Qualitative and Quantitative Aspects of the Biosynthesis of Ribonucleic Acid and of Protein in the Liver and the Lung of the Syrian Golden Hamster

By HANSPETER WITSCHI

*M.R.C. Group in Drug Toxicology, Department of Pharmacology, Faculty of Medicine,
Université de Montréal, Montréal, Que., Canada*

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1. The incorporation of orotic acid and of uridine into total RNA was measured *in vivo* in liver and lung of the Syrian golden hamster. Specific activities of total acid-soluble UMP were measured in both organs. An estimation of the rate of RNA biosynthesis showed that hamster lung synthesizes RNA at about one-half of the rate of that of hamster liver. 2. The apparent K_m and V_{max} values of a few enzymes involved in pyrimidine biosynthesis were measured in the 100000g supernatants of liver and lung. The apparent K_m values were very similar in both organs. From the estimated V_{max} , it was concluded that hamster lung cells have less capacity to metabolize orotic acid than have liver cells. 3. A time-response and a dose-response study showed that actinomycin D inhibits pulmonary RNA synthesis as efficiently as hepatic RNA synthesis. 4. Protein synthesis, measured as the incorporation of leucine, was inhibited in both organs 30min after a dose of 2mg of cycloheximide/kg. The dose-response patterns were similar in both liver and lung 3h after cycloheximide. 5. It is concluded that RNA and protein synthesis *in vivo* in hamster lung are very similar to the corresponding reactions in liver. Alterations of RNA and protein synthesis by toxic agents can therefore be evaluated in lung with a similar approach to that used to study the pathological biochemistry of liver.

Biochemical studies on the enzymology and metabolism of lung tissue are comparatively rare but more studies are being done on the non-respiratory function of this organ. A survey of the available data on pulmonary biochemistry reveals that the majority of work in this field has been done with rabbit lung (Reiss, 1966; Oppelt *et al.*, 1970; Matsubara & Tochino, 1971; Breyer, 1971; Hook *et al.*, 1972; Bend *et al.*, 1972). Other commonly used laboratory animals receive considerably less attention. There is, however, one species that is becoming increasingly popular for studying acute and chronic inhalation toxicology: the Syrian golden hamster. This animal provides a suitable model for studies on the development of bronchogenic carcinoma (Saffiotti *et al.*, 1968, 1972) and has also recently been used to evaluate the acute effects of air pollutants (Palmer *et al.*, 1971, 1972) and of tobacco-smoke and marijuana-smoke condensates (Okamoto *et al.*, 1972). Attempts have been made to examine the biochemistry of an isolated and defined cell population of the hamster respiratory tract (Kaufman *et al.*, 1972a,b). Therefore I decided to investigate some aspects of the hamster's normal pulmonary biochemistry *in vivo*. This paper reports studies on the incorporation of small-molecular precursors into pulmonary RNA and protein and compares the results with the same processes in the liver.

Experimental

Materials

The radioactive substances were purchased from New England Nuclear Corp., Waltham, Mass., U.S.A., and had the following specific radioactivities: [6-¹⁴C]orotic acid hydrate, 50–60Ci/mol; [carboxy-¹⁴C]orotic acid hydrate, 10.2Ci/mol; [2-¹⁴C]uridine, 54.1Ci/mol; DL-[1-¹⁴C]leucine, 20–30Ci/mol; DL-[4-¹⁴C]aspartic acid, 2.1Ci/mol; [carboxy-¹⁴C]orotidine 5'-monophosphate, 21Ci/mol; [2-¹⁴C]UMP, 24.1Ci/mol.

Special chemicals for the enzyme assays were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., except orotidine 5'-monophosphate (trilithium salt), which was from Calbiochem, Los Angeles, Calif., U.S.A., and cycloheximide (Actidione), from Upjohn Co., Kalamazoo, Mich., U.S.A. Actinomycin D was a gift from Merck, Sharp and Dohme, Pointe Claire, Que., Canada.

Animals

Non-inbred Syrian golden hamsters (80–100g) were purchased from the Canadian Breeding Farm and Laboratories Ltd., St. Constant, Que., Canada, and from ARS/Sprague-Dawley, Madison, Wis., U.S.A.

Administration of drugs and radioactive precursors

All injections were made intraperitoneally and injection volumes were 0.2–1.0 ml. Actinomycin D was dissolved in propylene glycol and 20-methyl-cholanthrene was dissolved in corn oil; all the other substances were dissolved in 0.15 M-NaCl.

