EFFECTS OF STREPTOZOTOCIN - INDUCED DIABETES ON THE CYTOPLASMIC REGULATION OF ADENYLATE CYCLASE AND CYCLIC AMP-PHOSPHODIESTERASE IN RELATION TO THE RAT LUNG ALVEOLAR TISSUE METABOLISM

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By

Anwilika Felix Ofulue

Department of Pathology

Faculty of Medicine

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ANWILIKA FELIX OFULUE

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To my mother

whose love for her children has inspired in us the pursuit of knowledge as a basis of happiness.

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ABSTRACT

Some aspects of rat lung alveolar metabolism during diabetes have been investigated. Total protein content was unaffected but the DNA concentration was lowered. The proten/DNA ratio was markedly increased, suggesting increased differentiation. Hydroxyproline content was increased. Glycogen content was depleted. There was a marked reduction in the phospholipid content of the tissues and of the surfactant complex. The amount of phosphatidylcholine did not change appreciably, but the contents of disaturated phosphatidylcholine and lyso-phosphatidylcholine were markedly depleted in the surfactant complex. Also there was a reduced amount of phosphatidylglycerol in the surfactant complex. However, there was no appreciable change in the phospholipid content in the residual fractions. Ultrastructural studies showed alterations in the alveolar Type II cells which implied that the depressed metabolic activity of these cells were due to defects in the normal function of the granular endoplasmic reticulum and of the mitochondria.

Cyclic AMP and calmodulin play vital roles in cellular regulation and metabolism. In order to elucidate the role of cyclic AMP and calcodulin in the changes observed in the meta-

bolic activities of the rat lung alveolar tissue, the effects of streptozotocin-induced diabetes on the cytoplasmic regulation of adenylate cyclase and cyclic AMP phosphodiesterase were investigated. Alveolar tissues were obtained from the peripheral areas of lungs, homogenized and centrifuged at high speed to isolate a particulate fraction rich in adenylate cyclase and cyclic AMP phosphodiesterase and a supernatant fraction also rich in cyclic AMP phosphodiesterase but contained the cytoplasmic activator(s) of the particulate adenylate cyclase. Basal adenylate cyclase activity in the particulate fraction was decreased, but its activation by the supernatant fraction was markedly increased. The decrease in basal adenylate cyclase was found to be due to a translocation of calmodulin, on which it appears to depend in the expression of its activity, into the cytoplasm. The activation of particulate adenylate cyclase by the supernatant fraction appeared to be independent of calmodulin in the supernatant fraction, but was found to be due to the increased amount of a 65,000 dalton protein. Cyclic AMP phosphodiesterase activity was depressed, both in the particulate and supernatant fractions. Calmodulin translocation from the particulate into the supernatant fraction accounted for the depressed activity of the Ca^{2+}-dependent cyclic AMP phosphodiesterase in the particulate fraction. A heat-stable inhibitor of Ca^{2+}-activatable cyclic AMP phosphodiesterase was observed in the lung alveolar tissue and the activity

increased during diabetes, thus accounted for the reduced activity of the Ca^{2+}-dependent cyclic AMP phosphodiesterase in the diabetic lung alveolar tissue homogenates.

It was concluded that the altered and/or uncoordinated activities of the enzymes and the endogenous modulators related to the cyclic AMP metabolism and of calmodulin may be the most significant factors responsible for the depressed metabolic activity of the rat lung alveolar tissue during diabetes mellitus.

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A. INTRODUCTION

The ability of any organ to maintain its integrity and uniqueness depends on the differentiated properties of its constituent cells[1]. The lung is no different; however, like any other organ or tissue of an animal, it has its own characteristic pattern of biochemical activities which is associated with its physiological functions and morphological features[2]. These biochemical activities can define the altered status of lung in disease[3]. In this approach, lung disease is viewed as a process which alters lung morphology and function either directly or through the mechanisms that maintain the functional state of the lung.

