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Pulmonary toxicity of a commercial lipid aerosol in guinea pigs

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Yale University

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Gail M. Sullivan
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ABSTRACT

Two cases of possible lipoid pneumonia associated with use of Pam cooking oil aerosol stimulated interest in an animal model for preliminary studies of the potential toxicity of this lipid spray.

Guinea pigs were exposed to commercial Pam in short and long, up to six months, term exposure studies following intubation and intratracheal injection of the oil or inhalation of a brief(15 seconds) spray. The long term sprayed animals demonstrated markedly poor growth vs. controls with non-significantly different food and water ingestion and without evidence of malabsorption.

Small amounts of oil were observed within alveoli and bronchioles of sprayed and intratracheally injected animals. Oil was also noted finely dispersed within monocytic cells. Focal mononuclear cell infiltrate, foamy histiocytes, and, in three animals, giant cells were present; these changes increased with greater exposure to the oil and were most evident in the intratracheally injected animals receiving several doses.

Marked eosinophilia of pulmonary lavage cells was noted in the animals sprayed for six months vs. controls, with no observed eosinophils. The tissue response to Pam was not thought to represent a hypersensitivity phenomenon, but rather a foreign body reaction and gradual pulmonary clearance of a relatively non-irritating oil.

These preliminary findings indicate the need for more detailed studies to determine the importance of lipid aerosols in human respiratory disease.

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INTRODUCTION

It has become increasingly apparent in recent years that the use of aerosol sprays, ubiquitous in the modern American home, is not without hazards even when label instructions are followed correctly. Pulmonary side-effects differ for atopic individuals, smokers, and those with chronic lung disease, particularly the elderly. In the last few years the use of aerosolized vegetable oils, ("Pam," "Golden Touch," "Cooking Ease," and Mazola No Stick"; see Table I), in domestic cooking has become popular. The amount of oil used in pan-frying is less than with usual methods and results in fewer calories for foods ordinarily fried. Also, these componds do not contain cholesterol.

F.D.A. (Food and Drug Administration), does not require inhalation toxicity studies of aerosol foods or cosmetics by companies, nor does it at present envision studying this issue. Aside from fluorocarbon propellent inhalation studies, few papers have been published in this area. Two patients from Salt Lake City, Utah with presumed lipoid pneumonia associated with heavy Pam use stimulated interest in studying pulmonary complications secondary to aerosol lipid exposure. Guinea pigs were employed as an animal model in short and long term inhalation experiments.

I. Two cases of Possible Lipoid Pneumonia

Case I. An active 82 year old white male was seen by his private physician for complaints of shortness of breath, mild cough productive of yellow sputum, 15 pound weight loss, and a feeling of "hotness on eating,"

all of recent onset. His dysnea in fact was so marked that he was unable to cross a room without significant symptoms. He denied hemoptysis, chest pain, night sweats, pedal edema or other problems. Past medical history included spontaneous pneumothorax age 66, abdominal aortic aneurysm with successful graft replacement age 68, and probable pulmonary emphysema noted on radiologic examination five years previously. The patient had smoked one pack per day most of his life. Vital signs were within normal limits and physical examination was unremarkable except for amphoric breathing in the left axilla. Chest radiograph at this time showed new findings of a 10 cm diameter poorly defined consolidation in the central portion of the left lung consistent with pneumonia and accentuated markings in the central portion of the right lung, thought to represent an interstitial pneumonic process. CBC, urinalysis, SMA-12 except for a total bilirubin of 1.4, (normal to 1.0), were normal. Intermediate strength tuberculin test was nonreactive, two sputums for acid-fast bacilli were negative, and sputum cultures grew normal flora. Microscopic examination of the sputum revealed polymorphonuclear leukocytes and macrophages filled with oil.

Bronchoscopy and lung biopsy were suggested but the patient declined these procedures. He remained afebrile and was given a trial of Tetracycline, 250 mgm qid x 6 days. Although the patient reported no clinical change, the chest radiographic abnormalities progressed with increased diameter of the left mid-lung lesion and greater prominence of the markings in the right lung field.

Two weeks later a four month history of daily Pam aerosolized cooking oil use was obtained. With poor vision due to cataracts, he

usually placed his face very near the skillet when spraying. He denied nose drops or other oil use. The patient was advised to avoid all aerosols and he returned in two weeks feeling better. The patient stated that he was able to take much deeper breaths. Repeat chest radiographs showed considerable resolution in both lungs. He was begun on Prednisone, 40 mgm daily x two days, then 20 mgm daily thereafter.

