Fatty Acid Synthesis by Subcellular Fractions of Lung Tissue

Abstract. *The mitochondria-rich fraction of the lung tissue is the most active subcellular fraction for the synthesis of long-chain fatty acids. This observation is contrary to what has been reported for the subcellular fractions of the liver. The high rate of fatty acid synthesis observed with the mitochondria-rich fraction of the lung may be related to the morphological transformation of mitochondria.*

Lung phospholipids are important for the stability of alveolar radius and the maintenance of normal gas distribution (*1*). Forty percent of the pulmonary surfactant phospholipid is lecithin (*2*). Very likely the lecithin molecule contains long-chain, saturated fatty acids in both its alpha and beta positions (*3*). Lung tissue is capable of incorporating long-chain fatty acids into phospholipids (*4*) and also of incorporating carbon-14 from glucose (*5*) and acetate (*5, 6*) into fatty acids of phospholipids. I report here the results of studies on long-chain fatty acid synthesis from acetate by subcellular fractions of the lung.

Lungs from caesarean-derived female rats (3 to 4 months old) of the Sprague-Dawley strain were perfused and washed with the homogenizing medium. The washed lung tissue was chopped and homogenized in 0.25*M* sucrose, 0.001*M* Mg-K-ethylenediaminetetraacetic acid (EDTA) solution. Homogenization was done in a Virtis-45 homogenizer for 3 minutes at 20,000 rev/min. The whole homogenate was centrifuged at 800*g* for 8 minutes to remove the nuclei and cell debris. Three fractions were then obtained by centrifuging at 6600*g* for 20 minutes (mitochondria-rich fraction) and 92,000*g* for 40 minutes (microsomes and supernatant). The conditions of incubation were a modification of those used for determination of fatty acid synthesis by liver subcellular fractions (*7*). The incubation mixture was 0.001*M* in Mg-K-EDTA, 0.015*M* in KF, and 0.1*M* in phosphate buffer adjusted to *p*H 7.4, before adding the homogenate fraction but after adding the following: 60 μmole of reduced glutathione, 90 μmole of adenosine triphosphate (ATP), 2.2 μmole of nicotinamide-adenine dinucleotide phosphate (NADP), 2.2 μmole of nicotinamide-adenine dinucleotide (NAD), 60 μmole of citrate, 0.75 μmole of coenzyme A (CoA), 11 μmole of acetate, 5.1 μmole of biotin, 90 μmole of glucose-6-P, 10 μmole of $MgCl_2$, 100 μmole of $KHCO_3$, and 88 μmole of glycylglycine in KOH buffer. The final volume was 2.5 ml, containing approximately 5 mg of homogenate protein. The gas phase was air. The above compounds were in the form of potassium salts except for NAD and NADP which were in the form of sodium salts.

Table 1. Acetate-1-C^{14} incorporation into long-chain fatty acids by subcellular fractions of rat lung. In the complete incubation mixture, the incorporation of acetate into long-chain fatty acids by the mitochondria-rich fraction after an initial lag (4 to 7 minutes) was linear for the entire 40 minutes. The rate was calculated for the period that the incorporation of acetate was linear. The results are expressed as nanomoles of acetate-1-C^{14} incorporated per minute per milligram of protein.

Fraction tested	Experiment No.					
	1	2	3	4*	5	6
Mitochondria-rich fraction (6,600*g*)	0.15	0.28	0.3	0.18	0.5	0.23
Microsomes (92,000*g*)		.04	.02	.008		.11
Supernatant	.002			.014	.007	.001
Whole				.06		.12
Mitochrondria-rich fraction + microsomes		.015	.2			
Mitochrondria-rich fraction + supernatant	.04				.072	

* Without EDTA and KF.