It has become increasingly apparent that diabetes mellitus adversely affects the mechanical and biochemical functions of the lung [4-10]. Specific receptors for insulin have been identified in membrane preparations from normal rat lungs[8]. These studies suggest that insulin may play a role in the metabolic processes of the lung. Based on extensive information concerning the role of cyclic AMP in the anabolic action of insulin in other tissues and homogeneous populations of cells[11], it would be surprising indeed if enzymes and endogeneous modulators related to the metabolism of the cyclic nucleotide in lung tissue did not play important regulatory roles in many aspects of the depressed pulmonary activity in

diabetes mellitus.

There is no better way to appreciate what insulin means to the metabolic processes which maintain lung structure and function than to study the effect of acute insulin deprivation. It therefore becomes necessary to study the metabolic dysfunctions of the lung that affect the maintenance of the alveolar structure and function, and to examine possible alterations in the cellular mechanism(s) regulating the lung functions.

B. LITERATURE REVIEW

1. MAINTENANCE OF ALVEOLAR STRUCTURE AND FUNCTION

The major function of the lung is to provide the living organism with oxygen from the air and to remove excess carbon dioxide from the bloodstream. To accomplish this, the lung has evolved as a complex structure which brings together the atmosphere and blood in a fashion which is finely regulated to insure maximum gaseous exchange. The ability of the lung to continue to function normally in this role critically depends on the inherent properties of its parenchymal cells[1].

The mature adult lung parenchyma consists of four major types of cells (endothelial, mesenchymal cells, alveolar Type I, and alveolar Type II cells) and a complex extracellular matrix composed of three categories of connective tissue (collagen, elastic fibres, and proteoglycans). The most prevalent parenchymal cells are the endothelial and mesenchymal cells, with the alveolar Type II cells next in total number. The alveolar Type I cells are the least in number, even though the vast majority of the alveolar surface area is lined by a continuous layer of these extremely flattened and distended squamous cells with a very rich capillary bed underneath. Between these squamous superficial cells at sporadic intervals are the cuboidal shaped Type II cells, and both together with

the capillary endothelium and the reticulin basement membranes form the blood-air barrier where effective gas exchange takes place. Collagen is the most abundant constituent of the extracellular matrix, comprising 60-65% of the total extracellular mass[1].

For the lung parenchyma to maintain its function, its constituent cells must be able to modify their local environment. The inherent properties of the lung are capable of responding to a multitude of factors. The cells of the parenchyma can respond to changes in their environment because they possess the ability to receive the information of local changes. Examples of this are the receptors for corticosteroids[12,13] and the cyclic nucleotide system[14,15]. In addition, local cell-to-cell interactions can modulate changes in the cells present in the parenchyma. For example, mediators produced by parenchymal mast cells may be important in the control of smooth muscle cells located around the alveolar duct[16]. Also the demonstration of nerve endings in the region of alveolar epithelial cells suggests the possibility that direct neural control may influence the rate of surfactant synthesis and secretion[3].

The ability of the Type II alveolar cells to synthesize, store and secrete surfactant[17,18] represent a classic example of the inherent ability of a lung parenchymal cell to

modify its local environment and those of neighbouring cells. Surfactant, a surface active material which is mainly composed of phospholipids and small amounts of proteins and carbohydrates, lowers the surface tension of the alveoli, stabilizes the air spaces, and enables the lung to retain air at low inflational pressures, thus preventing alveolar collapse during expiration and greatly reducing the inspirational force required to expand the lungs[19]. Alveolar collapse occurs at birth in children with inadequate synthesis and secretion of surfactant, the syndrome known as hyaline membrane disease or respiratory distress syndrome which is a leading cause of neonatal death in developed countries.