Evaluation of RNA and protein synthesis

In all these experiments food was withdrawn from the animals the evening before the experiment. The incorporation of orotic acid and of uridine into RNA, the specific radioactivity and concentration in tissue of total acid-soluble UMP, the incorporation of leucine into protein and the tissue concentrations of DNA, RNA, protein and UMP were measured as described previously (Witschi, 1972). In one experiment RNA was labelled *in vivo* for a 15 min period with orotic acid. The acid-insoluble pellet was hydrolysed with 0.3 M-KOH at 37°C for 24 h. After acidification, neutralization with KOH and removal of the KClO₃, the hydrolysate was evaporated to dryness under vacuum at 40°C and the residue was redissolved in 0.05 M-HCl. Samples of this solution containing 10–20 E_{260} units were separated on columns (9 mm × 50 mm) of Dowex-50 into UMP, GMP and a fraction containing AMP and CMP (Katz & Comb, 1963). The radioactivity in each fraction was then measured.

Enzyme assays

The activities of aspartate carbamoyltransferase (EC 2.1.3.2) and uridine kinase (EC 2.7.1.48) were measured as described before (Witschi, 1970), except that in the assay for uridine kinase 2.5 μ mol of ATP was present. Conditions for measuring orotidine 5'-phosphate decarboxylase (EC 4.1.1.23) activity were those described by Handschuhmacher (1960). The combined activities of orotidine 5'-phosphate pyrophosphorylase (EC 2.4.2.10) and decarboxylase were measured as described by Bresnick *et al.* (1971). In both procedures the ¹⁴CO₂ evolved was trapped in 0.5 ml of hyamine hydroxide as described by Fox (1971). For measuring the kinetic constants, liver or lung tissue from two to four animals was pooled, homogenized in 5–10 vol. of 0.15 M-KCl and centrifuged for 60 min at 102000 $g_{av.}$. The supernatant was aspirated to give the enzyme preparation. K_m and V_{max} were then determined for several different enzyme preparations and from the values obtained from three to six preparations the mean $\pm$ S.E.M. was calculated. For obtaining K_m and V_{max} , s/v was plotted against s and the best straight line through the points (for four to six substrate concentrations) was calculated by the method of least squares (Wilkinson, 1961).

In all enzyme assays product formation was linear

within the time-period examined and proportional to the amount of protein in the incubation mixture.

Radioactivity measurements

All liquid samples were mixed with 10 ml of Aquasol (New England Nuclear Corp.) and counted for radioactivity in a Packard Tri-Carb model 3375 liquid-scintillation spectrometer. Hyamine-trapped ¹⁴CO₂ or DEAE-cellulose paper squares were counted for radioactivity in toluene containing 4.2% of Liquifluor (New England Nuclear Corp.). The results were converted from c.p.m. either into d.p.m. by automatic external standardization or, in the enzyme assays, into nmol of substrate transformed as described previously (Witschi, 1970).

Presentation of results

Unless otherwise stated, all results are given as mean values $\pm$ S.E.M. with the number of animals or assays given in parentheses. Significance levels were estimated with the Student's *t* test (Snedecor & Cochran, 1967).

Results

Biosynthesis of RNA and protein in hamster liver and lung

The kinetics of the incorporation of orotic acid and of uridine into total cellular RNA in both liver and lung were measured (Fig. 1). Both precursors had approximately the same specific radioactivity; 5 μ Ci of either orotic acid or uridine was injected intraperitoneally. After an initial lag period of 4 min, incorporation into RNA was linear for the next 12 min. Uridine yielded higher specific radioactivities of RNA than did orotic acid. Both hepatic and pulmonary RNA were labelled almost identically by uridine, whereas orotic acid seemed to give somewhat higher specific radioactivities of liver RNA than of lung RNA.

In the same experiment I determined the specific radioactivity of an intracellular RNA precursor. Ideally the specific radioactivities of UTP and CTP ought to be measured, since these two nucleotides are the immediate precursors in RNA biosynthesis. Determination of the triphosphates, however, is not easy and necessitates an experimental set-up (Bucher & Swaffield, 1966) that would be quite difficult to adopt to studies on the lung. Hydrolysis of all tri- and di-phosphates to the monophosphates seemed to avoid some of the problems associated with determination of the triphosphates. I also verified that after a short incorporation period (15 min) essentially all radioactivity from orotic acid was present in the 2'(3')-UMP of liver and lung RNA: 93.3 $\pm$ 3% (4) in liver RNA and 96 $\pm$ 3% (3) in lung RNA.