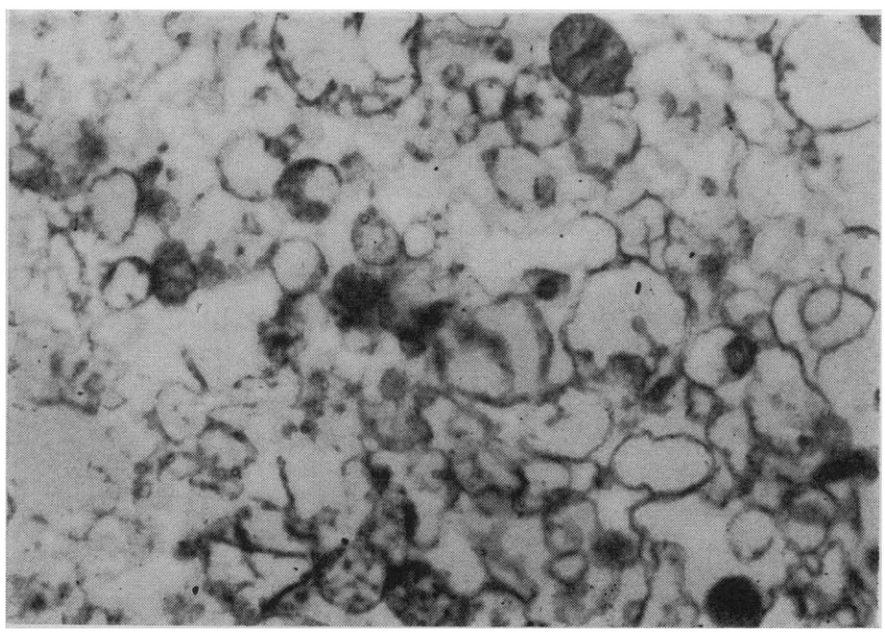

Fig. 1. Electron micrograph of the mitochondria-rich fraction, fixed in osmium tetroxide and embedded in epoxy resin (× 17,000).

The reaction mixture was incubated in 10-ml Erlenmeyer flasks in a water-bath shaker at 37°C. Samples were taken after 0, 5, 10, 20, and 40 minutes. At the end of each incubation period 4 ml of 20 percent alcoholic KOH was added and the samples were refluxed for 1 hour. The alcohol was then evaporated, 4 ml of water was added, and the samples extracted with ether to remove the nonsaponifiable lipids. After ether extraction the samples were acidified and twice extracted with heptane. The heptane extracts were combined, evaporated, and taken up with toluene for carbon-14 assay in a liquid scintillation spectrometer.

The subcellular fraction precipitated at 6600*g* (Fig. 1) was by far the most active fraction for the incorporation of acetate-1-C^{14} (Table 1) into long-chain fatty acids. By comparison, the amount of acetate incorporated into fatty acids by the other subcellular fractions was very small, and this may have been due to cross contamination during the fractionation procedure. Addition

of supernatant or microsomes to the mitochondria-rich fraction did not increase the incorporation of acetate-1-C^{14} into fatty acids. These experiments do not show whether the acetate-C^{14} incorporation represents the synthesis of new fatty acids or the elongation of preexisting fatty acids.

These results are contrary to those that have been reported for subcellular fractions of the liver, where the clear supernatant is the most active fraction for the incorporation of acetate-C^{14} into long-chain fatty acids (*8*).

Addition of DL-α-glycerophosphate to lung subcellular fractions did not alter the amount of acetate incorporated into fatty acids under our experimental conditions.

The amount of acetate-1-C^{14} incorporated into fatty acids by the mitochondria-rich fraction of lung tissue is higher than the amount usually reported for the liver mitochondria. (The possibility that the magnitude of the incorporation in our preparation is due to some minor component other than mitochondria cannot be excluded.) The high capacity of the mitochondria-rich fraction for incorporating acetate-1-C^{14} into fatty acids is consistent with the finding that, after the injection of acetate-1-C^{14} in vivo, lung tissue contained more fatty acids labeled with carbon-14 than did liver tissue (*9*).

One of the functions of lung mitochondria may be to provide the fatty acids of pulmonary surfactants. The high rate of fatty acid synthesis might be correlated with the observed morphological transformations of lung mitochondria (*3*).