He was seen two weeks later, feeling much improved. Chest radiograph showed some residual markings thought to be interstitial in nature and the Prednisone was reduced to 20 mgm every other day.

Later in the same year the patient experienced a transient ischemic episode with subsequent stroke and died a few months later from cardiorespiratory failure. There was no postmortem examination.

Case II. A 65 year old white male with COPD presented at the Veterans Administration Hospital, Salt Lake City, Utah with shortness of breath, dyspnea on walking short distances, and marked cough productive of scanty sputum, all progressive over the past few months. He denied other problems. Vital signs were within normal limits and physical exam was remarkable only for increased A-P diameter. Chest radiograph was read as consistent with pneumonia with a diffuse interstitial pattern. The patient's sputum was loaded with oil, free and within macrophages. No history of oil ingestion or nasal instillation was obtained; however, the patient used Pam "many" times a day, and sprayed into a pan already heating on the stove. The patient was advised to stop using the Pam spray and his symptoms disappeared along with gradual improvement of his chest radiograph.

These two cases do not prove pulmonary toxicity of Pam; they merely suggest that the potential of most oils to produce a pneumonic reaction when aspirated or administered in the form of nose drops may also be present when the oil is presented in aerosol form. In the first patient, viral pneumonia and bronchogenic carcinoma with overlying pneumonia are likely possibilities. The elderly age of the patient as well as the complaint of "hotness on eating" suggest consideration of gastro-esophageal sphincter incompetence and aspiration while supine. An aspiration pneumonia might be expected to produce a more fulminant reaction, however, and the resolution of symptoms and chest radiograph after cessation of the aerosolized oil is intriguing. Steroid therapy shortly thereafter also complicates interpretation. The oil in the patient's sputum is the most helpful finding; this is found in more than 95% of patients with lipoid pneumonia with rare false positives,[68,113] but aspiration cannot be completely excluded.

There is less information available on the second patient, but the story does suggest a lipoid pneumonia which may or may not have been due to the patient's frequent use of Pam. It should be noted that the patient followed labelled directions incorrectly by spraying into a heating, rather than cool pan. Both patients had chronic lung disease, mild, and were elderly. Both used thePam spray much more frequently than usually expected and the first patient held the can close to his face. These factors may be important in the pathogenesis of this hypothetical disease.

II. Aerosolized Cooking Oils

Pam, a product of Boyle-Midway Company, has been on the market for about 10 years. The company acquired the brand in 1971 from the original marketer at which time they greatly increasedadvertising and distribution. The category of this type of aerosol is in greater demand the past few years and, according to Boyle-Midway, Pam is easily the most popular aerosol in its class, with increasing popularity each year.[13]

Aerosol here refers to a product packaged under pressure and discharged from its container by this pressure. Aerosols contain three primary components: propellents, solvents, and active ingredients, as well as subsidiary substances such as perfumes, emolients, and flavors. Each component may have toxicity for humans. To discharge the contents the aerosol container has a hollow dipstick leading to a valve which is opened by depressing a nozzle. The propellents are gases at room temperature due to their low boiling points, have a high vapor pressure which provides propellent force, and are relatively inert, so that reaction with other ingredients in the container does not occur. The propellents as liquids exert pressure in the container which provides the force to drive the contents up the dipstick and out the open valve. Upon leaving the container the propellents abruptly becomed gaseous and thereby break-up the discharged ingredients and solvents into tiny droplets.[54]

The spray can of Pam when full contains approximately 3% ingredients and the rest fluorocarbon propellents (Freons* 11, 12, 114). As the can

*Tradename, E. I. Dupont de Nemours & Company

empties the percentage of ingredients increases; fluorocarbons are disproportionately lost in the process of discharging the contents. Fifty percent of the ingredients is commercial lecithin and fifty percent is soybean oil, a predominantly unsaturated vegetable oil. Commercial lecithin is composed of chemical lecithin, (phosphatidyl choline), phosphatidyl ethanolamine, and phosphatidyl inositol, (see Figure 1). The fatty acid composition of the commercial lecithin and soybean oil used in Pam is listed in Table II.

FDA classifies Pam as a food product; as commercial lecithin is on the GRAS list (Generally Regarded as Safe), no toxicological studies on the effects of ingestion of this product are necessary. FDA has not tested the possibility that this food may be inhaled. The company, Boyle-Midway, claims to have investigated this likelihood some time ago by spraying animals, but they state that the data on this is as yet "unavailable."[13] Other products of this type with additional ingredients such as artificial flavors and colors are still more suspect as regards pulmonary effects. If pulmonary disease is associated with use or abuse of these oil aerosols, it is not an obvious toxicity: no cases have been reported over the years. On the other hand, the sales pattern has changed enormously in the last few years and perhaps as more and more people are exposed to these aerosols those more susceptible to complications will appear with evidence of toxicity.