Another inherent property and critical secretory function of lung cells is the maintenance of the extracellular matrix of the alveolar septum. Functional studies have suggested that it is an important determinant of lung mechanics and structural stability[20]. The stability of the alveoli depends, in part, on the collagen comprising them. It has been suggested that collagen acts principally as a supporting framework for elastic tissue. In this concept, the major role of collagen would be to limit expansion. However, alterations of collagen by collagenase result in a marked increase in volume at high-distending pressures with no change in the distensibility or collapsibility in the range of normal pressures. Therefore a more likely concept is that both collagen

and elastin are important determinants of lung mechanics over most of the range of inflation; collagen produces the necessary stiffness which allows increasing recoil pressures at increasing lung volumes. The synthesis of the collagen found in the alveolus appears exclusively the function of the mesenchymal cells; however, the lung endothelial cells may synthesize basement membrane collagen[1]. It is now apparent that the alveolar septum is maintained in its normal state by several processes including the relative number of cells capable of collagen production and/or catabolism, and the modulation of the cellular control of collagen synthesis and destruction by cell-cell interactions, hormonal and humoral factors[21-23]. Abnormalities in any of these processes would apparently influence the functional characteristics of the lung. Factors external to lung which either directly or indirectly promote lung metabolic dysfunction appear to affect the maintenance of parenchymal lung collagen since lung elastic recoil is significantly decreased in young men with juvenile onset diabetes[4].

2. GLUCOSE UTILIZATION BY LUNG: ITS IMPLICATIONS IN DIABETES MELLITUS

Glucose has been shown to be an important substrate for lung tissue. In addition to serving as a precursor for lung glycogen, lactic acid, and lipids[24-27], glucose optimizes the

rates of lipid and protein synthesis in the lung[25,28,29]. It has been demonstrated that glucose is taken up by the lung in amounts comparable to those taken up by other organs and metabolized via similar pathways that have been described in other organs[29-31]. Such pathways are depicted in Fig. 1. The major fate of utilized glucose is conversion to lactate. This is of interest in that the lung is one of the most aerobic organs in the body. One possible explanation for the significant quantity of lactate production is that some lung cells, or portions of cells, are dependent upon glycolysis for ATP production[32]. However, while brain must use glucose for energy because of the characteristics of the blood-brain barrier, the lung does not utilize glucose as the major energy substrate[32]. This suggests that, in lung, fatty acids or possibly amino acids also serve this function. This is appreciable since significant amounts of glucose are converted to amino acids and protein in lung tissues[29,31].

The lung does not seem to have significant capacity to store glucose as glycogen or lipid, in contrast to liver and adipose tissue[29-32]. However, lung tissue does have an adequate pentose pathway activity. This pathway is concerned not primarily with the provision of energy, but rather with the production of pentose phosphates for DNA and RNA biosynthesis and with the generation of reducing equivalents for other pathways, such as fatty acid and phospholipid synthesis,

