERIMENTAL

Animals. Adult Wistar albino rats (Nutrition Research Laboratory's inbred strain) of known sex, age and weight (usually more than 150 g.), fed *ad libitum*, were used. All the tissue preparations used in any one experiment were obtained from the same liver.

Radioactive chemicals. [^{32}P]Phosphate was obtained from the Atomic Energy Establishment, Bombay, as orthophosphate dissolved in 0.9% NaCl buffered with

phosphate buffer to give a concentration of 1 mg. of P/ml. (specific radioactivity 1 mc/mg.). [$8\text{-}^{14}\text{C}$]Adenine, [$6\text{-}^{14}\text{C}$]orotic acid and [$2\text{-}^{14}\text{C}$]uracil were obtained from The Radiochemical Centre, Amersham, Bucks., and were dissolved in 0.9% NaCl to give concentrations of 2.54, 0.9 and 0.9 $\mu\text{moles/ml.}$ respectively; each of these solutions contained 9.1 $\mu\text{C/ml.}$

Buffers. The tissue preparations were incubated in one of the following buffers (Dawson, Elliott, Elliott & Jones, 1959): (a) Tyrode solution; (b) Krebs-Ringer original phosphate buffer; (c) Krebs-Ringer bicarbonate buffer. Glucose was added to the last two buffers to a final concentration of 15 mM, unless otherwise mentioned.

Preparation of liver slices. Slices were cut freehand with a razor blade. When perfused and unperfused slices were used in the same experiment, they were obtained from the same liver, one lobe of which was not perfused. Unless otherwise mentioned, the slices used were derived from perfused liver.

Preparation of liver-cell suspensions. Liver-cell suspensions were prepared by the method of Jacob & Bhargava (1962). When the effect of deletion of glucose from the incubation medium was studied, glucose was omitted from the perfusion fluid.

Incubations. The tissue preparation [50–150 mg. wet wt. (approx. 10–30 mg. dry wt.) of slices, or 6×10^6 – 25×10^6 hepatic-cord cells (approx. 9–38 mg. dry wt.) in suspension, per ml. of the incubation medium] was incubated in the desired buffer with the radioactive precursor in 25 ml. conical flasks in a water bath at 37° with shaking at 100 oscillations/min. All the incubations were commenced within 30 min. of killing the animal. Separate zero-time samples were run for each tissue preparation in every experiment.

Isolation of the total nucleic acids. The total nucleic acids (RNA + DNA) were isolated by one of the following three methods.

Method I. At the end of the desired period of incubation, ice-cold trichloroacetic acid was added to a final concentration of 10% (w/v). The precipitate was washed twice with 5% (w/v) trichloroacetic acid at 4° and the pH was brought to 4–5 by repeated washing in the cold with 5% (w/v) NaCl. Lipids were removed in the usual way and the residue was extracted thrice, each time with 1–2 ml. of 10% (w/v) NaCl at 98° for 30 min. Ethanol (3–4 vol.) was added to the NaCl extract and the mixture allowed to stand for 24–48 hr. at 3° (at –15° in some cases). The precipitated nucleic acid was washed twice with cold ethanol, dissolved in water and purified by reprecipitation with ethanol.

Method II. This method was the same as that employed by Schneider & Potter (1958); the tissue preparation was treated with cold 10% (v/v) perchloric acid and the nucleic acids were extracted by hot 10% NaCl directly (without the prior removal of lipids) from the washed and neutralized perchloric acid precipitate. The sodium nucleates were purified by two precipitations with 3–4 vol. of ethanol each time, as in method I.

Method III. This method was essentially the same as that employed by Hershey, Dixon & Chase (1953) and Iverson & Giese (1957). An equal volume of ethanol–ether (3:1, v/v) was added to the incubated samples. The mixture was quickly heated at 50° for 10 min. and centrifuged; the residue was washed once each with ethanol and ether, treated with 10% trichloroacetic acid in the cold, washed with 5% trichloroacetic acid, suspended in cold 10% NaCl (3–4 ml.) and neutralized with KOH. The mixture was centrifuged in the cold; the total nucleic acids were extracted from the sediment by hot 10% NaCl and purified as in method I.