Other commercial aerosol products have pulmonary effects. Hair sprays, associated with a sarcoid-like pulmonary lesion in beauticians,[8,9,34] caused diminished maximal expiratory flows at low lung volumes in non-users upon acute exposure[125] and significantly abnormal

pulmonary function tests in beauticians exposed chronically.[82] Ward in 1971[114] proposed a relationship between an aerosol deodorant spray and a granulomatous lung disease resembling sarcoid in two young patients. Cough, dyspnea, chest pain, and abnormal pulmonary function tests have been observed acutely after exposure to an aerosolized starch product.[30] Deaths due to pesticide spray inhalation are recorded each year in this country.[93] Acute lipoid pneumonitis with abscess formation has been reported following aspiration of a drying oil used in commercial enamel paint.[5] The aerosol toxicity described here has been seen with correct product use, outlined in labelled directions. Lipoid pneumonia has also been described in a young man who observed cooking fat fires regularly as a restaurant safety inspector. The inhaled animal and vegetable fats were in a submicronic form in this case.[80]

III. Experimental Lipoid Pneumonia

The first reports of lipoid pneumonia, or oil aspiration pneumonia as it was then called stem from the 1920's. Even earlier, in 1915, a brief study involving insufflation of various substances into the bronchi of dogs showed that both lecithin and egg yolk, which contains 8 to 10% phosphatides, produced a lobar type of consolidation with fibrin deposition, indistinguishable from lesions due to "avirulent pneumoncocci."[63,120] Lung cultures of the lesions were sterile in four of the six dogs used in the lecithin experiment.

In 1920 Guieysse-Pellissier[52] injected olive oil intratracheally into rabbits and dogs and found the alveoli plugged with polymorphonuclear leukocytes, eosinophils, and macrophages rich in oil droplets. Corper and Freed[25] two years later recorded mild proliferative pneumonitis seen

in rabbits and dogs following intratracheal injections of small amounts of olive oil and liquid petrolatum, which remained in the lungs for months. Chalmoogra oil, highly irritating, produced bronchopneumonia, consolidation, and abscess formation, which they attributed to easy hydrolysis to fatty acids in the lung.

Laughlen in 1925[65] reported oil aspiration pneumonia in infants and children following nasopharyngeal application of menthol and argyrol in a mineral oil base, a common nose drops treatment then for upper respiratory infections in children. He produced pneumonic lesions filled with oil by injecting 0.5 cc of menthol in abolene, a mineral oil, by tracheostomy into rabbits and also by instillation of the same material into their nasopharynx. Furthermore, he dyed the mixture with Scharlach R, an oil stain, prior to nasal applicaiton and at sacrifice found the dye widely distributed in the lungs with large and small droplets within alveoli and macrophages. The coalescence of vacuoles within the macrophages sometimes produced large clear spaces which pushed the nucleus eccentrically and simulated a "signet ring" cell.

Papers in the next decade primarily concerned experimental work and cases in children. In 1927 Pinkerton[86] discussed six cases of lipoid pneumonia derived from 290 consecutive autopsies of children and adults and was able to obtain in all a history of oil use: liquid petrolatum nose drops, cod liver oil. egg yolk, milk fat, or mineral oil ingestion. He observed that certain factors predisposed to the disease: infancy, lavage feeding, cleft palate, convulsions, depressed mental status, and other neurological problems associated with aspiration. He described the pathological sequence as progression of the oil gravity-wise against

ineffective ciliary action and without inciting a cough reflex, phagocytosis of the smaller oil droplets, and macrophage encirclement of the larger oil masses with formation of giant cells and collagen deposition. Clearance of the oil-laden macrophages was assumed to be via the lymphatics: he found oil in lymph nodes and in one case, the spleen. Removal of the oil from the alveoli appeared to be a slow process and a nodular fibrotic reaction was considered the final result of continued exposure to oily agents.

