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Palmitate Incorporation in the Lungs of Dogs with Granulomatous Disease (39467)

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Previous investigation of an animal model of diffuse granulomatous lung disease has shown that moderate hypoxia results in a dramatic fall in oxygen utilization and a rise in lactate production by the diseased lung (1-4). Newman and Naimark have found that severe hypoxia reduces palmitate incorporation by normal lung (5). Their study prompted us to test the effects of moderate hypoxia upon this metabolic activity in the model of granulomatous disease.

Methods. Pulmonary granulomatous disease was produced by intravenous injection in dogs of 0.3 ml/kg complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) on 2 successive days (1). The dogs were studied 3-4 weeks after injection when the disease reaches its maximum extent. Eight normal and eight diseased dogs were studied according to an identical protocol. The dog breathed the appropriate gas mixture (F_{IO_2} 0.14-0.25) until he attained a steady level of ventilation. Five-tenths millicurie in 1.3 mg of crystalline [1-^{14}C]palmitic acid (New England Nuclear Corp., Boston, Mass.) were then given intravenously. Arterial blood samples were collected from the femoral artery at 20, 40, and 60 min for measurement of oxygen and carbon dioxide tension and pH using an Instrumentation Laboratory blood gas analyzer. At 1 hr after administration of labeled palmitate, the dog was exsanguinated by transection of the femoral arteries.

The lungs were promptly removed, weighed, and immersed in 2:1 (v/v) chloroform-methanol at −20°, then homogenized, stirred, and filtered. The filtrate was washed with 0.37% KCl (20 ml/100 ml filtrate), centrifuged, and the supernatant was collected. The washing was repeated twice with 0.74% KCl-chloroform-methanol solution in a volume ratio of 47:3:48, respectively. The washed lipid extract was dried and redissolved with petroleum ether to a volume of 500 ml. This comprised the stock solution from which all subsequent analyses were made. Four milliliters of the stock solution were dried under nitrogen, redissolved in chloroform, and put through 10-cm Kimax columns containing 2 g of silicic acid (325 mesh). Three subsequent washings of 10 ml of methanol eluted the phospholipids which were collected in preweighed vials, dried under nitrogen, and reweighed. Total lipid recovery from the columns, as determined by radioactivity recovered, was 80% for both the normal and diseased specimens.

Additional aliquots of the stock solution were similarly prepared for counting and fractionation. Counting was done with a Packard Tri-Carb liquid scintillation counter. Separation was done by thin-layer chromatography with silica gel H, using chloroform-methanol-water-glacial acetic acid, 80:25:4:1 (v/v) as the solvent. The plates were stained with iodine vapor and the spots were scraped separately for counting. In addition, the fatty acid composition of phosphatidyl choline was determined in four diseased and four control specimens by gas chromatography.

For autoradiographic studies two control and two diseased dogs were studied breathing room air according to the protocol previously described. One hour after infusion of 2 mCi/kg palmitic acid 9-10 ^{3}H (New England Nuclear Corp., Boston, Mass.) the lungs were removed and fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.6). Samples were postfixed in 1% osmium tetroxide, dehydrated in graded acetone solutions, and embedded in Araldite resin. One-micron-thick sections were dipped in Kodak Nuclear Track emulsion (NTB-2), air-dried, and incubated for 2, 4, and 6 weeks. The

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autoradiographs were developed in Kodak D-19 and examined by phase contrast and dark-field microscopy. For electron microscopic studies ultrathin sections were cut on a Sorval MT-2 microtome, stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop 101.

Results. The data on lung weight and phospholipid content are summarized in Table I. On the average, there was a twofold increase in the lung weight to body weight ratio in the diseased dogs. This was attributable to the increase in weight of the diseased lung since body weight did not fall by more than 10% during the development of the pulmonary lesions. The larger standard deviations in the diseased group reflect the variability in extent of disease in different animals. Since phospholipid content increased linearly with increasing lung weight, as shown in Fig. 1, the phospholipid content per unit lung weight was, on the average, identical in both groups. The three diseased lungs which group with the normals in Fig. 1 showed features on gross examination characteristic of the granulomatous process. The lungs have a dark raspberry color and are filled with nodules which vary in size from 1 to 10 mm in diameter. Microscopically, the lesions are seen to be typical noncaseating granulomata (1). There are, in addition, increased numbers of free alveolar macrophages. Electron microscopy reveals an increased number of Type II pneumocytes lining all alveolar air spaces (Fig. 2). This proliferation of alveolar Type II cells is not localized to regions with granulomata, but is found as well in otherwise unaffected parts of the lung.