Fig. 1. Kinetics of incorporation of orotic acid or uridine into total liver and lung RNA

At zero time male hamsters received [^{14}C]orotic acid ($5\mu\text{Ci}$ per animal; sp. radioactivity 52.7Ci/mol) or [^{14}C]uridine ($5\mu\text{Ci}$ per animal; sp. radioactivity 60Ci/mol) intraperitoneally. The animals were killed at the times indicated on the abscissa and the specific radioactivities of total liver and lung RNA were determined. Each point represents the mean value from four animals. Orotic acid: ○, lung; ●, liver. Uridine: □, lung; ■, liver.

Therefore determination of the specific radioactivity and cellular concentration of total acid-soluble UMP was considered to give satisfactory information about the pool sizes of immediate RNA precursors. This has been found by others (Feigelson & Feigelson, 1966; Yu & Feigelson, 1969).

The data are presented in Fig. 2. The specific radioactivity of the UMP after injection of orotic acid rose linearly with time in both liver and lung. With uridine the increase in specific radioactivity was less regular in the two organs. The data were analysed in two ways: if it is assumed that the labelled molecules entering RNA during the incorporation period remain in RNA and if the specific radioactivity of the immediate precursor during this time rises linearly, the biosynthetic rate per unit time can be estimated as follows (Bucher & Swaffield, 1969):

$$\frac{\text{d.p.m. in RNA/unit of tissue}}{0.5 \times \text{d.p.m./}\mu\text{mol of UMP}} = \mu\text{mol of UMP incorporated into RNA/unit of tissue}$$

Fig. 2. Kinetics of labelling of the total acid-soluble UMP in liver and lung after injection of orotic acid or uridine

Data were obtained from the same animals as in Fig. 1. At zero time, all animals received either [^{14}C]orotic acid or [^{14}C]uridine intraperitoneally ($5\mu\text{Ci}$ per animal; same sp. radioactivities as in Fig. 1). The animals were killed at the time-interval indicated on the abscissa and the specific radioactivity of total acid-soluble UMP was determined as described by Feigelson & Feigelson (1966). Orotic acid: ○, lung; ●, liver. Uridine: □, lung; ■, liver.

These calculations were done by taking the values for the specific radioactivities of both RNA and UMP measured 8 min after the injection of either orotic acid or uridine. The reason for selecting this time-point was that in all experiments the specific radioactivity of UMP seemed to increase linearly up to this time. Values for total amount of RNA per unit of tissue, i.e. mg of RNA/mg of DNA, were taken from Table 1. The results are presented in Table 2.

A second way of estimating the rate of biosynthesis has been used previously to analyse data in which the specific radioactivity of UMP did not increase in a linear way (Witschi, 1972). In this approach the d.p.m. in RNA/mg of DNA and also the d.p.m. in total acid-soluble UMP/mg of DNA were calculated from the corresponding specific radioactivities and the concentrations of RNA and UMP in liver and lung. The values for mg of DNA, RNA, protein and nmol of total acid-soluble UMP per g of organ weight and per mg of DNA are given in Table 1. The sum of the d.p.m. in RNA and UMP then

Table 1. *Composition of hamster liver and lung*

The concentration of DNA/g wet wt. was determined with diphenylamine, that of RNA with orcinol, and that of protein by a modified biuret method. For the determination of protein in the 100000g supernatant the tissue was homogenized either with a Teflon-glass homogenizer as in the enzyme assays or by combining mechanical shear with ultrasound (Polytron homogenizer). The concentration of total acid-soluble UMP was determined as described earlier (Witschi, 1972). Reference substances were calf thymus DNA, yeast RNA and bovine serum albumin. The molar extinction coefficient of UMP in 0.05M-HCl was taken to be $9600\text{M}^{-1}\cdot\text{cm}^{-1}$ at 260nm (Katz & Comb, 1963).