A year later Pinkerton[87] conducted an investigation of the effects of several "sterile" oils injected into the tracheas of puppies and rabbits. He stated that animal, vegetable, and mineral oils were associated with different reactionsin the lung. Vegetable oils, with the exception of chalmoogra and peanut oils which caused acute necrosis, remained in the alveoli up to 90 days with very little tissue response. These mild vegetable oils were iodized poppy seed and sesame oil, (Lipiodol and Iodopin, respectively, used in bronchography), and olive oil. The animal oils caused marked consolidation in a few days but seemed to clear rapidly leaving scar tissue. The reaction to mineral oil was in between these, with gradual formation of giant cells and fibrosis around large masses of free oil. A nearly inert, foreign insoluble substance, it appeared to be poorly cleared by the lungs. Pinkerton suggested that reaction to the oils depended upon the amount of free fatty acids (FFA) originally present and the rapidity with which they were formed by hydrolysis in the lung. The vegetable oils had very little FFA initially and were apparently nothydrolysed before clearance.

Patterson in 1938[83] studied the effects of vegetable, animal, and

mineral oils, saturated and unsaturated fatty acids, cholesterol, and phosphatidyl choline when injected intratracheally into rats and rabbits. Fifty-nine days after a single 1 cc dose of a "watery" alcohol-solubilized emulsion of lecithin in rats, the lungs showed a marked peribronchial cellular response of polymorphonuclear leukocytes and oil-laden macrophages, and hyperplasia of peribronchial lymphoid tissue. The fatty material was finely emulsified and phagocytized by macrophages in groups around the bronchioles. Vegetable oils as a group produced less reaction than the others and seemed to be removed rapidly from the lung. Arachis and olive oils, however, caused a nodular reaction of oil-filled macrophages and the arachis oil was also associated with fibrinous alveolar exudate. Patterson essentially agreed with Pinkerton; he concluded that the pulmonary reaction to all oils was similar with slow phagocytosis and removal by macrophages. He proposed that the slower the clearance, the greater likelihood of a foreign body reaction with giant cells and fibrosis. The unsaturated fatty acids appeared to be both more finely emulsified and more easily phagocytized than saturated ones. Lecithin was considered by this author to be "especially potent" in producing experimental lipoid pneumonia.

Burrows and Johnston[19] in 1925 injected corn oil, ("mazola"), subcutaneously into several animals and observed that the vegetable oil was not absorbed. Binet[11] working at the same time found that various vegetable oils persisted in toto after subcutaneous injection, whereas animal oils completely disappeared. He concluded that metabolism of these substances in the tissues of laboratory animals was exceedingly slow and postulated on the basis of the histological picture that

mononuclear digestion of the oil formed the major clearance route.

Athough the pulmonary toxicity of free fatty acids, with adverse effects increasing with degree of unsaturation, is considered greater than that of neutral fat, this concept is borrowed form toxicity studies with other tissues and definitive work in the lungs is absent. Effects of homologous FFA and neutral fats on pulmonary vasculature have been examined in animal models of the fat embolism syndrome, consisting classically of the triad of neurologic dysfunction, respiratory insufficiency, and petechiae in a traumatized fracture patient. Peltier[85] in 1956 injected human bone marrow fats intravenously into rabbits and observed more acute and severe changes with FFA than with neutral fats as determined by LD_{50} and histologic findings. Following injection of the neutral fats, (mineral oil, triolein, or human fat), the animals died apparently from acute right heart failure secondary to obstruction of the arterioles of the pulmonary vascular bed. Following saponified human fat, oleic, linoleic, or linolenic acid injection, the rabbits died as a result of massive hemorrhage into the lung, which Peltier attributed to chemical action of FFA upon capillary endothelium, with subsequent exudation and hemorrhage. His results are analogous to those of other workers[62,91,109] and to Pinkerton's[87] findings with fatty acids in the lungs: tissue toxicity appeared to be a function of the degree of unsaturation, that is, their "chemical activity."[85]

Other investigators have postulated that neutral fat, arrested in the pulmonary micorcirculation, is acted upon by lung lipases to release high concentrations of FFA which then produce a chemical

pneumonitis.[53,62] FFA are toxic to various cells, including erythrocytes.[50,62] Pulmonary parenchymal tissue, too, is probably more susceptible to the increased "chemical activity" of unsaturated FFA.

III. Iodized Vegetable Oils in Bronchography

Other experimental studies of vegetable oilsin the lungs grew out of observations that the iodized vegetable oils used in bronchography could be retained in the lungs and could cause lipoid pneumonia as well as an acute reaction to the iodine.[32] First employed in 1922,[101] Lipiodol, a 40% iodized poppy seed oil, was initially considered nontoxic. Wright in 1935[123] reported a case where iodized rapeseed oil had been instilled a year prior to death; the oil was seen on chest radiograph just before demise and was histologically present in bronchiectatic cavities in the area of an obstructing carcinoma. A dense reticulated mass formed by many oil-filled phagocytic cells and a granulomatous region in the pleura were attributed to retention of the oil. Rabinovitch and Lederer[89] at the same time described the case of an elderly man with lipoid pneumonia at autopsy with recent iodized poppy seed oil bronchography. Information on mineral oil or other oil use was not mentioned. Granuloma formation following Lipiodol bronchography was reported following Lipiodol bronchography was reported by Brody[14] and Sheldon[99] in 1943.