Autoradiographic studies reveal the developed silver grains clustered in the Type II alveolar epithelial cells in the diseased lung (Fig. 3). There is no concentration of silver grains over the granulomata or alveolar macrophages at 1 hr after infusion of labeled palmitate (Fig. 4). In the normal controls, the palmitate was similarly associated with the Type II alveolar pneumocyte and not with other normal cell types.

The uptake of labeled palmitate in total lung lipids was no different in diseased and normal groups, even during moderate hypoxia (Fig. 5). Nor was there any correlation between lipid counts per minute per lipid weight and P_{AO_2}, P_{aCO_2} or pH in either the normal or diseased groups.

There was no difference in the preferential incorporation of the label into the phosphatidyl choline fraction of phospholipids, being 57 ± 9% for diseased and 48 ± 13% for controls and no significant correlation between incorporation and P_{aO_2}, (Fig. 6). Accordingly, the data are summarized in Table II with reference only to the presence or absence of disease. The increase in phos-

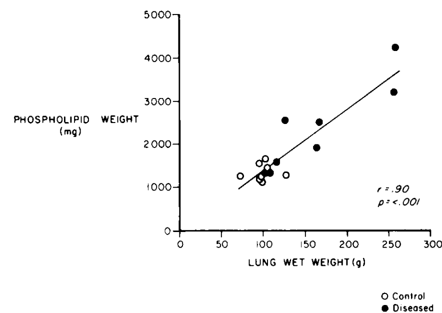

FIG. 1. Correlation of phospholipid content and lung wet weight in eight control (○) and eight diseased (●) dogs.

TABLE I. BODY, WET LUNG, AND PHOSPHOLIPID WEIGHTS (MEAN ± SD) IN EIGHT CONTROL AND EIGHT DISEASED DOGS.[a]

	Units	Controls	Diseased	*P*
Body weight	kg	12 ± 2	10 ± 2	NS
Lung wet weight	g	97 ± 17	162 ± 63	<.02
Phospholipid weight	g	1.3 ± 0.2	2.3 ± 1.0	<.02
Lung wet weight / Body weight	%	0.8 ± 0.1	1.7 ± 0.8	<.02
Phospholipid weight / Lung wet weight	%	1.4 ± 0.3	1.4 ± 0.3	NS

[a] The means are compared by the Student's *t* test of Fisher.

