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Lipid Metabolism by Rat Lung *in vitro*

EFFECT OF STARVATION AND RE-FEEDING ON UTILIZATION OF [U-^{14}C]GLUCOSE BY LUNG SLICES

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1. The incorporation of [U-^{14}C]glucose into several lipid components of lung and liver slices, and the activities of glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), 'malic' enzyme (EC 1.1.1.40) and NADP–isocitrate dehydrogenase (EC 1.1.1.42) of the cell cytosol were examined in normal, starved and re-fed rats. 2. Lipogenesis and the activities of these enzymes in liver were decreased markedly in rats starved for 72h. Re-feeding starved rats on a fat-free diet for 72h resulted in the well documented hyperlipogenic response in liver, particularly in its ability to convert glucose into neutral lipid, and increased activities of glucose 6-phosphate dehydrogenase, 'malic' enzyme and 6-phosphogluconate dehydrogenase to values approx. 700, 470 and 250% of controls respectively. 3. Approx. 70% of the total label in lung lipids was present in the phospholipid fraction. Hydrolysis of lung phospholipids revealed that lipogenesis from glucose was considerable, with approx. 40% of the total phospholipid radioactivity present in the fatty acid fraction. 4. Incorporation of glucose into total lung lipids was decreased by approx. 40% in lung slices of starved rats and was returned to control values on re-feeding. Although phospholipid synthesis from glucose was decreased in lung slices of starved rats, the decrease proportionally was greater for the fatty acid fraction (approx. 50%) as compared with the glycerol fraction (approx. 25%). 5. The activities of lung glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP–isocitrate dehydrogenase were not affected by the dietary alterations. 'Malic' enzyme activity was not detected in lung cytosol preparations. 6. The results are discussed in relation to the surface-active lining layer (surfactant) of the lung.

Lipid metabolism in the mammalian lung is directed primarily toward the synthesis of phospholipids. This synthetic activity gains physiological significance with the observations that (1) phospholipids, predominantly (dipalmitoyl) phosphatidylcholine, are major components of the surface-active material (surfactant) that lines the alveolar membrane (Klaus, Clements & Havel, 1961) and serves to lower and stabilize surface forces during the respiratory cycle, and (2) phospholipids are involved in the maintenance of the structural integrity of the lung (Kennedy, 1961).

Various studies have demonstrated that lung tissue is capable of using acetate and palmitate for the synthesis of glycerides and phospholipids (Lands, 1957; Havel, Felts & Van Duyne, 1962; Nasr & Heinemann, 1965; Felts, 1965; Buckingham, Heinemann, Sommers & McNary, 1966; Salisbury-Murphy, Rubinstein & Beck, 1966). In addition, glucose and glycerol may serve as precursors for the synthesis of lung phospholipids (Lands, 1957; Felts, 1965; Salisbury-Murphy *et al.* 1966; Wolfe, Anhalt, Beck & Rubinstein, 1970). Phospholipids in the lung are associated physiologically with the surfactant system and factors altering normal patterns of metabolism conceivably could affect the mechanical properties of the lung. Recent reports suggest that surface-active properties of lungs are affected considerably by the nutritional state of the animal (Garbagni, Coppo, Grassini & Cardellino, 1968; Faridy, 1970).

It is well documented that lipid metabolism in a number of tissues responds to a variety of nutritional conditions. A systematic study of the effect of altered nutrition on lung lipid metabolism, however, has not been reported. Accordingly, the present studies examined the utilization of [U-^{14}C]glucose by lung slices and the activities of four cytoplasmic

NADP-linked enzymes from normal, starved and re-fed rats. These parameters were examined also in liver tissue to evaluate comparatively with lung the metabolic changes resulting from starvation and re-feeding. It was considered that such studies could provide information on possible underlying mechanisms for the changes in pulmonary surface-active properties observed by Garbagni *et al.* (1968) and Faridy (1970) in animals that had been deprived of food, water, or both.

EXPERIMENTAL

Animals. Male Long–Evans Hooded rats averaging 250–300g body weight were used in all experiments. This strain was chosen because of its low incidence of respiratory infection (Stratman & Conejeros, 1969). All rats were housed individually in stainless steel cages with raised wire floors. Temperature, relative humidity and light were controlled at 22°C, 50% and a 12h light–dark cycle, respectively.

Diets. A control diet similar to that described by Leveille & O'Hea (1967) consisted of 18% casein (vitamin-free), 67.5% glucose monohydrate, 4% cellulose, 5% corn oil, 0.3% L-cystine, 0.2% choline chloride (70% mixture), 1% vitamin mixture (Harper, 1959) and 4% mineral mixture (Salt mixture USP XIV; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.). The fat-free diet was identical with the control diet with the exception that all the corn oil was replaced by an equal amount of glucose monohydrate.