	Amount per g wet wt.		Amount per mg of DNA	
	Liver	Lung	Liver	Lung
DNA (mg)	3.05 ± 0.13 (10)	6.18 ± 0.31 (10)		
RNA (mg)	8.42 ± 0.39 (10)	2.72 ± 0.13 (10)	2.76	0.44
Protein (mg)	157 ± 3 (19)	141 ± 5 (19)	51.5	22.8
Supernatant protein (mg)				
Teflon-glass	90.3 ± 4.9 (4)	58.9 ± 2.9 (4)	29.6	9.5
Polytron	90.9 ± 3.7 (4)	76.2 ± 11.0 (4)	29.8	12.3
UMP (nmol)	565 ± 24 (8)	1316 ± 99 (7)	185	213

Table 2. *Incorporation of radioactive precursors into RNA relative to pool size of total acid-soluble UMP*

The rate of biosynthesis of hepatic and pulmonary RNA was calculated by the two procedures outlined in the Results section. The values for the first 8min after injection of radioactive precursor (0-8) are given in parentheses as μmol of UMP/min per unit of tissue.

% of total UMP incorporated into RNA/unit of tissue				
	Time after injection (min)	Liver	Lung	Liver:lung
^[14C] Orotate	4	25.6	10.0	2.56
	8	32.2	14.5	2.22
	12	34.4	15.2	2.26
	16	39.0	16.2	2.41
	(0-8)	(0.022)	(0.009)	(2.44)
^[14C] Uridine	4	25.3	8.9	2.84
	8	37.8	15.8	2.39
	12	42.3	20.8	2.03
	16	46.1	26.9	1.71
	(0-8)	(0.028)	(0.010)	(2.80)

represents the total radioactivity in UMP/mg of DNA and this value is proportional to the number of cells. The amount of radioactivity in the RNA/mg of DNA is then expressed as a percentage of the total radioactivity in UMP/mg of DNA. These values, given in Table 2, can serve as some relative estimate of the rate of biosynthesis. By using both methods of calculation it becomes obvious that hamster lung is about half as effective as liver in incorporating uridine or orotic acid into RNA.

An analysis of some of the enzymes involved in pyrimidine biosynthesis showed that the apparent K_m values for uridine kinase, orotidine 5'-phosphate

decarboxylase and orotidine 5'-phosphate pyrophosphorylase were very similar in high-speed supernatants from liver and lung and only the apparent K_m for aspartate carbamoyltransferase was higher in lung. The values found agree reasonably well with data reported in the literature (Table 3). However, greater differences between liver and lung became obvious for the apparent V_{max} values (Table 4). All kinetic data were obtained from measurements in which high-speed supernatants of lung and liver homogenates were used as enzyme sources. Product formation was then calculated per mg of protein. In all these experiments both lung and liver were

Table 3. *Apparent K_m for some enzymes of pyrimidine biosynthesis in liver and lung*

Kinetic constants were determined in the 100000g supernatant from liver and lung by plotting s/v against s (Wilkinson, 1961). Values are means $\pm$ S.E.M. and the numbers of assays are given in parentheses.

Enzyme and substrate	$10^6 \times$ Apparent K_m (M)		$10^6 \times$ Reported K_m (M)
	Liver	Lung	
Uridine kinase: Uridine (ATP, 2.5mM)	190 $\pm$ 36 (5)	153 $\pm$ 10 (5)	Ehrlich ascites-tumour cells; 48 (Sköld, 1960)
Aspartate carbamoyltransferase: Aspartic acid (carbamoyl phosphate, 6.2mM)	1520 $\pm$ 160 (4)	6780 $\pm$ 400 (4)	Rat liver, 530 (Bresnick, 1963)
Carbamoyl phosphate (aspartic acid, 4.7mM)	—	106 $\pm$ 40 (4)	Rat liver, 250 (Bresnick, 1963)
Orotidine 5'-phosphate pyrophosphorylase and decarboxylase: Orotate (5-phosphorylribose 1-pyrophosphate, 0.37mM)	5.08 $\pm$ 1.0 (6)	8.58 $\pm$ 2.2 (5)	Rat liver, 3.4 (Hoffman & Sweeney, 1973)
Orotidine 5'-phosphate decarboxylase: Orotidine 5'-monophosphate	1.64 (2)	1.2 $\pm$ 0.1 (3)	Rat liver, 4.5 (Creasey & Handschuhmacher, 1961) and 0.7 (Hoffman & Sweeney, 1973)

Table 4. *Apparent V_{max} for some enzymes of pyrimidine biosynthesis in hamster liver and lung*

Apparent V_{max} values were calculated as nmol of substrate transformed/min per mg of protein under the assay conditions stated in the Experimental section. Values are means $\pm$ S.E.M. and the numbers of assays are given in parentheses. By multiplying this value by the amount of protein found in the 100000g supernatant from 1 g of tissue or per mg of DNA (see Table 1) the V_{max} /g of organ or V_{max} /unit of tissue was obtained.