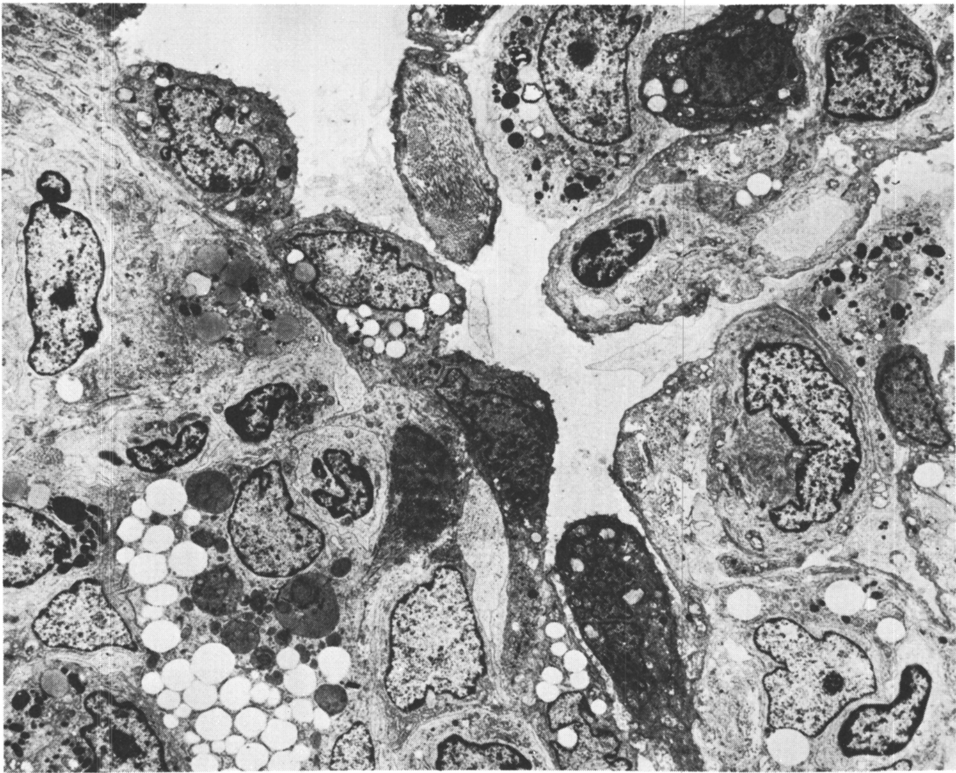

Fig. 2. This electron micrograph shows a portion of a granulomatous lesion encroaching on an air space. The lesion is made up of epithelioid cells containing fat, autophagosomes, and phagocytized material. The surface is lined by four adjacent Type II pneumocytes in different stages of development (×3000).

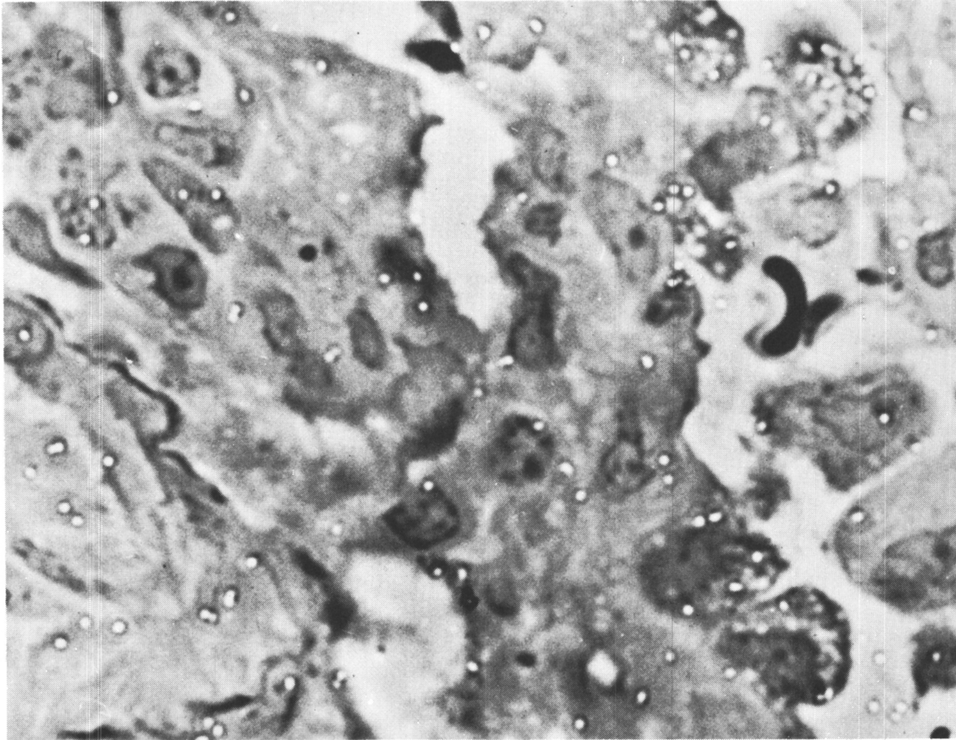

Fig. 3. In this autoradiograph examined in a phase contrast microscope, the developed silver grains are out of the plane of focus and are therefore seen as bright spots rather than dark grains. This micrograph shows a granulomatous lesion lined by Type II pneumocytes containing a concentration of silver grains. The dispersed grains in the rest of the field indicate the background level. (×750).

