Glucose Metabolism in Lung Slices of Late Fetal, Newborn, and Adult Rats[1] (36703)

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(Introduced by Donald Massaro)

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At the onset of ventilation there is an abrupt change in the blood flow and oxygen tension to which the lung is exposed (1). In addition, there are probably new metabolic demands on the organ due to the new work of ventilation (2). The major energy substrate of the developing rat and human fetus is thought to be glucose (3, 4). Virtually no data are available, however, on glucose metabolism by the lung before and after the onset of ventilation, despite the known morphologic changes that occur at this time (5). In this report we present data concerning the metabolism of D-glucose-^{14}C by lung slices prepared from late fetal, neonatal, and adult rats.

Materials and Methods. Pregnant Sprague-Dawley rats of specified delivery date and adult rats of either sex were obtained from Charles River Breeding Farms, Winchester, MA. Animals were examined at 22 days gestation, at 12–18 hr after birth, and in the adult state, 250–300 g. The rats were fed food and water *ad libitum*. They were anesthetized with intraperitoneal pentobarbital (60 mg/kg) and sacrificed by opening of the chest and exsanguination through a carotid artery, followed by perfusion of the lungs through the right side of the heart with physiologic saline until the perfusate was clear. The lungs were quickly removed and placed in ice-cold saline solution. Slices were cut 1 mm thick with a McIlwain tissue chopper (Brinkmann Instruments, Inc.), with care taken to avoid major vessels and bronchi. The slices were blotted one time, then weighed.

[1] This work was supported by the Veterans Administration.

Two hundred mg of lung slices were placed in 3.0 ml Krebs–Ringer bicarbonate medium (KRB) (6) in 25 ml Erlenmeyer flasks, with glucose, 1 mg/ml. In one series of experiments 0.025 ml of D-glucose-U-^{14}C (0.09 μmoles; 0.42 μCi) was added to each flask. In another series of experiments 0.025 ml of either D-glucose-1-^{14}C (0.06 μmoles; 0.42 μCi) or D-glucose-6-^{14}C (0.09 μmoles; 0.42 μCi) was added to each flask. Flasks were run in triplicate with and without tissue; and in addition flasks were prepared with tissue, and 0.5 vol of 0.1 *N* HCl was added at the end of gassing for determination of base line lactic acid values. Incubation, $^{14}CO_2$ collection, and determination of radioactivity were done as described previously (7).

The acidified tissue specimens were brought to a constant dry weight in a Boekel oven at 60° for 48–72 hr in aluminum foil boats, and dry weights of the tissue were calculated. Glycogen content was determined by the method of Lo, Russell, and Taylor (8), with an aliquot of the glycogen solution taken for radioactivity determination. Glucose was measured by the glucose oxidase method (9). Glucose uptake was calculated from the difference in glucose levels in the media of flasks with and without tissue. Lactic acid was determined enzymatically (10) in the supernatant fluids from flasks with tissue that had been killed without incubations, and from flasks with tissue that had been incubated, and lactic acid production was calculated by difference. Hexose monophosphate shunt activity was determined as done previously (7) with a method proposed by Katz and Wood (11).

All radioactive glucose was obtained from

TABLE I. Glucose Uptake, Lactic Acid Production, and Percentage of Radioactivity of D-Glucose-U-^{14}C Appearing in CO_2 and Glycogen.[a]

	Fetal (3)	Newborn (4)	Adult (4)
Glucose uptake (μmoles/100 mg dry wt/hr)	7.00 ± 0.15	8.19 ± 1.50	7.47 ± 0.60
Lactic acid production (μmoles/100 mg dry wt/hr)	10.90 ± 2.2[b]	8.54 ± 1.50	7.08 ± 0.46[b]
Glucose-^{14}C (%) taken up into $^{14}CO_2$	35.0 ± 6.6	33.8 ± 11.9	36.3 ± 6.5
Glucose-^{14}C (%) taken up into ^{14}C-glycogen	1.9 ± 1.1	1.3 ± 1.0	0.8 ± 0.2

[a] After gassing with 95% O_2–5% CO_2, 200 mg lung slices were incubated 2 hr at 38° in Krebs–Ringer bicarbonate medium. 0.025 ml D-glucose-U-^{14}C (0.09 μmoles; 0.42 μCi) and glucose-^{12}C were added to a final glucose concentration of 1 mg/ml. Reactions were stopped with 0.1 *N* HCl. Results were determined as described under Materials and Methods. Numbers in parentheses are number of experiments in each group. Values are means ± 1 SD.