Enzyme (and substrate)	Apparent V_{max} in liver			Apparent V_{max} in lung		
	nmol/min per mg of protein	nmol/min per g of tissue	nmol/min per unit of tissue	nmol/min per mg of protein	nmol/min per g of tissue	nmol/min per unit of tissue
Uridine kinase (uridine)	0.70 $\pm$ 0.05 (5)	63.2	20.72	1.77 $\pm$ 0.13 (5)	104.05	16.81
Aspartate carbamoyltransferase (aspartic acid)	2.63 $\pm$ 0.36 (4)	237.5	77.8	6.03 $\pm$ 1.76 (4) 4.55 $\pm$ 1.50 (4)	355.2 268.0*	57.3 43.2*
Orotidine 5'-phosphate pyrophosphorylase and decarboxylase (orotate)	0.118 $\pm$ 0.008 (6)	10.66	3.49	0.028 $\pm$ 0.014 (5)	1.65	0.27
Orotidine 5'-phosphate decarboxylase (orotidine 5'-phosphate)	0.220 (2)	19.9	6.51	0.048 $\pm$ 0.026 (3)	2.83	0.46

* The substrate is carbamoyl phosphate.

homogenized with a glass-Teflon homogenizer. From 1 g of liver 90.3mg of protein was obtained in the 100000g supernatant, whereas from 1 g of lung only 58.9mg of protein was obtained. This could be due to incomplete homogenization of the fibrous lung tissue; the experiment was therefore repeated by

using a homogenizer that combines mechanical shear with ultrasound (Polytron homogenizer). Under these conditions 1 g of liver yielded 90.9mg of protein in the supernatant, compared with 76.2mg for lung. With both methods lung has 2-3 times less protein in the 100000g supernatant/mg of DNA than

has liver (Table 1). If the apparent $V_{\max}$ value given in Table 4 is multiplied by the amount of 100000g supernatant protein derived from 1g of organ it becomes apparent that lung could transform per g wet wt. as much or more uridine and aspartic acid than does liver. Lung would, however, be inferior in metabolizing orotic acid or orotidine 5'-monophosphate. If the same data are calculated per mg of DNA the differences between lung and liver become even larger. All these considerations, of course, still necessitate the assumption that all enzyme activity or at least most of it was extracted in both organs into 100000g supernatant; the validity of this assumption remains to be proved.

The incorporation of leucine into total hepatic and pulmonary protein was determined for the first 16min after an injection of radioactive leucine. The specific radioactivities of both pulmonary and hepatic protein increased linearly during this time-period.

Studies with actinomycin D and cycloheximide

The effects of various amounts of actinomycin D on a 15min incorporation time of orotic acid into RNA were examined. A high dose of actinomycin D (5mg/kg) produced a significant decrease in labelling of RNA in liver and lung as early as 30min after administration; the effect was still present 8h after

actinomycin D. A dose-response study was done 4h after the injection of various amounts of actinomycin D and the results are given in Table 5(a). All but the lowest doses of actinomycin D significantly inhibited the incorporation of orotic acid into RNA in both organs in a dose-dependent manner. It was also found that actinomycin D (5mg/kg) increased somewhat the total acid-soluble UMP in liver but did not alter its specific radioactivity (Table 5b). The percentage of the total radioactive UMP incorporated into RNA was, however, substantially less in liver and lung of the treated animals compared with the controls (Table 5b).

The effects of cycloheximide on the incorporation of leucine into total liver and lung protein were measured at various times (Table 6a). Shortly after cycloheximide injection, protein synthesis was greatly inhibited in both organs, but recovered within the next 7h. A dose-response study done 3h after cycloheximide injection showed that the smallest dose of cycloheximide to inhibit protein synthesis significantly was 1 mg/kg (Table 6b).