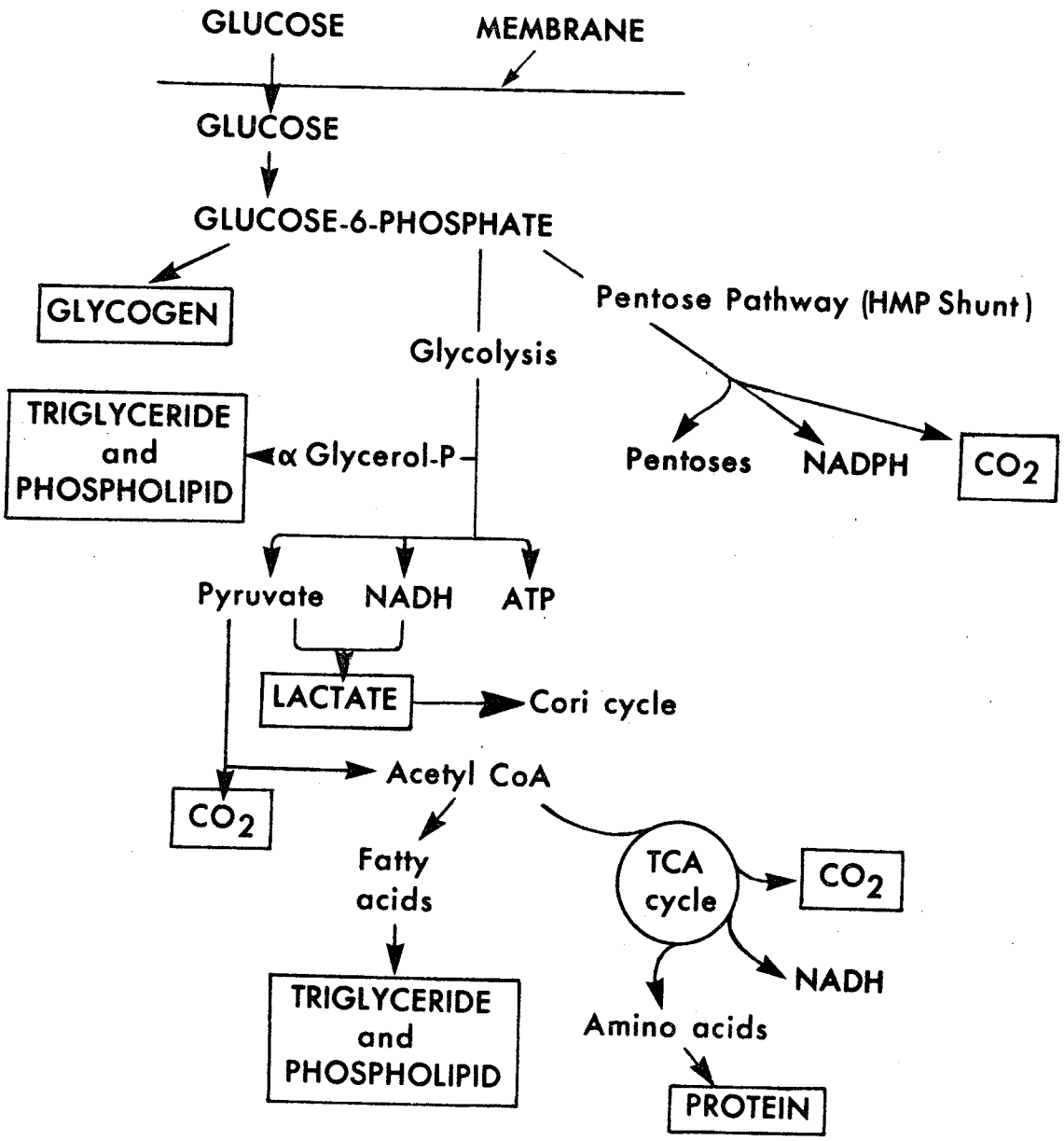

Fig. 1 Different pathways for glucose utilization

that require NADPH rather than NADH as an essential cofactor. Moreover, NADPH in some tissues, especially the erythrocyte, is essential to maintain glutathione in a reduced state and to prevent harmful oxidation, such as lipid peroxidation. It is possible that NADPH production in the lung may help to protect it from oxidants, but to date the evidence is indirect[32].

The effect of insulin on glucose transport into cells was elegantly demonstrated by Levine et al[33]. Subsequent investigations[34] indicated that glucose transport is enhanced by insulin. Recent interests have therefore focused on the possibility that glucose transport and metabolism in the lung is hormonally regulated. It has been suggested that the lung is freely permeable to glucose[24] since the addition of insulin to lung slices in vitro failed to stimulate glucose utilization. On the other hand, Weber and Visscher[26] have demonstrated an increased glucose utilization and lactate production in the presence of insulin. Morishige et al[8] also found that in the diabetic rat lung, glucose oxidation was markedly decreased and was restored to normal values with insulin treatment. Lactate production by diabetic lung slices in vitro was found to be significantly elevated, suggesting that there is a reduced flow along the pathways leading from pyruvate. The activity of the pentose pathway, which is the major pathway of glucose oxidation in lung tissue[25] was normal in the diabetic lung, suggesting that the intracellular