For the same tissue preparation the yield of the total nucleic acids (or of the RNA fraction obtained from the total nucleic acids as described in the next section) per unit weight of liver, in the various experiments, was slightly lower when method III was used than that obtained with methods I or II.

Separation of the RNA fraction from the total nucleic acids. RNA was obtained in the hydrolysed form by treatment of the total nucleic acid fraction with 0.3 N-KOH (0.2 ml./mg. of nucleic acid) at 37° for 18 hr. (for only 5 hr. in those experiments in which it was intended to determine the specific activity of the constituent nucleotides), followed by adjustment of the pH to 4 in the cold with 0.6 N-perchloric acid and removal of the DNA-KClO₄ precipitate by centrifugation in the cold; the precipitate was washed twice with cold distilled water and the combined supernatants were used for the estimation of RNA, for the determination of radioactivity in RNA, and for the separation and determination of radioactivity of the constituent nucleotides.

In some experiments in which DNA was not removed from the total nucleic acid preparation, the specific activity values for different time points for the same tissue preparation are comparable; the values obtained for one tissue preparation cannot be strictly compared with those obtained for the other tissue preparation, but the values may be taken as indicative of the extent of incorporation in the tissue preparation.

The yield of total nucleic acids or of RNA per unit weight

of liver was significantly less in the cell suspensions than in the slices for every method of extraction used; this may be partly due to a loss of the cells other than the hepatic-cord cells from liver during the preparation of the suspensions.

Base analysis. A known amount of the total nucleic acid fraction was hydrolysed with 72% (v/v) perchloric acid (0.016 ml./mg. of nucleic acids) at 100° for 1 hr. (Marshak & Vogel, 1951). The hydrolysate was diluted with water and centrifuged. The bases were determined according to the method of Wyatt (1951).

Separation of nucleotides. The ribonucleotides obtained by alkaline hydrolysis were separated by paper electrophoresis at 4° in citrate buffer, pH 3.5, at a potential gradient of 14 v/cm. in a horizontal chamber (Davidson & Smellie, 1952a).

Estimation of nucleic acids. The amount of nucleic acid in the various extracts was determined by measurement of the extinction at 260 m μ in either a Beckmann or a Hilger-Watts ultraviolet spectrophotometer. An extinction value of 1.0 in the neutralized RNA fraction after alkaline hydrolysis was taken to correspond to 33 μ g. of RNA/ml. In solutions containing unhydrolysed mixed nucleic acids (RNA + DNA) an extinction value of 1.0 was taken to correspond to 40 μ g. of total nucleic acid/ml.

Measurement of radioactivity. A measured volume of the solution containing a known amount of nucleic acid, base or nucleotide was plated on an aluminium planchet (2 cm.²). The radioactivity was measured in either a Philips or an Ekco end-window Geiger-Müller counter, each with 6 and 10% counting efficiency for ¹⁴C and ³²P respectively for a counting area of the size of the planchets used. All the radioactivities for ³²P-containing samples have been corrected for decay to an initial specific activity of 1 mc/mg. of P.

In experiments with radioactive adenine, uracil or orotic acid as the precursor, no radioactivity was found in the RNA or the total nucleic acids from the zero-time samples. In experiments with radioactive phosphate, correction was made for the small amount of radioactivity (less than 100 counts/min./mg. of nucleic acid) usually present in the zero-time samples.

Concentration of cells in the suspensions. This was determined on a haemocytometer.

RESULTS

Since liver-cell suspensions have not been used previously for studies on nucleic acid metabolism, and provide a virtually new material for most biochemical studies, it was considered worth while to investigate the extent of the reproducibility of the results obtained with this tissue preparation. Therefore a series of experiments was performed with each isotopically labelled precursor.