Discussion

RNA and protein synthesis seem to proceed similarly in hamster lung and liver. In both organs the incorporation of intraperitoneally injected precursors into macromolecules (RNA and protein)

Table 5. *Effect of actinomycin D on RNA synthesis in liver and lung*

Hamsters were given various doses of actinomycin D intraperitoneally in (a) and 5mg/kg in (b); the control group received 0.15M-NaCl. After 4h the animals were given 12.5 μ Ci of [14 C]orotic acid (sp. radioactivity 57.7 Ci/nmol) and killed 15min later. The specific radioactivities of liver and lung RNA and UMP were determined. The significance of the difference (P) between controls and treated group is given. The numbers of animals are given in parentheses.

(a) Dose-response

Dose of actinomycin D (mg/kg)	RNA synthesis (d.p.m./mg of RNA)	
	Liver	Lung
None	5902 $\pm$ 603 (10)	3554 $\pm$ 297 (10)
0.31	3636 $\pm$ 903 (5); ($P > 0.05$)	2736 $\pm$ 683 (5); ($P > 0.05$)
0.63	2542 $\pm$ 365 (4); ($P < 0.05$)	2066 $\pm$ 213 (4); ($P < 0.05$)
1.25	1512 $\pm$ 116 (5); ($P < 0.05$)	1446 $\pm$ 213 (5); ($P < 0.05$)
2.50	903 $\pm$ 94 (5); ($P < 0.05$)	1206 $\pm$ 395 (5); ($P < 0.05$)
5.00	553 $\pm$ 85 (4); ($P < 0.05$)	770 $\pm$ 39 (4); ($P < 0.05$)

(b) Rate of biosynthesis

	RNA synthesis			
	Liver: controls	Liver: actinomycin D	Lung: controls	Lung: actinomycin D
d.p.m./mg of RNA	4319 $\pm$ 435 (5)	$P < 0.01$ 620 $\pm$ 226 (5)	3456 $\pm$ 496 (5)	$P < 0.01$ 790 $\pm$ 260 (5)
d.p.m./nmol of UMP	106 $\pm$ 11 (5)	$P > 0.05$ 92 $\pm$ 5 (5)	27 $\pm$ 4 (5)	$P > 0.05$ 30 $\pm$ 4 (5)
nmol of UMP/g of tissue	528 $\pm$ 22 (6)	$P > 0.05$ 891 $\pm$ 142 (5)	1482 $\pm$ 163 (6)	$P > 0.05$ 1328 $\pm$ 211 (5)
% of total UMP in RNA	34	4.6	14	3

Table 6. *Effects of cycloheximide on incorporation of leucine into liver and lung protein*

Hamsters were injected intraperitoneally with cycloheximide. The incorporation of [^{14}C]leucine ($10\mu\text{Ci}$ per animal; sp. radioactivity 21.3Ci/mol) was measured after an incorporation time of 15 min (Witschi & Aldridge, 1968). The numbers of animals, and statistical significance (P) where appropriate, are given in parentheses.

(a) Time-response

Time (h) after dose (2mg/kg)	Incorporation (d.p.m./mg of protein)			
	Liver		Lung	
	Controls	Treated	Controls	Treated
0.5	791 ± 28 (4)	189 ± 28 (4)*	386 ± 43 (4)	96 ± 19 (4)*
1.5	730 ± 75 (4)	403 ± 31 (4)*	348 ± 34 (4)	224 ± 24 (4)*
7	824 ± 99 (4)	881 ± 39 (4)	320 ± 49 (4)	401 ± 51 (4)
24	879 ± 81 (4)	971 ± 56 (4)	251 ± 31 (4)	322 ± 16 (4)

(b) Dose-response

(b) Dose (mg/kg)	Incorporation (d.p.m./mg of protein)	
	Liver	Lung
None	836 ± 39 (7)	356 ± 11 (7)
0.5	702 ± 55 (5); ($P > 0.05$)	391 ± 27 (5); ($P > 0.05$)
1.0	400 ± 65 (5); ($P < 0.01$)	202 ± 30 (5); ($P < 0.01$)
2.0	215 ± 63 (5); ($P < 0.01$)	101 ± 32 (5); ($P < 0.01$)
4.0	88 ± 16 (5); ($P < 0.01$)	41 ± 5 (5); ($P < 0.01$)

* Significantly different from controls.

follows linear kinetics over short incorporation periods. An estimate of the rate of biosynthesis *in vivo* showed that in lung RNA synthesis proceeds at about half the speed of that in liver. Time-response and dose-response to actinomycin D and cycloheximide were identical in the two tissues. Lung therefore takes up those two inhibitors as readily as does liver; also it can be inferred from the data that the toxicologically active concentrations in lung are very similar to those in liver.