Experimental design. All rats were fed *ad lib.* on the control diet for 1–2 weeks. Groups of five to eight rats were subsequently assigned at random to the following treatments: (1) consumption *ad lib.* of the control diet; (2) 72h starvation; (3) 72h starvation followed by re-feeding with the fat-free diet for 24h; and (4) 72h starvation followed by re-feeding with the fat-free diet for 72h. Body-weight changes and food consumption were recorded daily. Water was supplied *ad lib.* to all treatment groups.

Techniques with tissue slices in vitro. The rats were killed by intraperitoneal sodium pentobarbitol injection (Halatal solution; Jensen–Salsbery Laboratories, Kansas City, Mo., U.S.A.) (3mg/kg body wt.) and rapid exsanguination via a carotid artery and thoracotomy. Blood was removed from the lung by perfusing the pulmonary vasculature via the right ventricle with calcium-free Krebs–Ringer bicarbonate buffer (Umbreit, Burris & Stauffer, 1957) while the heart was still actively beating. After perfusion, the lungs and heart were carefully removed *en bloc.* The lungs were trimmed of extraneous tissue, blotted and quickly weighed to the nearest 10mg. Tissue slices (approx. 90–110mg) were prepared with a Stadie–Riggs hand microtome. Visceral pleura, major vessels and large bronchi were excluded from the slices. The slices were incubated in replicate for 2h in 50ml reaction flasks (Kontes Glass Co., Vineland, N.J., U.S.A.), fitted with a scintillation vial on the side arm. Boiled tissue was also incubated and served as a blank. The incubation medium (4ml) contained 20μmol of glucose, 2μCi of [U-^{14}C]glucose (specific radioactivity 15.6μCi/μmol; New England Nuclear Corp., Boston, Mass., U.S.A.), and calcium-free Krebs–Ringer bicarbonate buffer, pH7.4. The specific radioactivity of glucose in the incubation medium was 0.1μCi/μmol. The flasks were gassed initially for 5min with O_2+CO_2 (95:5) and then shaken in a reciprocating water bath (90 strokes/min) at 37°C. At the end of the incubation period (2h) 0.2ml of Hyamine hydroxide (Packard Instrument Co., Downer's Grove, Ill., U.S.A.) was added to a fluted circle of Whatman no. 1 filter paper in the scintillation vials. The reaction was terminated by injecting 0.5ml of 0.2M-H_2SO_4 into the medium. Shaking was continued for 1h to collect the liberated CO_2. All $^{14}CO_2$ measurements were made by removing scintillation vials from the reaction flasks, allowing the filter paper to dry and adding 15ml of scintillation fluid (Buhler, 1962). Trapping efficiency for $^{14}CO_2$ was better than 90%, as determined with a standard solution of $NaH^{14}CO_3$.

Isolation and determination of lipid fractions. The tissues were rinsed three times in 0.9% NaCl and lipids were extracted by the method of Folch, Lees & Sloane-Stanley (1957). Non-lipid radioactivity was removed from the lipid extract by employing the 'salty wash' procedure (Folch *et al.* 1957). Extracted lipids were concentrated to dryness under nitrogen, resuspended to a known volume in chloroform–methanol (2:1, v/v), and divided into portions. Phospholipids were separated from neutral lipids by subjecting a portion from each lipid sample to silicic acid chromatography (Bio-Sil A, 100–200 mesh; Bio-Rad Laboratories, Richmond, Calif., U.S.A.) by using the method of Hirsch & Ahrens (1958). Free fatty acids were separated from neutral lipids by the method of Kelley (1968). For determination of radioactivity, total lipids and phospholipids were also separated by t.l.c. The plates were prepared with silica gel HR (extra pure; E. Merck A.-G., Darmstadt, Germany) and developed in a solvent system of light petroleum (b.p. 30–60°C)–diethyl ether–acetic acid (90:10:1, by vol.). After detection by iodine vapour, areas containing the lipids were aspirated into scintillation vials for radioactivity counting. Mild hydrolysis of the phospholipids collected from the silicic acid columns was carried out in 10ml graduated test tubes fitted with water-jacketed condensers. Refluxing was performed for 30min at 67°C in 2ml of methanolic 3.75% (w/v) KOH. After the addition of 1ml of 2M-HCl, fatty acids were extracted twice with 5ml portions of light petroleum (b.p. 30–60°C) and transferred into scintillation vials. The volume of the remaining aqueous phase containing the phospholipid glycerol was recorded and a portion transferred for radioassay. The efficiency of the hydrolysis was found to be 97% when known amounts of ($\beta\gamma$-dipalmitoyl) phosphatidylcholine (A-grade; Calbiochem, Los Angeles, Calif., U.S.A.) were submitted to the same procedure. All radioactive lipid samples were counted in toluene–Triton X-100 (2:1, v/v) scintillant containing 4g of 2,5-diphenyloxazole and 0.1g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l. Radioactivity counting was performed in a Nuclear–Chicago scintillation spectrometer and counting efficiency was determined by the channels-ratio method.