Incorporation of [³²P]phosphate. The specific activities of RNA derived from the cell suspensions and the tissue slices incubated with [³²P]phosphate in a series of experiments are given in Table 1. The incorporation of [³²P]phosphate into the RNA of the cell suspensions was generally of the same order as that in the tissue slices; the incorporation in the former was, in most experiments, more than

Table 1. Variation in the incorporation of [^{32}P]phosphate into the RNA of rat-liver slices and cell suspensions

Incubations were carried out in 3 ml. of Krebs–Ringer bicarbonate buffer. The [^{32}P]phosphate concentration (including the phosphate of the buffer) was $33.3\text{ }\mu\text{C}$ ($69.8\text{ }\mu\text{g. of P}$)/ml. The mixed nucleic acids (RNA + DNA) from which the RNA was obtained in the hydrolysed form were isolated by method I (see the text).

Expt. no.	Period of incubation.....	Specific activity of RNA (counts/min./mg.)							
		Cell suspensions				Perfused liver slices			
		60 min.	90 min.	120 min.	180 min.	60 min.	90 min.	120 min.	180 min.
1		1520	—	2920	3690	3490	—	7380	9240
2		1430	—	3360	3140	—	—	—	—
3		7150	—	14600	19600	7050	—	15500	27400
						9060†	—	—	27800†
4*		—	3060	—	7540	—	8380	—	13400
5		—	4170	—	6500	—	5080	—	5400
6		—	3700	—	9780	—	8710	—	31800
7		—	970	—	1460	—	1540	—	2440
8		—	5140	—	65200	—	6120	—	43800
9		—	3840	—	8360	—	3240	—	—

* Incubations were carried out in 2 ml. The [^{32}P]phosphate concentration was $50\text{ }\mu\text{C}$ ($86.5\text{ }\mu\text{g. of P}$)/ml.

† Unperfused liver slices.

half (and in some considerably higher than) that in the latter. The variation from experiment to experiment in the extent of incorporation obtained at any one time point in the cell suspensions was also of the same order as that in the tissue slices. The incorporation in the cell suspensions usually proceeded linearly for 90–180 min. and then fell, as in the perfused tissue slices. In some experiments there was a lag, occasionally of considerable magnitude (Expt. 8 in Table 1), in the incorporation in both the tissue preparations. The ratio of the incorporation in the slices and in the cell suspensions was usually of the same order for the different time points in any one experiment.

In the above experiments the RNA fraction was obtained by alkaline hydrolysis of the total nucleic acid fraction isolated by method I. This method has been shown to give no detectable radioactive non-nucleic acid phosphorus-containing compounds ('concomitant phosphorus'; Davidson, Frazer & Hutchison, 1951; Davidson & Smellie, 1952b) in the RNA fraction from rat-liver microsomes labelled with [^{32}P]phosphate *in vivo* (Bhargava, Simkin & Work, 1958), and very little of such compounds, if any, in the RNA fraction from unperfused whole rat liver labelled similarly (Davidson & Smellie, 1952b). The presence of 'concomitant phosphorus' in the RNA fraction could not, however, be completely ruled out in the present experiments, which used cell suspensions and perfused slices, and in which the incorporation of [^{32}P]phosphate was studied *in vitro*. Therefore in three experiments (Table 2) the ribonucleotides

were separated by electrophoresis and their specific activities determined. In all instances the specific activity of RNA calculated from that of the nucleotides was between 66 and 100% of that determined on the RNA fraction before hydrolysis. Insufficient 'concomitant phosphorus' was present to vitiate the results of Table 1.