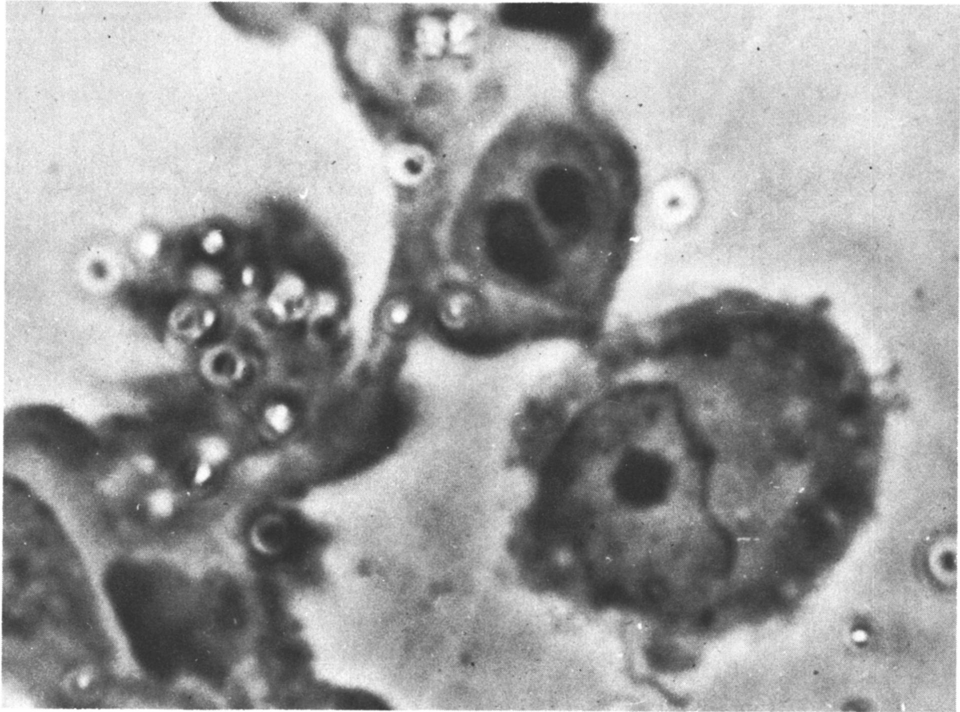

FIG. 4. In this autoradiograph examined by phase contrast at high magnification, the developed silver grains are seen as bright rings which are concentrated over a single Type II pneumocyte. An alveolar macrophage and an intracapillary polymorphonuclear leukocyte have no associated grains. (×2000).

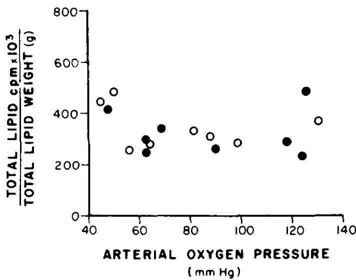

FIG. 5. Uptake of [1-^{14}C] palmitate in total lung lipids in eight control (○) and eight diseased (●) dogs.

pholipid counts in the diseased lung reflects the increase in lung weight; the specific activity, expressed as phospholipid counts per unit phospholipid weight, being no different in the groups.

Separation of the fatty acid constituents of the phosphatidyl choline fraction by gas chromatography in four control and four diseased dogs showed the C16 chain (palmitate) comprised, on the average, 59 and 57%, respectively, of the total fatty acids. Radioactivity recovered from the phosphatidyl choline fatty acids was almost exclusively confined to palmitate in both the normal (98%) and the diseased (94%) specimens.

The total neutral lipid count per gram

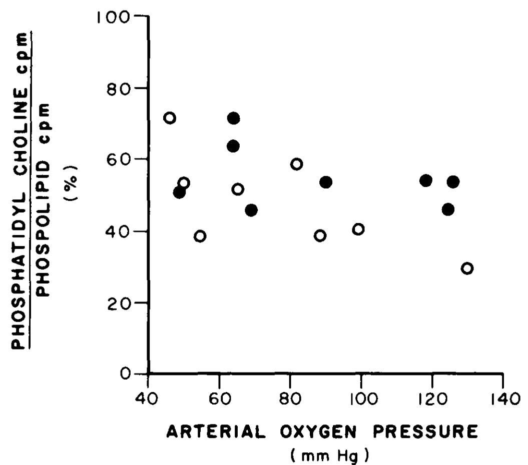

FIG. 6. Incorporation of [1-^{14}C] palmitate into phosphatidyl choline fraction of lung phospholipids in eight control (○) and eight diseased (●) dogs.