Assay of enzyme activities. Portions of lung and liver tissue were homogenized in 9vol. of chilled 0.14M-KCl, pH7.4, containing 1mM-EDTA. The homogenates were

centrifuged for 20min at 22500*g* in a refrigerated centrifuge maintained at 2°C. Portions of the cell-free supernatant were centrifuged again at 105000*g* for 1h at 2°C and the resulting clear supernatant used for enzyme analyses. D-Glucose 6-phosphate–NADP oxidoreductase (EC 1.1.1.49), hereafter referred to as glucose 6-phosphate dehydrogenase, and 6-phospho-D-gluconate–NADP oxidoreductase (EC 1.1.1.44), hereafter referred to as 6-phosphogluconate dehydrogenase, were assayed separately by the method of Glock & McLean (1953*a*). L-Malate–NADP oxidoreductase (decarboxylating) (EC 1.1.1.40), hereafter referred to as 'malic' enzyme, and *threo*-D_S-isocitrate–NADP oxidoreductase (decarboxylating) (EC 1.1.1.42), hereafter referred to as NADP–isocitrate dehydrogenase, were assayed by the procedures of Ochoa (1955*a*,*b*) respectively. The experimental cuvette contained glycylglycine buffer, pH7.4 (75μmol), $MnCl_2$ (6μmol), $NADP^+$ (1μmol), either L-malate or DL-isocitrate (10μmol) and cell-free extract in a total volume of 3.0ml for the assays of 'malic' enzyme and NADP–isocitrate dehydrogenase. All assays were performed by measuring the E_{340} in a Beckman Kintrac VII recording spectrophotometer equipped with a circulating water bath maintained at 25°C. One unit of enzyme activity is defined as that catalysing the formation of 1μmol of NADPH/min under the assay conditions described.

Protein determination. Soluble protein remaining after centrifugation at 22500*g* for 20min was determined with a modified biuret reagent (Mokrasch & McGilvery, 1956).

Glycogen determination. Liver and lung glycogen contents were determined in replicate by the method of Lo, Russell & Taylor (1970).

RESULTS

Response of rat lung constituents to alterations in diet. The results presented in Table 1 indicate that relative lung size and soluble protein, total lipid and glycogen contents are relatively stable to the changes in nutritional state examined in the present studies. Similar results for the liver (not shown) indicated marked compositional changes; for example, liver glycogen decreased from a normal value of 4.0 to 0.05% after starvation for 72h and was increased to 8.1% after re-feeding for 24h with the fat-free diet.

Utilization of [U-^{14}C]*glucose by lung and liver slices.* The utilization of [U-^{14}C]glucose by rat lung slices as assessed by its oxidation to $^{14}CO_2$ and incorporation into several lipid components is shown in Table 2. The major portion of the total lipid radioactivity (approx. 70%) was present in the phospholipid fraction. Glucose utilization was decreased by approx. 40% in lung slices of starved rats and subsequently returned to control values on re-feeding.

The distribution of the radioactivity in the phospholipid fraction between the fatty acid and glycerol moieties is shown in Table 3. The results show that for control rats there was a considerable incorporation of glucose into the phospholipid fatty acid fraction, representing approx. 40% of the total phospholipid radioactivity. Phospholipid synthesis from glucose was decreased in the starved rats although the decrease was proportionally greater in the fatty acid fraction (approx. 50%) as compared with the glyceride glycerol fraction (approx. 25%). Re-feeding for 24h caused [U-^{14}C]-glucose incorporation into the fatty acid and glycerol portions of lung phospholipids to return to control values.

The results showing the effect of starvation and re-feeding on [U-^{14}C]glucose utilization by rat liver slices are presented in Table 4. Unlike lung tissue, most of the total lipid radioactivity in liver was present in the neutral lipid fraction. Starvation decreased glucose incorporation into liver lipids whereas re-feeding for 72h on the fat-free diet enhanced greatly the conversion of glucose into neutral lipid.

Response of rat liver and lung enzyme activities to alterations in diet. The effects of starvation and re-feeding on several rat liver and lung NADP-linked enzyme activities are shown in Tables 5 and 6 respectively. Enzyme activity is expressed in terms of concentration (units/g of tissue) and as total activity (units/100g body wt.) to assist in the interpretation of the results, since size and composition of the liver were affected considerably by the dietary alterations. Starving rats for 72h decreased the activities on a body-weight basis of all the liver enzymes studied. Re-feeding starved

Table 1. *Effect of starvation and re-feeding on rat lung constituents*

The values are given as means±S.E.M. of eight observations each. Lung weight is expressed in terms of body weight before starvation. The re-fed rats were fed with the fat-free diet for the time specified after 72h of starvation.