Incorporation of [^{14}C]adenine. The results of a series of experiments in which the incorporation of [^{14}C]adenine into the RNA or the total nucleic acids of liver cells in suspension and of liver slices was studied under various conditions are given in Table 3; the results of a typical experiment are illustrated in Fig. 1. No incorporation into the RNA or the total nucleic acids was obtained in the cell suspensions when the suspending medium was Tyrode solution (Expts. 1–3 in Table 3), whereas Krebs–Ringer original phosphate buffer gave excellent incorporation in every experiment (Expts. 4–10 in Table 3). The cell suspensions differed in this respect from the perfused tissue slices, which incorporated [^{14}C]adenine to about the same extent in both the buffers. The reason for the lack of any incorporation of [^{14}C]adenine into the RNA of the cell suspensions incubated in Tyrode solution is not fully clear; since the two incubation media differed significantly only in the concentration of phosphate, which (as P) is over 50-fold higher in Krebs–Ringer original phosphate buffer than in Tyrode solution, it seems possible that the incorporation of adenine into the RNA of liver cells in suspension requires the presence of a high concentration of extracellular phosphate. The concentration of phosphate (as P,

Table 2. *Distribution of radioactivity between the different nucleotides obtained by the hydrolysis of RNA derived from rat-liver slices and cell suspensions incubated with [³²P]phosphate*

Expts. 1, 2 and 3 are Expts. 6, 8 and 9 respectively of Table 1; details are given in Table 1. The base ratio for the RNA of unperfused rat liver reported by Fleck & Munro (1962) is: guanine, 100; adenine, 56; uracil, 56; cytosine, 83.

Expt. no.	Type of preparation	Period of incubation (min.)	Specific activity of RNA (counts/min./mg.)			Specific activity of the nucleotides (counts/min./mg.)				Base ratio of RNA			
			Observed	Calc. from specific activities of the nucleotides		Guanilylic acid	Adenylic acid	Uridylic acid	Cytidylic acid	Guanine	Adenine	Uracil	Cytosine
1	Perfused liver slices	90	8710	6320		6080	6550	4450	6430	—	—	—	—
		180	9780	10200*		11000	14100	6670	—	100	50	44	—
2	Perfused liver slices	90	6120	6120*		3640	—	7510	8040	100	—	56	82
		180	43800	37100		31400	41600	44200	30300	100	50	53	71
	Cell suspension	90	5140	5590		3600	8820	6320	4890	100	44	53	73
		180	65200	52400		47600	74400	54400	34300	100	45	41	81
3	Perfused liver slices	90	3240	2240		1620	3090	1860	2300	100	—	50	—
		90	3840	3930		2230	5650	3480	4460	100	—	45	77
	Cell suspension	180	8360	5480		2930	7190	6070	6120	—	—	—	—

* For these calculations the specific activity of cytidylic acid was assumed to be the same as that of uridylic acid in Expt. 1, and that of adenylic acid one and a half times that of guanylic acid in Expt. 2.

Table 3. Incorporation of [8-¹⁴C]adenine into the nucleic acids of rat-liver slices and cell suspensions

Details of methods I and II are given in the text. Incubations were carried out in 2 ml. of either Tyrode solution (TYR) or Krebs-Ringer original phosphate buffer (KRP). The [¹⁴C]adenine concentration was 0.46 μ C (0.13 μ -mole)/ml.

Expt. no.	Incubation medium	Method of isolation of total nucleic acid fraction	Nucleic acid preparation	Period of incubation...	Specific activity of nucleic acid preparation (counts/min./mg.)							
					Cell suspensions				Perfused liver slices			
					90 min.	120 min.	180 min.	240 min.	90 min.	120 min.	180 min.	240 min.
1	TYR	I	RNA		—	0	0	—	—	135	153	—
2	TYR	I	RNA+DNA		—	0	0	—	—	325	548	—
3	TYR	I	RNA+DNA		—	0	0	0	—	338	402	435
4	TYR	II	RNA+DNA		—	0	0	0	—	452	491	640
5	KRP	II	RNA		55	—	168	—	344	—	406	—
6	KRP	II	RNA		—	194	576	1270	—	402	634	820
7	KRP*	II	RNA		—	145	388	404	—	410	641	855
8	KRP	I	RNA		—	66	—	935	—	348	—	540
9	KRP	II	RNA		—	95	—	1470	—	500	—	707
10	KRP	I	RNA		—	81	153	653	—	432	669	—
11	KRP	II	RNA+DNA		19†	39	80	382	124†	362	497	498
12	KRP	II	RNA+DNA		—	175	—	899	—	432	—	606
13	KRP	II	RNA+DNA		—	289	—	438	—	1020	—	1210