Gowar and Gilmour[48] two years before had found many nodules of epithelial cells, some with foreign body giant cells, surrounding oil droplets in the lungs of rabbits which had received intrabronchial injections of iodized poppy seed oil. Chesterman[20] produced lipoid

pneumonia in cats injected with the same oil. In 1949 Storrs et al[108] described a large granulomatous lesion enveloping oil droplets in a surgical lung specimen removed three weeks after bronchography with Iodochloral (iodized and chlorinated peanut oil). Chemical analysis of tissue from the granuloma contained 300 times the iodine content of adjacent normal tissue.

With this evidence of granulomatous reaction to the iodized vegetable oils, Dunbar et al in 1959[32] investigated the fate of Lipiodol in 25 rats injected intrabronchially. They used a stain which reacted differentially with Lipiodol and found some oil retention, although most was cleared by four months, the endpoint of their study. Minimal necrosis at one month which resolved by four months and minimal fibrosis at four months were noted. They concluded that Lipiodol was not without pulmonary toxicity.

Felton in 1953 [39] examined randomly selected, surgically resected lungs and lymph nodes from 34 patients who had undergone bronchography 46 to 1,404 days prior to surgery. Twenty-three of the lungs showed residual microscopic oil after differential stain for iodized oil. Six of these, (16% of total specimens), had granulomas consisting of oil droplets surrounded by epitheloid cells and foreign body giant cells encapsulated within fibrous connective tissue. The lesions varied from microscopic nodules within fairly normal lung tissue to dense granulomas visible grossly as sub-pleural, well-circumscribed, firm, yellow-white lesions. No Lipiodol was found in lymphatics or lymph nodes and Felton concluded that lymphatic clearance was unlikely: dye not involved in the cellular reaction was probably phagocytized

and eliminated tracheobronchially. Perhaps bronchial obstruction was a factor in suboptimal clearance of the oil in the granulomatous lungs. It is not known whether the lesions described were of any clinical significance.

Reports of granuloma formation following bronchography with vegetable oils have continued to appear.[103] The pathogenesis of these lesions in humans is ill-defined: animal experiments with the same iodized oils have not revealed as extensive lesions. The effect of the iodine in addition to that of the oil has not been differentiated. The importance of poor drainage and coexisting respiratory disease is unknown. Nevertheless, inflammatory reaction to these compounds is present in certain individuals, especially when the dye is retained for long periods, i.e., months to years.

V. Clinical Lipoid Pneumonia

As the above studies reveal, the pathological definition of lipoid pneumonia is not a simple one. Type of oil, length and type of exposure, and previous or coexisting disease in the lungs affect the pathological findings as well as the clinical picture. Attempts over the years have been made to untangle the various clinical presentations and correlate them with some sort of pathogenesis, but data on the basic mechanisms is lacking. Nothing is known about the 'mediators' of inflammatory changes initiated by oils in the lung. The disease was first recognized in infants and children,[58,65,86] but after numerous autopsy series it became clear that adult cases were more common. The incidence of the disease varies, from 41 in

3,500 [43] (1%) to 14% of 389 [113] consecutive autopties.

Ikeda in 1937 [58] and Sodeman and Stuart in 1946 [104] developed criteria for the diagnosis of lipoid pneumonia. They differentiated two major types: "infantile," an acute process, from "adult," a chronic granulomatous infiltration. Both types could be seen at any age. The association of cod liver and other animal vitamin oils which produced fulminant disease, with children, and of mineral oil laxatives and nose drops which caused often asymptomatic, slowly progressive lesions, with adults, led to these misleading designations. The term "paraffinoma," employed by Ikeda,[58] described a localized oil granuloma nearly always due to mineral oil which was clinically, radiologically, and grossly very similar to bronchogenic carcinoma.