Dietary status ...	Fed control diet *ad lib.*	Starved 72h	Re-fed 24h	Re-fed 72h
Lung wt. (g)	1.39±0.10	1.21±0.04	1.14±0.24	1.28±0.07
Lung wt. (g/100g body wt.)	0.40±0.02	0.40±0.01	0.40±0.02	0.38±0.02
Lung soluble protein (mg/100mg)	6.08±0.11	5.99±0.21	6.36±0.23	6.08±0.32
Lung lipid (mg/100mg)	4.30±0.19	4.24±0.28	4.02±0.45	3.66±0.09
Lung glycogen (mg/100mg)	0.11±0.01	0.09±0.01	0.10±0.02	0.11±0.02

Table 2. *Utilization of* [U-^{14}C]*glucose by rat lung slices*

The values are given as means±S.E.M. of five observations each. Each value represents nmol of glucose oxidized to $^{14}CO_2$ or its incorporation into the lipid component specified/2h per 100mg of lung at 37°C. The re-fed rats were fed with the fat-free diet for the time specified after 72h of starvation.

	Glucose utilized (nmol/2h per 100mg)			
Dietary status ...	Fed control diet *ad lib.*	Starved 72h	Re-fed 24h	Re-fed 72h
Total lipid	75.6± 7.6	44.4± 7.7	62.1± 6.1	66.5±13.4
Phospholipid	52.1±10.5	34.8± 7.1	47.3± 6.0	47.8±12.5
Neutral lipid	16.8± 3.0	6.7± 0.6	11.5± 0.7	14.5± 1.4
Free fatty acids	1.4± 0.3	0.6± 0.2	1.3± 0.2	1.7± 0.3
Non-saponifiable lipids	1.4± 0.4	0.4± 0.1	0.7± 0.1	1.2± 0.4
CO_2	214.8±27.8	138.7±15.7	211.9±17.5	212.9±20.4

Table 3. *Distribution of radioactivity in the phospholipid fraction from lung slices incubated with* [U-^{14}C]*glucose*

The values are given as means±S.E.M. of five observations each. Each value represents nmol of glucose incorporated into phospholipids, phospholipid glycerol and phospholipid fatty acids/2h per 100mg of lung at 37°C. The re-fed rats were fed with the fat-free diet for the time specified after 72h of starvation.

	Glucose incorporated (nmol/2h per 100mg)			
Dietary status ...	Fed control diet *ad lib.*	Starved 72h	Re-fed 24h	Re-fed 72h
Phospholipids	52.1±10.5	34.8±7.1	47.3±6.0	47.8±12.5
Phospholipid glycerol	33.1± 5.2	24.8±4.0	31.2±2.8	31.0± 6.5
Phospholipid fatty acids	20.7± 4.1	9.2±3.5	15.9±3.4	15.7± 6.1

Table 4. *Utilization of* [U-^{14}C]*glucose by rat liver slices*

The values are given as means±S.E.M. of five observations each. Each value represents nmol of glucose oxidized to $^{14}CO_2$ or its incorporation into the lipid component specified/2h per 100mg of lung at 37°C. The re-fed rats were fed with the fat-free diet for the time specified after 72h of starvation.

	Glucose utilized (nmol/2h per 100mg)			
Dietary status ...	Fed control diet *ad lib.*	Starved 72h	Re-fed 24h	Re-fed 72h
Total lipid	30.7 ±8.3	16.9 ± 4.7	49.4 ±7.3	102.4 ±15.9
Phospholipid	11.9 ±2.6	8.7 ± 2.4	20.5 ±3.2	21.2 ± 4.2
Neutral lipid	17.7 ±5.8	8.6 ± 2.1	28.5 ±4.9	79.9 ±14.8
Free fatty acids	0.04±0.02	0.07± 0.02	0.18±0.06	0.22± 0.09
Non-saponifiable lipids	0.22±0.13	0.07± 0.06	0.98±0.27	3.36± 1.72
CO_2	38.9 ±6.8	54.2 ±11.9	58.5 ±8.4	80.6 ± 7.2

rats for 72h on the fat-free diet resulted in increased activities on a body-weight basis of glucose 6-phosphate dehydrogenase, 'malic' enzyme and 6-phosphogluconate dehydrogenase to values approx. 700, 470 and 250% of the controls respectively. NADP–isocitrate dehydrogenase activity on a body-weight basis was not elevated above control values on re-feeding. The results show also for rat liver the rate-limiting characteristic of glucose 6-phosphate dehydrogenase activity as compared with 6-phosphogluconate dehydrogenase activity in the hexose monophosphate pathway. Glucose 6-phosphate dehydrogenase activity normally is lower than 6-phosphogluconate dehydrogenase activity in rat liver and generally it is the activity of the rate-limiting enzyme of a particular metabolic pathway that shows the greatest proportional increase during conditions associated with increased metabolite flux through that pathway. The nature of the response of the liver enzymes studied (Table 5) to the dietary alterations has been discussed in relation to the