* Without glucose.

† 60 min.

Fig. 1. Incorporation of [¹⁴C]adenine into the RNA of rat-liver-cell suspensions (O) and perfused liver slices (Δ). Incubations were carried out in 2 ml. of Krebs-Ringer original phosphate buffer. The [¹⁴C]adenine concentration was 0.40 μ C (0.13 μ mole)/ml. RNA was obtained in the hydrolysed form by treatment of the total nucleic acid fraction (isolated by method II) with alkali as described in the text.

including the radioactive phosphate added) in Krebs-Ringer bicarbonate buffer, which was used for studies on the incorporation of [³²P]phosphate and which gave good incorporation of the precursor into the RNA of the perfused slices as well as of the cell suspensions, was also much (sevenfold) higher than in Tyrode solution.

Omission of glucose from Krebs-Ringer original phosphate buffer had no effect on the incorporation of adenine into the RNA of the tissue slices (which are known to be able to use their store of glycogen), but decreased the incorporation in the cell suspensions by nearly 25% at 2 hr. and by over 70% at 4 hr. (Expt. 5 in Table 3).

Method II gave 30–60% greater radioactivity in RNA than did method I (Expts. 3 and 6 in Table 3), suggesting that a small amount of RNA that gets labelled faster than the bulk of the cellular RNA is lost in both the tissue preparations during the removal of lipids from the trichloroacetic acid precipitate in method I.

The perfused tissue slices exhibited at most a slight lag (Expt. 8 in Table 3), after which the incorporation proceeded almost linearly up to 2–4 hr.; in most experiments the rate of incorporation fell after 3 hr. In the cell suspensions, however, in almost all the experiments, the lag appeared to extend up to 4 hr., the maximum period of incubation tried. Up to 3 hr. the incorporation in the slices was always more (usually several-fold) than that in the cell suspensions, but by the end of the

Table 4. *Distribution of radioactivity between the different bases obtained on hydrolysis of the total nucleic acids derived from rat-liver slices and cell suspensions incubated with labelled precursors for 4 hr.*

Details of Expts. 1 and 2 are as given for Expt. 9 of Table 3 and Expt. 5 of Table 5 respectively; in Expt. 1 the two tissue preparations were derived from two different animals.

Expt. no.	Tissue preparation	Precursor	Specific activity of total nucleic acids (counts/min./mg.)	Specific activity of bases (counts/min./mg.)				
				Guanine	Adenine	Cytosine	Uracil	Thymine
1	Perfused liver slices	[¹⁴ C]Adenine	1020*	777	9060	0	0	0
	Cell suspension	[¹⁴ C]Adenine	899	744	6370	0	0	0
2	Perfused liver slices	[¹⁴ C]Orotic acid	2480	0	0	4920	11100	0
	Cell suspension	[¹⁴ C]Orotic acid	127	0	0	347	656	0

* From slices incubated for 2 hr.

Table 5. *Incorporation of [6-¹⁴C]orotic acid into the nucleic acids of rat-liver slices and cell suspensions*

Details of methods I and II are given in the text. Incubations were carried out in 2.05 ml. of either Tyrode solution (TYR) or Krebs-Ringer original phosphate buffer (KRP). The [¹⁴C]orotic acid concentration was 0.67 μ C (0.066 μ mole)/ml.