TABLE II. INCORPORATION OF [1-^{14}C]PALMITATE INTO LUNG PHOSPHOLIPIDS OF EIGHT CONTROL AND EIGHT DISEASED DOGS (MEAN ± SD).[a]

	Units	Controls	Diseased	*P*
Total Injectate Dose	cpm × 10^6	43 ± 14	38 ± 20	NS
Total Phospholipid Counts	cpm × 10^3	488 ± 213	794 ± 238	<.02
Phospholipid counts / Phospholipid weight	cpm × 10^3 / g	369 ± 135	380 ± 166	NS
Phosphatidyl choline counts / Phospholipid counts	%	48 ± 14	55 ± 9	NS

[a] The means are compared by the Student's *t* test of Fisher.

neutral lipid weight was 127 ± 58 in normal lungs and 103 ± 29 in the diseased lungs. Of the total neutral lipid counts per minute, the breakdown as a percent found in cholesterol, fatty acids, triglycerides, and cholesterol esters was 14, 9, 54, and 14, respectively, for normals and 18, 3, 59, and 9 for the diseased. The differences in breakdown between normal and diseased were not statistically significant.

Discussion. The present study confirms earlier observations that palmitate is incorporated preferentially into the phosphatidyl choline fraction of the phospholipids of the lung. Our results in normal lungs demonstrate that the incorporation of the fatty acid occurs in the Type II alveolar pneumocyte, as has been shown previously by one of us (H.O.H.) as well as by others (6, 7). Surprisingly, the presence of granulomatous disease does not interfere with the incorporation of palmitate. The increase in lung weight in this disease results from an increase in cell mass; there are no differences in the ratios of protein weight: wet weight or wet weight:dry weight between diseased lungs and normal lungs (4).

Since there is a proliferation of epithelioid and mononuclear inflammatory cells in the granulomas which are not known to incorporate palmitate one might expect there would be a reduction in the specific activity of labeled palmitate (counts per minute per gram of phospholipid) in the diseased lung. Rather, there is an increased uptake of palmitate in proportion to the increase in weight of the lung in the diseased group. The possibility that this behavior of the diseased lung might be due to a reduction in the circulating levels of palmitate was considered, but measurement revealed no differences in plasma concentrations of palmitate or albumin between the diseased and control dogs. Since we have identified an increase in numbers of Type II alveolar pneumocytes in the diseased lungs, and found by autoradiographic study that labeled palmitate is preferentially incorporated into these cells, we conclude that the enhanced incorporation of fatty acid in the diseased lung is the result of metabolic activity of the Type II alveolar cells. The autoradiographic studies identify primarily palmitate incorporation into phospholipids rather than neutral lipids, since acetone used in the preparation of these tissues may have extracted the latter. Elsbach (8) and Mason and co-workers (9) have shown that the alveolar macrophage incorporates palmitate into phospholipids and these cells are increased in adjuvant induced pulmonary disease. Yet, Young and Tierney (10) and Thomas and Rhoades (11) have reported that it requires more than 6-hr after intravenous infusion of radioactive palmitate before the peak of specific activity is found on the surface of the alveoli in normal lungs. In addition, the report of Askin and Kuhn (7) confirms that in normal lungs the label first appears in the Type II alveolar cells. Hence, it seems unlikely that the label would be found in the free alveolar cells in the present study where the lungs were harvested 1 hr after palmitate infusion. This time was chosen because other studies have shown that the maximal uptake of palmitate in normal lung occurs within this period of time (4, 5, 11).

Whereas Newman and Naimark (5) demonstrated an effect of severe hypoxia on

palmitate incorporation by normal lung in the rat at an arterial tension of 29 mm Hg, the present study failed to demonstrate such an effect over a range of 40–140 mm Hg in the dog. We believe this difference reflects the level of hypoxia studied. While fatty acid incorporation appears to be relatively unaffected by moderate hypoxia, dramatic changes in pulmonary oxygen utilization and lactate production have been previously demonstrated during hypoxia in this disease model (1-4).

Summary. Moderate hypoxia did not influence the pulmonary incorporation of an intravenous dose of [1-^{14}C]palmitate either in dogs with experimentally produced granulomatous disease or in normal controls. The lung weight in the diseased animals, was, on the average, double that of the controls. There was a proportionate increase in uptake of the radioactive label at 1 hr after infusion in the diseased lungs, hence the specific activity of labeled palmitate (counts per minute per gram of phospholipid) was no different in the two groups. Moreover, half the radioactivity of the phospholipids was recovered in palmitate separated from the phosphatidyl choline fraction in both diseased and normal lungs. Anatomic studies demonstrated increased numbers of Type II pneumocytes lining all alveolar air spaces in the diseased lung. Autoradiographic studies indicated the presence of labeled palmitate in the Type II cells, but not in the inflammatory cells of the granulomata. We conclude that the increased palmitate uptake in this disease in accounted for by the metabolic activity of the Type II pneumocytes.

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