Table 5. *Effect of starvation and re-feeding on rat liver NADP-linked dehydrogenase activities*

The values are given as means±S.E.M. of six observations each. One unit represents enzyme activity catalysing the formation of 1 μmol of NADPH/min at 25°C. A pre-starvation body weight was used for expressing enzyme activity on a body-weight basis. The re-fed rats were fed with the fat-free diet for the time specified after 72h of starvation.

	Glucose 6-phosphate dehydrogenase		6-Phosphogluconate dehydrogenase		'Malic' enzyme		NADP–isocitrate dehydrogenase	
Dietary status	(units/g)	(units/100g body wt.)	(units/g)	(units/100g body wt.)	(units/g)	(units/100g body wt.)	(units/g)	(units/100g body wt.)
Control diet *ad lib.*	3.8±0.3	15.1±0.1	6.9±0.6	27.9±2.8	2.4±0.3	9.5±1.4	18.2±1.6	71.2±7.0
Starved 72h	1.7±0.1	4.1±0.3	3.8±0.1	9.1±0.7	1.1±0.1	2.7±0.2	18.6±0.8	40.7±0.5
Re-fed 24h	3.4±0.6	15.6±3.0	4.1±0.3	18.9±1.6	1.9±0.1	8.6±0.6	13.3±0.6	59.9±2.5
Re-fed 72h	23.3±2.4	104.8±8.5	15.3±0.8	69.5±3.8	9.9±1.9	44.5±8.1	16.5±0.8	72.9±2.4

Table 6. *Effect of starvation and re-feeding on rat lung NADP-linked dehydrogenase activities*

The values are given as means±S.E.M. of six observations each. One unit represents enzyme activity catalysing the formation of 1 μmol of NADPH/min at 25°C. A pre-starvation body weight was used for expressing enzyme activity on a body-weight basis. The re-fed rats were fed with the fat-free diet for the time specified after 72h of starvation.

	Glucose 6-phosphate dehydrogenase		6-Phosphogluconate dehydrogenase		NADP–isocitrate dehydrogenase	
Dietary status	(units/g)	(units/100g body wt.)	(units/g)	(units/100g body wt.)	(units/g)	(units/100g body wt.)
Control diet *ad lib.*	2.1±0.1	0.89±0.07	2.3±0.1	0.98±0.07	2.5±0.1	1.04±0.05
Starved 72h	2.1±0.1	0.80±0.02	2.1±0.1	0.80±0.09	2.7±0.1	0.99±0.03
Re-fed 24h	2.2±0.2	0.93±0.11	2.1±0.1	0.90±0.06	2.9±0.2	1.14±0.07
Re-fed 72h	2.2±0.1	0.84±0.04	2.3±0.1	0.86±0.04	2.5±0.1	0.94±0.07

'hyperlipogenesis' observed when starved rats are re-fed on diets high in carbohydrate (Tepperman & Tepperman, 1958*a*,*b*, 1964).

Unlike the response observed for rat liver, however, the activities of lung glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP–isocitrate dehydrogenase were not affected by starvation and re-feeding (Table 6). The results indicate that neither glucose 6-phosphate dehydrogenase activity nor 6-phosphogluconate dehydrogenase activity was rate-limiting with respect to each other in rat lung. 'Malic' enzyme activity was not detected in lung cytosol preparations, suggesting that the 'transhydrogenation' cycle proposed by Ballard & Hanson (1967) has little functional significance in this tissue.

DISCUSSION

The rapid rate of phospholipid turnover in the mammalian lung (Tierney, Clements & Trahan, 1967; Thomas & Rhoades, 1970) and the preferential incorporation of acetate and palmitate into phospholipids (as compared with liver) suggest an active involvement of the lung in phospholipid synthesis (Buçkingham *et al.* 1966; Heinemann & Fishman, 1969). This active lipid metabolism gains physiological significance when the enormous surface area and the phospholipid content of the surface-active lining layer (surfactant) of lung are considered.

The metabolism of glucose by rabbit lung slices has been examined by Felts (1965). His results show that up to 99% of the total phospholipid radioactivity incorporated from incubations with [U-^{14}C]glucose was present in the phospholipid glycerol fraction. The extremely small amount of radioactivity from glucose in the phospholipid fatty acid fraction suggested that glucose functioned predominantly in lung lipid metabolism by providing an acceptor molecule, L-α-glycerophosphate, for the esterification of long-chain fatty acids (Felts, 1965). The metabolism of glucose by lung has been considered also with respect to providing a source of NADPH, via the hexose monophosphate pathway, for the reductive biosynthesis of fatty acids.