supply of substrate was limiting. These investigators also showed that insulin interacted in a specific manner with receptors in a particulate lung preparation. Fricke and Longmore[35] have now demonstrated that the rat lung contains a hexose transport system that is stimulated by insulin and depressed in diabetes. The transport system has several features which strongly suggest the presence of a carrier-mediated transport process, which includes an uptake process that follows Michaelis-Menten type kinetics with saturation at high substrate levels.

Moxley and Longmore[36] have shown that glucose incorporation into lipids was increased by insulin treatment and decreased in experimental diabetes. This finding is very significant since it has been suggested[37] that lung glycogen plays a role in phospholipid synthesis. Fig. 2 shows the metabolic pathways by which glycogen or glucose may be incorporated into both the glycerol and fatty acid portions of the phospholipid molecule. Pulmonary glycogen levels vary considerably during tissue development[38-40]. Accumulation of glycogen is abundant in the undifferentiated cuboidal cells throughout most of the gestation period, depleting rapidly around birth, then accumulating again by the fifth day postpartum to an adult level. The postnatal accumulation, however, is in the mesenchymal cells instead of the epithelial cells and correlates to a rapid mitotic activity of the mesenchymal

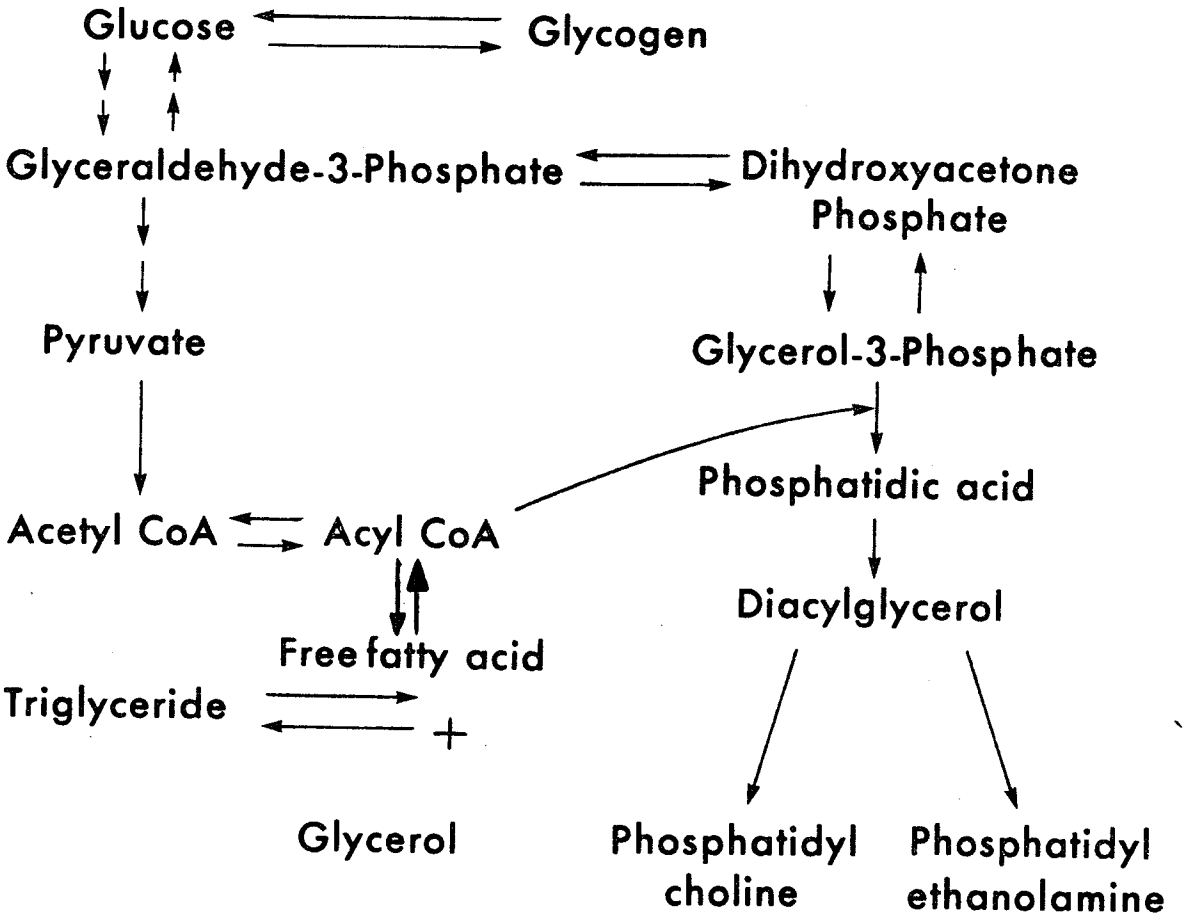

Fig. 2 Scheme by which glucose can be converted into phosphatidyl choline. Single arrowhead indicates one-step reactions and double arrow heads indicate multiple step reactions.

tissue[41]. Thus it has been suggested that glycogen provides energy for rapid cellular multiplication; the glycogen accumulating during developmental periods when there is rapid cellular multiplication and depleting when differentiation occurs. The rapid prenatal fall in fetal lung glycogen content is coincident with the differentiation of epithelial cells, and increase in pulmonary phospholipid content, and the appearance of lamellar bodies in the Type II cells[42,43]. The neonatal depletion of glycogen in lungs is apparently due to an enhanced glycogen phosphorylase a activity[38,40] and appears to provide the substrate for phospholipid synthesis[44]. It has been suggested that insulin may play a role in the short term regulation of the incorporation of glucose into the mammalian pulmonary surfactant complex[36].