Expt. no.	Incubation medium	Method of isolation of total nucleic acid fraction	Nucleic acid preparation	Period of incubation...	Specific activity of nucleic acid preparation (counts/min./mg.)							
					Cell suspensions				Perfused liver slices			
					90 min.	120 min.	180 min.	240 min.	90 min.	120 min.	180 min.	240 min.
1	TYR	I	RNA + DNA		0	0	0	—	143††	441†	707†	—
2	TYR	I	RNA		0	—	0	—	61	—	185	—
	TYR	I	RNA		—	—	—	—	290†	—	613†	—
3	KRP	II	RNA		0	—	0	—	384	—	439	—
4	TYR	II	RNA		—	0	—	0	—	161	—	236
	KRP	II	RNA		—	0	—	0	—	695	—	1000
	KRP*	II	RNA		—	0	—	0	—	470	—	1340
5	KRP	II	RNA + DNA		—	—	71	127	—	1940	2260	2480
6	TYR	II	RNA + DNA		—	—	—	—	—	450	—	570
	TYR	II	RNA + DNA		—	—	—	—	—	576†	—	836†
	KRP	II	RNA + DNA		—	—	—	—	—	281†	—	673†

* Without glucose.

† 60 min.

‡ Unperfused liver slices.

fourth hour of incubation the cell suspensions had incorporated, in most experiments, almost as much (and, in some, considerably more) labelled precursor into their RNA (or the total nucleic acids) than was incorporated by the tissue slices.

The specific activities of adenine and guanine obtained by the acid hydrolysis of the total nucleic acid fraction derived from the cell suspensions and the perfused tissue slices that had been incubated with [¹⁴C]adenine were, in one experiment, approx. 700 and 80% (for adenine and guanine respectively) of the specific activity of undegraded mixed nucleic acid preparation for the cell suspensions, and

approx. 900 and 70% respectively for the tissue slices (Table 4). In either case the observed radioactivity in the purine bases accounted for the entire radioactivity contained in the unhydrolysed total nucleic acid fraction.

Incorporation of [6-¹⁴C]orotic acid. In most experiments [¹⁴C]orotic acid was not incorporated to any measurable extent into the RNA or into the total nucleic acid fraction of the cell suspensions, in contrast with the perfused (or unperfused) tissue slices, which incorporated this precursor to a considerable extent (Table 5). A slight incorporation (one-twentieth to one-thirtieth of that in the

tissue slices) was obtained in the cell suspension only in one experiment, after incubation for 3 hr.; in this experiment (Expt. 5 in Table 5) the incorporation in the tissue slices was exceptionally high, in fact the highest obtained in all the experiments.

The incorporation of labelled orotic acid in the perfused liver slices, either into RNA or into the total nucleic acid fraction, was lower than that in the unperfused tissue slices when the medium of incubation was Tyrode solution (Expts. 2 and 6 in Table 5). The incorporation of orotic acid into RNA, unlike that of adenine, was much lower in the perfused tissue slices incubated in Tyrode solution than in the slices incubated in Krebs-Ringer original phosphate buffer (Expt. 4 in Table 5), whereas the incorporation in the unperfused tissue slices was slightly more in Tyrode solution than in Krebs-Ringer original phosphate buffer (Expt. 6 in Table 5). These observations show that perfusion results in a loss of some factor (or condition) required for maximal incorporation of orotic acid into the tissue RNA, which factor is partially provided by (or can be partly replaced by) Krebs-Ringer original phosphate buffer but not by Tyrode solution. The nature of this factor is not fully clear; it may be phosphate. The incorporation of orotic acid (like that of adenine) in the perfused slices was not affected by the omission of glucose from Krebs-Ringer original phosphate buffer (Expt. 4 in Table 5).