The presence in lung tissue of hexose monophosphate pathway activity has been demonstrated spectrophotometrically (Glock & McLean, 1953*b*) histochemically (Tyler & Pearce, 1965), and with radioisotopes by using specifically labelled [^{14}C]glucose (Felts, 1965; Salisbury-Murphy *et al.* 1966).

Our experiments have indicated for lung slices of normal rats that approx. 40% of the total phospholipid radioactivity from incubation with [U-^{14}C]glucose was present in the phospholipid fatty acid fraction. These findings are in contrast with work reported by Felts (1965), which indicates an apparent lack of lipogenesis from glucose. Part of this discrepancy possibly could be attributed to the lability of lung slices. Preliminary experiments in our laboratory have indicated that lung slices are extremely labile with respect to metabolic activity unless certain precautions are observed. For example, conversion of glucose into phospholipid fatty acids was essentially abolished if freshly cut slices were kept in chilled calcium-free Krebs–Ringer bicarbonate buffer before being placed in the gassed incubation medium maintained at 37°C. For this reason the tissues were handled as rapidly as possible after their isolation. Replicate slices generally gave glucose incorporation values within 10% of each other. Additions of insulin to the incubation medium did not alter the rate of glucose uptake by lung slices (R. W. Scholz & R. A. Rhoades, unpublished work). This apparent insensitivity of lung toward insulin with respect to glucose uptake has been observed by others (Felts, 1965; Salisbury-Murphy *et al.* 1966). Glucose, rather than acetate, was selected for examining lipid synthesis in the present studies, for the reasons discussed by Favarger (1965). Liver tissue was selected for comparisons of lipid metabolism with lung because of the well documented response of the liver enzyme activities to the dietary alterations employed in these studies.

Lipogenesis in rat liver and adipose tissue is affected considerably by the nutritional state of the animal. In general, starvation depresses incorporation of acetate or glucose into fatty acids whereas re-feeding (particularly with a diet devoid of fat) greatly accelerates the lipogenic process (Masoro, Chaikoff, Chernick & Felts, 1950; Tepperman & Tepperman, 1958*a*,*b*; Allman, Hubbard & Gibson, 1963; Jomain & Hanson, 1969). Further, it is generally accepted that fatty acid biosynthesis in the cell cytosol is supported in part by an available supply of reducing equivalents in the form of NADPH. The relative contributions of the NADP-linked enzyme systems, as assessed by their activities *in vitro*, to the NADPH supply varies between tissues and animal species. Thus in rat adipose tissue, both hexose monophosphate pathway dehydrogenase activity and 'malic' enzyme activity contribute significantly to the supply of NADPH (Young, Shrago & Lardy, 1964; Flatt & Ball, 1964; Ballard & Hanson, 1967). In chicken liver, 'malic' enzyme activity is considered to supply most of the NADPH required for fatty acid biosynthesis (O'Hea & Leveille, 1968) whereas NADP–isocitrate dehydrogenase activity has been reported to be an important NADPH-generating system in the lactating bovine mammary gland (Bauman, Brown & Davis, 1970). The activities of the NADP-linked enzymes in these tissues have been shown to change in parallel with the fluctuations in lipogenesis caused by starvation and re-feeding (Tepperman & Tepperman, 1958*a*,*b*, 1964, 1970; Young *et al.* 1964; Allman *et al.* 1963; Leveille & Hanson, 1966; Johnson & Sassoon, 1967; Jomain & Hanson, 1969; Novello, Gumaa & McLean, 1969; Fabry, Kleinfeld, Tepperman & Tepperman, 1970).

The increased activities observed in the present studies for the NADP-linked enzymes in liver would support the enhanced lipogenesis in this tissue after re-feeding of starved rats. A lack of an adaptive response, however, has been observed for the activities of enzymes supplying NADPH in the lung. Since the lung is not considered an active site for triglyceride synthesis or storage, it appears likely that it would not engage actively in the synthesis of fatty acids for this purpose. The adaptive enzyme response therefore would not assume the physiological significance in the pulmonary system that it would for liver or adipose tissue.