3. PULMONARY LIPID METABOLISM:-- SURFACTANT PRODUCTION

It has become increasingly apparent that lipid metabolism in the lung is of considerable importance in maintaining the structural and functional integrity of the normal alveoli. The alveoli in the lung are lined by surfactant, a surface-active material, whose major components are dipalmitoylphosphatidylcholine (dipalmitoyl-PC) and phosphatidylglycerol (PG). These phospholipids are relatively rare in mammalian systems. The lung is, therefore, rather unusual in containing relatively large amounts of these two phospholipids and it

might as well contain a unique system for their synthesis[45].

Like virtually all naturally occurring phospholipids, lung phospholipid synthesis requires fatty acids and glycerol-3-phosphate or dihydroxy-acetone phosphate. The glycerol-3-phosphate (the backbone of the phospholipid molecule) may arise either from glucose via dihydroxyacetone phosphate, which is formed as an intermediate in the glycolytic breakdown of glucose, or from glycerol[37]. Under normal circumstances, the uptake of free fatty acids from the circulation is probably a major source of fatty acid for the lung. The uptake of palmitic acid from the bloodstream by the lung was shown many years ago[46]. A second external source of fatty acid is represented by circulating lipoproteins, either very low density lipoproteins synthesized in the liver or chylomicrons originating from the intestine[46]. Lipoprotein lipase has been found in the lung[47], presumably on the capillary wall, but the enzyme could also function outside the pulmonary circulation to hydrolyze circulating triglycerides to provide fatty acids to be used by the lung. The uptake of fatty acids is probably not rate limiting nor selective, but is dependent on the concentration of fatty acids in the perfusate and is altered by the distribution of pulmonary blood flow[48,49]. The lung also has a great potential for fatty acid synthesis[50]. Acetyl-CoA carboxylase and fatty acid synthetase, two enzyme systems involved in the de novo synthesis of fatty acids, are both present in the lung cytosol[51].