In most experiments the rate of incorporation of orotic acid in both perfused and unperfused tissue slices fell after 2-3 hr.; in some the incorporation was linear up to 4 hr. (the maximum period of incubation tried) (Table 5).

The specific activities of cytosine and uracil

obtained by the acid hydrolysis of the total nucleic acid fraction in the only experiment in which any detectable incorporation of orotic acid in the cell suspension was obtained were approx. 275 and 500% respectively of the specific activity of the undegraded mixed nucleic acid preparation for the cell suspension, and approx. 200 and 450% respectively for the tissue slices (Table 4). In either case no radioactivity was detected in the purine bases and in thymine, and the observed radioactivity in the two pyrimidine bases accounted for the entire radioactivity contained in the unhydrolysed total nucleic acid fraction.

Incorporation of [2-¹⁴C]uracil. [¹⁴C]Uracil was, on an average, incorporated to a smaller extent than orotic acid into the RNA (or the total nucleic acid fraction) of perfused liver slices (Table 6; cf. Table 5), in accordance with previous observations (Heidelberger, Leibman, Harbers & Bhargava, 1957). The cell suspensions incorporated uracil to a significantly greater extent than the tissue slices, the incorporation at 4 hr. in the former being as much as 3-7 times that in the latter (Expts. 1-4 and 6 in Table 6) in five out of a series of six experiments; in the remaining experiment (Expt. 5 in Table 6) the incorporation in the two tissue preparations was nearly equal. The incorporation in unperfused tissue slices (Expt 1 in Table 6) was 20-25% less than that in the perfused tissue slices. In all the tissue preparations there was a considerable initial lag, and the rate of incorporation increased with time up to 4 hr. (the period of incubation tried).

The extent of incorporation of uracil into the total nucleic acid fraction from cell suspensions was about the same when the nucleic acids were obtained by method II as when they were isolated by method III.

Table 6. *Incorporation of [2-¹⁴C]uracil into the nucleic acids of rat-liver slices and cell suspensions*

Details of methods II and III are given in the text. Incubations were carried out in 2 ml. of Krebs-Ringer original phosphate buffer. The [¹⁴C]uracil concentration was 0.46 μ C (0.045 μ mole)/ml.

Expt. no.	Method of isolation of total nucleic acid fraction	Nucleic acid preparation	Period of incubation...	Specific activity of nucleic acid preparation (counts/min./mg.)			
				Cell suspensions		Perfused liver slices	
				120 min.	240 min.	120 min.	240 min.
1	III	RNA		54	669	31	213
	III	RNA		—	—	29*	162*
2	III	RNA + DNA		24	244	14	89
3	III	RNA		32	366	38	90
4	III	RNA + DNA		—	1210	—	—
	II	RNA + DNA		—	1270	—	—
5	II	RNA		44	523	—	600
6	II	RNA		—	850	—	123

* Unperfused liver slices.

hepatic cells after dispersion of the liver tissue could be due either to damage to the hepatic cells during the process of dispersion of the tissue (i.e. to a change not intrinsic to disorganization) or to an alteration in the level of biological organization of the liver cells on dispersion, leading to metabolic changes intrinsic to such disorganization. Although the former possibility cannot be ruled out, it seems unlikely for the reasons discussed elsewhere (Jacob & Bhargava, 1962; Iype & Bhargava, 1965). Significant differences were also found between the perfused and the unperfused slices with regard to the incorporation of pyrimidines into RNA. In general, the behaviour of the perfused slices, e.g. the preferential utilization of uracil compared with orotic acid, was in between that of the unperfused slices and the cell suspensions.

The base ratio of RNA derived from the cell suspensions was found to be about the same as that of the RNA of the perfused tissue slices (Fleck & Munro, 1962; Table 2). This is to be expected, as in the liver tissue the RNA of cells other than the hepatic cells is unlikely to represent more than a small fraction of the RNA of the hepatic cells (Iype & Bhargava, 1963).

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