The present studies demonstrate a considerably lower incorporation of glucose into lung phospholipids in starved rats (72h) as compared with fed controls. Although glucose carbon was incorporated into both the fatty acid and glycerol moieties of lung phospholipids, starvation decreased in particular its incorporation into the fatty acid fraction. These results suggest similarities in the control of lipogenesis in lung tissue, as have been reported for liver and adipose tissue (Bortz & Lynen, 1963; Wieland & Weiss, 1963; Dorsey & Porter, 1968; Pande & Mead, 1968). In this context Salisbury-Murphy *et al.* (1966) have reported that palmitic acid depresses glucose incorporation into phospholipid fatty acids of lung slices when added to the incubation media. Since circulating concentrations of fatty acids are increased in the fasting state, these results possibly could account for the lowered content of phosphatidylcholine and altered surface-active properties of lungs of starved rats (Faridy, 1970). The relative importance of the regulation of lipogenesis in lung by fatty acids, however, remains to be elucidated.

The precise role of lung fatty acid synthesis *de novo* remains obscure. Reports indicate that lung tissue actively incorporates acetate into fatty acids

(Felts, 1965; Nasr & Heinemann, 1965; Buckingham *et al.* 1966), but this does not preclude an elongation of existing fatty acyl-CoA derivatives rather than synthesis *de novo*. The present studies and those of Salisbury-Murphy *et al.* (1966) demonstrate a marked capacity of lung tissue to utilize glucose for phospholipid fatty acid synthesis. Obtaining quantitative information on the net contribution of lung tissue to fatty acid synthesis will be difficult, however, until concentrations of precursor pools are determined. The acetate pool in lung, for example, is considerably smaller than in most other tissues (Favarger & Gerlach, 1955). The low content of lung glycogen, and its relative stability to starvation and re-feeding (Table 1), cast doubt on the significance of this metabolite as an important contributor to a fatty acid precursor pool.

There are reports suggesting that circulatory fatty acids may be trapped by the lung before their incorporation into pulmonary phospholipids (Harlan, Said & Banerjee, 1966; Buckingham *et al.* 1966). These findings may have significance in view of the fact that lipolytic activity has been demonstrated in the perfused rabbit lung (Heinemann, 1961). Nutritional states that alter the circulating concentration of fatty acids, either in the form of albumin–fatty acid complexes or as triglycerides bound to lipoproteins or chylomicrons, conceivably could result in aberrations in the maintenance of the surface active material of the lung. The use of alterations in diet as a model for studying possible mechanisms associated with the control of lipid metabolism in lung warrants further investigation.

Before meaningful studies on the control of lipid metabolism in lung tissue can be undertaken, however, there is need for determining the subcellular locations that are quantitatively important in the synthesis of fatty acids. Evidence has appeared suggesting that mitochondria are active in surfactant synthesis in general (Klaus, Reiss, Tooley, Piel & Clements, 1962) and in fatty acid synthesis in particular (Tombropoulos, 1964). The heterogeneous cell population found in mammalian lung, and the associated divergent metabolic activities (Heinemann & Fishman, 1969), may suggest that there are several independent subcellular sites and mechanisms for lipogenesis. Perhaps this one characteristic exemplifies more than any other the complex nature of this tissue, and indicates the possible obstacles that may be encountered in suggesting unified mechanisms for metabolic control. The failure to detect in lung cytosol preparations significant 'malic' enzyme activity suggests the lack of a functionally significant 'transhydrogenation cycle'. Additional experiments designed to determine the presence of citrate-cleavage and acetyl-CoA carboxylase enzyme activities in lung possibly could provide evidence for an active cytoplasmic fatty acid-synthetic system in this tissue.