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Microvibrations in Man and Dolphin

Abstract. *Microvibrations were recorded from the dorsal body surface of a bottle-nosed dolphin* (Tursiops truncatus) *while it swam in water and while it lay on a foam rubber mattress in an air environment. Unlike poikilothermic water-living animals which do not manifest microvibrations, this homeothermic mammal has 13-cycle per second microvibrations similar to those of man and other homeotherms. For comparative purposes, microvibrations of 11 cycles per second were recorded from the arm of a man while lying on the same mattress. The nature and origin of these microvibrations is discussed in relation to physiological tremors and shivering.*

Fine, tremor-like vibrations, invisible to the human eye, which occur at all times over the entire body surface of warm-blooded animals, have been studied extensively by Rohracher (*1*). These have been referred to as microvibrations (*1*) and minor-tremor (*2*). In relaxed, awake humans the amplitude of the microvibrations ranges from 1 to 5 μ. During sleep their amplitude is greatly reduced, and during muscular activity it is enhanced. Interruption of the motor innervation to a given body part results in almost complete disappearance of microvibrations in that area (*2*). Because of these characteristics there has been a tendency to associate microvibrations with processes underlying muscle tonus. The frequency of microvibrations in humans ranges from 7 to 13 cy/sec, values which are comparable to those of fine, finger tremors (*3–5*), "physiological tremors" (*6*), the grosser tremors of shivering (*7, 8*), and to the alpha rhythm of the electroencephalogram (*4, 5*). However, the lack of an exact correspondence between finger tremors and alpha waves over the motor area of the brain led Jasper and Andrews (*5*) to conclude that they were not interdependent. Also it seems unlikely that there is any close correspondence between body microvibrations and alpha waves, since the latter vary in frequency and phase over different regions of the head.

Rohracher proposed that microvibrations may vary as a function of body temperature regulation in homeothermic organisms. In humans, changing the ambient temperature from 25°C to 4.5°C caused an increase in the frequency of microvibrations, but a decrease in amplitude. Studies of wide varieties of animals showed that microvibrations occur in all warm-blooded animals, but do not occur in poikilothermic animals (*1*). It was observed recently that the snake (*Python molurus*) can increase its temperature by muscular contraction, but these contractions are of sufficiently large amplitude to be visible and are quite slow, of the order of one per 2 seconds to one per 8 seconds (*9*).

To further clarify the problem of possible relations between microvibrations and temperature constancy in warm-blooded animals, it seemed relevant to determine whether mammals adapted to living in the water show microvibrations, and if so, how these change when the animals are active and inactive in the water and how they vary from a water environment to an air environment.

Microvibrations were studied on the body surface of a female, bottle-nosed dolphin (*Tursiops truncatus*), which weighed approximately 112 kg. Measurements of microvibration were made with a special piezo-electric transducer coupled to a Grass P5C preamplifier and a Brush inkwriting recorder. The overall frequency-response characteristics of this system were good from 1.5 to 100 cy/sec at one-half amplitude, a range amply broad for the purpose. The transducer had no resonance effect in the frequency range of the microvibrations recorded.

In a preliminary experiment the dolphin was removed from the water and placed on a stretcher. The vibration transducer was fixed with vaseline on different parts of the body surface. At all points tested, microvibrations appeared with a frequency of about 13 cy/sec and with considerably greater amplitude than that of the resting human under similar circumstances.

A few weeks later a more detailed study was attempted. The transducer was mounted in a water-proof container held in place by a suction cup. With this unit on the back of the dolphin, halfway between the blowhole and the dorsal fin, records were obtained with the dolphin moving slowly in water about 38 cm in depth. The dolphin was then removed briefly to an air environment, and records were obtained while it was lying moistened on thick layers of rubber foam. Subsequently, recordings were made from the right forearm of a human subject while lying on the rubber foam mattress.

Figure 1 shows sample records of