In agreement with animal experiments, animal oils in humans produce more acute reactions of pulmonary edema and necrosis and even extensive fibrosis or death.[104] Mineral oil lesions have been the most thoroughly studied. The course here may be subacute, progressive and protracted, or marked by recurrent superimposed bacterial bronchopneumonias often terminating fatally. Most often it is asymptomatic and indolent, or with nonspecific symptoms of dyspnea, cough, chest pain, yellow or purulent sputum, hemoptysis, and accompanying low-grade bacterial infection.[104] The patient with lipoid pneumonia is frequently discovered on routine radiologic examination or at autopsy. Signs if present are usually non-specific: râles, wheezing, rhonchi, and areas of bronchial breathing and dullness. The radiologic picture is also quite varied. Localized density or widespread diffuse interstitial appearance ("ground glass") can be seen.[72] As in other aspiration

moieties the bases are more often involved and the right greater than the left. The localized "paraffinoma" is difficult to distinguish from carcinoma of the lung, as mentioned.[17,61] Early diffuse lipoid pneumonia is believed to have characteristic linear interstitial changes and "acinar consolidations" due to aspirated oil filling the fine air spaces.[117] This acinar pattern can sometimes be seen in the periphery of consolidated lesions; it is also found in pulmonary hemosiderosis, pulmonary alveolar proteinosis, tuberculosis, pulmonary edema, and many other conditions. Very gradual change in the radiograph over months with radiologic findings out of proportion to the clinical presentation is perhaps the only "typical" finding for mineral oil pneumonitis.[72]

Pulmonary function tests in patients with extensive involvement demonstrate reduced vital capacity and decreased compliance. The restrictive ventilatory dysfunction is due to replacement of air spaces with fat-laden inflammatory tissue.[26,72] Bronchography is negative unless secondary infection is present. Sputum examination, unstained or stained with fat dyes, shows oil-laden macrophages or excessive free oil.[37] Sputum was positive in 56 out of 57 patients in one series[113] and is generally considered to yield false negatives extremely rarely. Several early morning collections before mouth cleansing or breakfast and following a few days of fat-free diet is recommended for highest yield.[37,68]

The clinical pictures of lipoid pneumonia can be summarized: an acute pneumonitis, often misdiagnosed as acute bronchopneumonia as well as exacerbated by superimposed bacterial infection; a chronic

granulomatous lesion with cough, chest pain, weight loss, and night sweats simulating tuberculosis or carcinoma of the lung; and a very slowly progressive, often asymptomatic diffuse interstitial process. Chest radiograph, sputum examination, and lung aspiration biopsy are the most reliable diagnostic criteria.

The histopathologic findings associated with these clinical descriptions are similar to those outlined above in animals. Grossly the lung is heavy with irregular, often confluent areas of induration which are yellow-white if the oil aspiration has been recent.[97] Both gross and microscopic appearance may resemble tuberculous caseous consolidation or sarcoid granulomas.

Theories as to why fats seem to produce different reactions in the lungs have already been alluded to; the actual pathogenesis of exogenous lipoid pneumonia is unknown. Pinkerton,[86,87] Sante,[98] and others believe that the intense acute reaction to animal oils is due to their higher free fatty acid content. In addition, their position in the alveoli favors oxidation to these, presumably more irritating, compounds. Others hypothesize that the more unsaturated, the easier lung lipases can hydrolyze an oil to FFA. Animal oils are apparently cleared more rapidly by macrophages than mineral oil, which is attributed to easy saponification of the animal oils. The problem with mineral oil is presumed to be this extremely slow clearance; not saponified, it is thought to be undigestable by the phagocytes. Sante[98] and Miller et al[72] attribute both the diffuse interstitial lesions and dense consolidations of mineral oil to its physical presence: small quantities administered over a long period of time

gradually accumulate, providing a nidus for foreign body reaction, and overwhelm the lung by a mass effect. The different distributions in the lung are related to the different ways mineral oil is administered and aspirated.

Volk et al[112,113] hypothesize that macrophages can disintegrate and set free the intracellular lipid droplets. The liberated material may then be re-aspirated from the upper bronchial tree, thus producing a vicious cycle. According to them, a recycling process could account for the chronicity of lipoid pneumonia, in which progression of pulmonary changes is sometimes seen after oral or nasal intake of the lipid has been discontinued.

As regards the vegetable oils, most workers argue that their relative lack of toxicity is due to absence of FFA; however, increased fatty acid content would be expected with deteriorated oil, as with the animal fats. Why certain vegetable oil--chalmoogra, peanut, arachis, and occasionally olive--should produce intense inflammatory reaction is perhaps a result of particular components, as yet undetermined, of these oils. The combination of fairly rapid clearance and slow breakdown may account for the usual mild response. In individuals with impaired clearance mechanisms, ciliary, cellular, or obstructive, greater toxicity might be expected and possibly this is the case in the rare patient who developes lipoid pneumonia secondary to iodized vegetable oils. In all the various oils the relative importance of lymphatic vs. tracheobronchial clearance mechanisms has not been determined.