[b] Significant difference between groups ($p < .02$).

New England Nuclear Corp., Boston, MA. Reagents for the enzymatic determination of glucose and lactic acid were obtained from Sigma Chemical Company, St. Louis, MO; and hydroxide of Hyamine from Packard Instrument Co., Des Plaines, IL. Statistical calculations were performed by conventional methods (12).

Results and Discussion. The results of our studies of glucose uptake, lactic acid production, and amount of radioactive glucose converted to CO_2 and glycogen are seen in Table I. We found no significant difference in glucose uptake in the three groups of animals that we studied. Villee (13) reported an early decrease followed by an increase in glucose uptake with maturation in human lung slices. There may be a decline in glucose uptake in rat lung also, but the decline has reached a plateau by the time the animals reach 22 days of age.

It is of interest that the value we obtained for lactic acid production in adult rat lung slices, 7.08 ± 0.46 μmoles/100 mg dry wt/hr, is similar to the 5.7 ± 0.38 μmoles/hr 100 mg dry wt reported by Tierney (14) in a similar system. The decrease in aerobic lactic acid production found in our experiments calls to mind the decreased activity of hexokinase and phosphofructokinase previously described in the liver of rats with maturation (3).

We found a decreasing amount of radioactivity being incorporated into glycogen, and a decreasing amount of stored glycogen, in slices in progressively older animals (Tables I and II). A smaller amount of stored glycogen has been observed in earlier studies in adult, compared to fetal lung (15). Lung glycogen in the fetus evidently serves mainly for the nutrition of rapidly growing lung tissue. Glycogen in the fetal heart and liver, in addition to serving for the energy requirements of those organs, apparently also serves as an important part of the fetal and neonatal defense against hypoxia (15).

Previous studies of the hexosemonophosphate pathway in developing lung have been very few. Villee and Loring (16) concluded that there is no change in the relative amounts of glucose metabolized via the glycolytic and hexosemonophosphate pathways in human fetal lung tissues from 8 to 25 weeks gestational age. They used, however, the method of $^{14}CO_2$ ratios from glucose labeled in the one and six positions (16). Histochem-

TABLE II. Glycogen Content of Lungs.[a]

Series	Glycogen (mg/100 mg dry wt)
Fetal (3)	3.42 ± 2.14
Newborn (4)	1.63 ± 0.47
Adult (4)	1.19 ± 0.19

[a] The tissue was the same as that incubated with D-glucose-U-^{14}C, as described for Table I. The acidified tissue was dried and analyzed for glycogen as described under Materials and Methods. Number of experiments, each with three replicate flasks, is shown in parentheses. Values are means ± 1 SD.

ical techniques have suggested an increase in pentose pathway activity in the lung toward the end of fetal development (17). We found a decrease in hexosemonophosphate pathway activity with maturation: calculated glucose metabolism via the pentose pathway was 15.3 $\pm$ 3.7% ($N = 4$) in late fetal lung, and 6.2 $\pm$ 2.5% ($N = 4$) in adult lung. Our data call to mind the decline in enzymes of the pentose pathway reported in the developing liver in rats (3). This change may reflect a decreased need for sugars and NADPH for growth in the adult, compared to the late fetal rat lung.

Summary. We have studied several aspects of glucose metabolism in lung slices of late fetal, neonatal, and adult rats. There was no significant difference in glucose uptake or in CO_2 production among the three groups. Lactic acid production was 10.9 $\pm$ 2.2, 8.5 $\pm$ 1.5, and 7.1 $\pm$ 0.5 μmoles/100 mg dry wt/hr; 15.3 $\pm$ 3.7% of glucose was metabolized via the hexosemonophosphate pathway in lungs of late fetal animals and 6.2 $\pm$ 2.5% in those of adults.

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Received Apr. 19, 1972. P.S.E.B.M., 1972, Vol. 141.