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REFERENCES

Allman, D. W., Hubbard, D. D. & Gibson, D. M. (1963). *J. Lipid Res.* **6**, 63.
Ballard, F. J. & Hanson, R. W. (1967). *J. Lipid Res.* **8**, 73.
Bauman, D. E., Brown, R. E. & Davis, C. L. (1970). *Archs Biochem. Biophys.* **140**, 237.
Bortz, W. M. & Lynen, F. (1963). *Biochem. Z.* **337**, 505.
Buckingham, S., Heinemann, H. O., Sommers, S. C. & McNary, W. F. (1966). *Am. J. Path.* **48**, 1027.
Buhler, D. R. (1962). *Analyt. Biochem.* **4**, 413.
Dorsey, J. A. & Porter, J. W. (1968). *J. biol. Chem.* **243**, 3512.
Fabry, P., Kleinfeld, R., Tepperman, H. M. & Tepperman, J. (1970). *Proc. Soc. exp. Biol. Med.* **133**, 577.
Faridy, E. E. (1970). *J. appl. Physiol.* **29**, 493.
Favarger, P. (1965). In *Handbook of Physiology*, section 5, p. 19. Ed. by Renold, A. E. & Cahill, G. F., jun. Baltimore: Waverly Press Inc.
Favarger, P. & Gerlach, J. (1955). *Helv. physiol. Acta*, **13**, 91.
Felts, J. M. (1965). *Medna thorac.* **22**, 89.
Flatt, J. P. & Ball, E. G. (1964). *J. biol. Chem.* **239**, 675.
Folch, J. P., Lees, N. P. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Garbagni, R., Coppo, F., Grassini, G. & Cardellino, G. (1968). *Respiration*, **25**, 458.
Glock, G. E. & McLean, P. (1953*a*). *Biochem. J.* **55**, 400.
Glock, G. E. & McLean, P. (1953*b*). *Biochem. J.* **56**, 171.
Harlan, W. R., jun., Said, S. I. & Banerjee, C. M. (1966). *Am. Rev. resp. Dis.* **94**, 938.
Harper, A. E. (1959). *J. Nutr.* **68**, 405.
Havel, R. J., Felts, J. M. & Van Duyne, C. M. (1962). *J. Lipid Res.* **3**, 297.
Heinemann, H. O. (1961). *Am. J. Physiol.* **201**, 607.
Heinemann, H. O. & Fishman, A. P. (1969). *Physiol. Rev.* **49**, 1.
Hirsch, J. & Ahrens, E. H., jun. (1958). *J. biol. Chem.* **233**, 311.
Johnson, B. C. & Sassoon, H. F. (1967). In *Advances in Enzyme Regulation*, vol. 5, p. 93. Ed. by Weber, G. New York: Pergamon Press Inc.
Jomain, M. & Hanson, R. W. (1969). *J. Lipid Res.* **10**, 674.
Kelley, T. P. (1968). *J. Lipid Res.* **9**, 799.
Kennedy, E. P. (1961). *Harvey Lect.* **57**, 143.
Klaus, M., Reiss, O. K., Tooley, W. H., Piel, C. & Clements, J. A. (1962). *Science, N.Y.*, **137**, 750.
Klaus, M. H., Clements, J. A. & Havel, R. J. (1961). *Proc. natn. Acad. Sci. U.S.A.* **47**, 1858.
Lands, W. E. M. (1957). *J. biol. Chem.* **231**, 883.

Leveille, G. A. & Hanson, R. W. (1966). *J. Lipid Res.* **7**, 46.

Leveille, G. A. & O'Hea, E. K. (1967). *J. Nutr.* **93**, 541.

Lo, S., Russell, J. C. & Taylor, A. W. (1970). *J. appl. Physiol.* **28**, 234.

Masoro, E. J., Chaikoff, I. L., Chernick, S. S. & Felts, J. M. (1950). *J. biol. Chem.* **185**, 845.

Mokrasch, L. G. & McGilvery, R. W. (1956). *J. biol. Chem.* **221**, 909.

Nasr, K. & Heinemann, H. O. (1965). *Am. J. Physiol.* **208**, 118.

Novello, F., Gumaa, J. A. & McLean, P. (1969). *Biochem. J.* **111**, 713.

Ochoa, S. (1955*a*). In *Methods in Enzymology*, vol. 1, p. 739. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Ochoa, S. (1955*b*). In *Methods in Enzymology*, vol. 1, p. 699. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

O'Hea, E. K. & Leveille, G. A. (1968). *Comp. Biochem. Physiol.* **26**, 111.

Pande, W. V. & Mead, J. F. (1968). *J. biol. Chem.* **243**, 6180.

Salisbury-Murphy, S., Rubinstein, D. & Beck, J. C. (1966). *Am. J. Physiol.* **211**, 988.

Stratman, S. L. & Conejeros, N. (1969). *Lab. Anim. Care*, **19**, 742.

Tepperman, H. M. & Tepperman, J. (1958*b*). *Diabetes*, **7**, 478.

Tepperman, H. M. & Tepperman, J. (1964). *Am. J. Physiol.* **206**, 357.

Tepperman, J. & Tepperman, H. M. (1958*a*). *Am. J. Physiol.* **193**, 55.

Tepperman, J. & Tepperman, H. M. (1970). *Fedn Proc. Fedn Am. Socs exp. Biol.* **29**, 1284.

Thomas, T., jun. & Rhoades, R. A. (1970). *Am. J. Physiol.* **219**, 1535.

Tierney, D. F., Clements, J. A. & Trahan, H. J. (1967). *Am. J. Physiol.* **213**, 671.

Tombropoulos, E. G. (1964). *Science, N.Y.*, **146**, 1180.

Tyler, W. S. & Pearce, G. E. (1965). *Thorax*, **20**, 149.

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, p. 149. Minneapolis: Burgess Publishing Co.

Wieland, O. & Weiss, L. (1963). *Biochem. biophys. Res. Commun.* **13**, 26.

Wolfe, B. M. J., Anhalt, B., Beck, J. C. & Rubinstein, D. (1970). *Can. J. Biochem.* **48**, 170.

Young, J. W., Shrago, E. & Lardy, H. A. (1964). *Biochemistry, Easton*, **3**, 1687.