Another question has been posed: can chronic pulmonary exposure

to mineral or other oils result in neoplasm?[36] Examples of lipoid pneumonia simultaneous or adjacent to carcinoma have rarely been reported,[17,49,61,98,122] so a direct etiologic relationship is unlikely. Evidence does suggest that certain lung carcinomas, especially adenocarcinoma, may arise in areas of epithelial proliferation accompanying pulmonary fibrosis.[124] Pulmonary fibrosis due to mineral oil could thus contribute secondarily to the genesis of malignancy; in one case this appeared to be so.[17] This concern is of particular importance to workers exposed occupationally to mineral oil mist generated by rapidly moving machinery lubricated with oil. More direct complications of lipoid pneumonia are fibrosis, contraction and narrowing of the bronchial tree, compensatory emphysema, and moderately severe restrictive and obstructive ventilatory dysfunction similar to that found in chronic bronchitis.[117]

Treatment of diagnosed lipoid pneumonia consists of discontinuing exposure, correction of underlying defects predisposing to or causing aspiration, and resection if disabling symptoms persist and disease is localized. Most researchers feel that discontinuing oil use leads to stabilization and slow resolution of lesions, but residual pulmonary fibrosis is not unusual.[72] Prednisone therapy is questionable. Cases of histologically demonstrated reversal of lesions following steroid therapy are reported as well as unresponsive. ones.[4]

VI. Surfactant and Endogenous Lipoid Pneumonia

In addition to soybean oil, Pam contains phospholipids. These

same compounds are important constituents of pulmonary surfactant, of which dipalmitoyl lecithin (DPL) is the major surface active component.[77] Phosphatidyl choline in human surfactant is completely saturated,[16] whereas commercial lecithin is 77.8% unsaturated.

Endogenous lipoid pneumonia is a rare chronic inflammatory process involving a segment or a lobe, secondary to obstruction of a bronchus. Alveolar macrophages in this disease, compared with those from control patients with non-obstructing endobronchial tumors, are larger, contain large, lipid-filled vacuoles with a "foamy" appearance, and upon culturing in vitro yield lipid material very high in DPL.[21] Similar material is not obtained from cultured control macrophages. Whether endogenous lipids are responsible for this disease or macrophages important in the normal catabolism of the surface-lining layer of the lung is unknown.

Gross et al[51] injected a small amount of homologous lipid, extracted from animal lungs of the same species, into the tracheas of rats and guinea pigs: all of the animals exhibited a chronic pneumonitis resembling kerosene inhalation, and several died. No assessment of the sterility of the injected material was attempted.
Early lesions showed proliferation of alveolar cells and reticulin with thickening of alveolar walls and obliteration of air spaces. These changes progressed at 7 days to diffuse alveolar fibrosis and desquamation of cells in the air spaces. By the end of the second week much of the pneumonitis had resolved, though irregular scar-like cellular foci remained. At four weeks the lesions appeared fairly

uniform from animal to amimal and showed widely separated scars. This histology is similar to reactions secondary to heterologous animal fats in the lungs, in humans and experimental animals. It is interesting to note that no cells with vacuolated cytoplasm, as are customarily found in endogenous lipoid pneumonia, were observed with any stain. Resolution with mild scarring after one dose might not be seen with repetitive doses.

Before the development of continuous positive airway pressure ventilatory devices, much research effort was directed towards developing aerosolized DPL. In 1964 DPL was administered in microaerosol form to infants with respiratory distress syndrome.[94] Synthetic DPL was dispersed into water and distributed in particles of mean diameter 0.25 μm. The small series yielded equivocal results and research in the area was abandoned, but one wonders whether this technique might have become an occupational hazard to health personnel in nurseries where lecithin micro-droplets were generated.

VII. Fluorocarbon Propellents

The fluorocarbon propellents (see Table III) which are responsible for aerosol generation in Pam are not without their own toxicity. These fluorochlorohydrocarbons are identified by a number following the name, e.g., Freon 114, which designates the number of halogen and carbon atoms.* Freon 12 was the first to be synthesized in 1930

*This is the Dupont numbering system. The first digit on the right indicates the number of fluorine atoms, the second digit from the right is one more than the number of hydrogen atoms, and the third digit from the right, omitted if zero, is one less than the number of carbon atoms.

by Migdley and Henne[71] and was introduced as a refridgerant gas less toxic via inhalation than sulfur dioxide or ammonia. Aerosol insecticides using Freon 12 were developed during World War II and sold on the civilian market by 1947.[35] Since then the uses of cosmetic, pharmaceutical, and household aerosols have grown exponentially; in 1972 the world production, half of it in th United States, of the two major gases, Freon 12 and Freon 11, was 0.5 and 0.3 megatons, respectively.[54] The non-fluorinated aerosol propellents such as propane and isobutane are quite flammable and this accounts for much of the popularity of the non-flammable Freons.