Another similarity between the two organs was found by measuring the apparent K_m of several enzymes involved in pyrimidine biosynthesis; it seems that these enzymes are very similar if not identical in both liver and lung. This conclusion agrees with recent observations on rabbit microsomal preparations: the mixed-function oxidase system of the lung seems to be very similar to that of the liver (Bend *et al.*, 1972). On the other hand, it is likely that definite quantitative differences in pyrimidine biosynthesis between hamster liver and lung exist.

As outlined in the Results section, the values measured for the apparent $V_{\max}$ can be multiplied by the amount of protein derived from 1 g of tissue to give some indication as to how much substrate a unit weight of tissue might be capable of metabolizing *in vitro* under the conditions of my assay. Liver has apparently greater activities of orotidine 5'-phos-

phatede carboxylase and orotidine 5'-phosphate pyrophosphorylase than lung. The difference between the two organs becomes even larger if the apparent $V_{\max}$ values are calculated per mg of DNA instead of per g of tissue. Since a calculated apparent $V_{\max}$ /mg of DNA is proportional to the number of cells present per unit weight it seems that lung cells have considerably less capacity to metabolize orotic acid than have liver cells. This conclusion is derived from an analysis *in vitro* of the kinetic constants of a few selected enzymes only and must therefore be viewed with caution. Nevertheless, the data on both the incorporation of orotic acid and of uridine into RNA *in vivo* agree with this conclusion. Although we are by no means sure of the extent to which the data presented in Table 2 represent maximal possible rates of RNA biosynthesis, they nevertheless show that hamster lung has less capacity to synthesize RNA *in vivo* than has hamster liver.

This finding was somewhat surprising, since I had found earlier that RNA synthesis in rat lung seems to proceed at least at the same rate as in liver (Witschi, 1972). The reason for this species difference is not clear at the moment. It has to be realized, however, that the growth curves of the two species are quite different (data supplied by ARS/Sprague-Dawley). Rats (150–200g) used in the previous study are 7–8 weeks old and will still double their body weight

by the time they are 11 weeks old. Hamsters (80–100 g) are between 6 and 8 weeks old and will only attain a weight of about 120 g at the age of 11 weeks. This difference in the growth curves could explain the finding that rat lung (which presumably is still increasing its cell number, cell size or both) shows a more active RNA synthesis relative to liver, whereas in hamster lung this is not the case.

Another interesting difference between hamster and rat was also noted: rat liver takes up orotic acid most avidly. As a result, hepatic RNA is about 20-fold more highly labelled than pulmonary RNA, despite practically identical rates of biosynthesis (Witschi, 1972). Hamster liver does not have such a great affinity for orotic acid. The reason for this remains at the moment unclear. There is also a difference in the action of actinomycin D on the metabolism of rat liver as compared with hamster liver. Yu & Feigelson (1969) report that actinomycin D inhibited in rat liver the incorporation of orotic acid into RNA. This was accompanied by a substantial decrease in the specific radioactivity of the acid-soluble precursor pool. The direct effect of actinomycin D on RNA synthesis was therefore presumably smaller than the data on the incorporation of orotic acid into RNA alone would suggest. In my experiments with hamster liver I observed only a very small and statistically not significant decrease in the specific radioactivity of UMP. It can be concluded that actinomycin D in hamsters interferes with RNA synthesis solely at the level of transcription of RNA on the DNA template. The increase in total acid-soluble UMP observed after actinomycin D is probably due to a breakdown of nuclear RNA; such an effect of actinomycin D has been shown to occur in rat liver (Stewart & Farber, 1968).

We can conclude that some aspects of basic cellular biochemistry in hamster lung are remarkably similar to the corresponding reactions in liver. The pathological biochemistry of lung tissue with regard to RNA and protein synthesis can therefore be studied essentially along the same lines and with similar methods as for toxic liver damage. Diseases of the lung produced by chemicals are among the major health problems of our times (Bates, 1972). A better understanding of normal and pathological biochemistry of the lung is therefore of more than pure theoretical interest.

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