Originally the fluoroalkane gases were considered biologically inert. In the 1960's reports occurred of sudden deaths among teenagers who attempted to "turn on" by inhaling the propellent gases from aerosols.[126] The sudden nature of these "sniffing deaths" and the lack of autopsy findings implicated a cardiac mechanism, such as an arrhythmia, in the pathogenesis. Also during this time were numerous reports form the United Kingdom of an increase in sudden, unexpected deaths of asthmatics.[59,105,106] After epidemiological and clinical studies the British investigators decided that this excessive mortality was due to ventricular arrhythmias following over-use of pressurized aerosol bronchodilators.

Animal studies since then have researched the fluorocarbon blood livel at which toxicity, primarily cardiac, occurs. Taylor and Harris[110] found that mice pretreated with three breaths of a fluoroalkane gas, (Freons 12, 11, or 114), and then exposed to an otherwise well-tolerated

degree of hypoxia developed fatal AV block and profound sinus bradycardia. In addition, they observed that the hypoxic challenge could be given as late as 15 minutes after the brief exposure to Freon with the same cardiac depression, which implied that it was not secondary to displacement of pulmonary alveolar oxygen by Freon. In their original experiments a bronchodilator was used; since then they have obtained similar results in more than 200 mice using eight commonly used household and cosmetic aerosols, an aerosol toy, and commercially available pure Freons 12, 11, and 114. Similar experiments in dogs and monkeys revealed that this toxic sensitization by Freon on the heart disappeared more rapidly--in 1½ to 3 minutes--than in mice.

In awake or anesthetized monkeys, inhalation of 30% Freon 12, 9% Freon 114, and 61% oxygen caused ventricular premature beats, bigeminy, or tachycardia in all, without lowering arterial oxygen tension or pH or raising arterial CO_2 tension.[55] Kilen and Harris exposed rat left ventricular papillary muscle in a well-oxygenated muscle bath to Freon 12; they observed depression of myocardial contractility which was linearly dose-dependent. Freon 12 caused a similar decrease in myocardial contractility in four human papillary muscles.[56] In dogs and cats the arterial blood levels of Freon 12 rose linearly with its inspired concentration, while myocardial contractility and arterial blood pressure decreased in a dose-dependent fashion, and total peripheral resistance declined.[54] Harris and his coworkers conclude that Freon 12 is directly toxic to vascular and heart muscle. However, the doses

used were much greater than those expected to result from usual aerosol use.

Aside from cardiac toxicity, fluorocarbon damage to the lungs of experimental animals has been reported by only one group of investigators, Aviado et al.[2,3,15,28,42,115] They find that several propellents, including Freons 11, 114, and 12, can cause changes in pulmonary function in rats, dogs, and monkeys. Bronchoconstriction and dilation, decreased respiratory minute volume and tidal volume, and increased pulmonary resistance are variously observed. The concentrations of fluorocarbon used were high: 10% and 20% in air, whereas an asthmatic using a a bronchodilator aerosol, for example, would in a single breath inhale a concentration of about 2%. An individual spraying Pam for several seconds even in a poorly ventilated kitchen would probably not experience this high a concentration. In addition, many of the numbers generated in Aviado's pulmonary toxicity studies, although suggestive of a trend, are not significant. There have been a few reports of decreased airway conductance in healthy and asthmatic subjects after inhalation of fluorocarbons, but these changes have not produced symptoms[18,107] or have produced only slight cough and wheezing.[44]

Experiments with humans, both normal volunteers and asthmatics with histories of heavy aerosol use, to determine the peak blood levels of fluorocarbons after single breaths, several repeated breaths, and single breaths over a period of hours of commercial bronchodilators have demonstrated a wide range of levels and sparked a lively controversy over the safety of aerosol bronchodilators.[29,31,76,84,102,119]

Difficulty in interpreting much of the work stems from extrapolation of levels which cause definite toxicity in experimental animals to humans. Also, variation among human subjects in absorption and elimination of the fluorocarbons--as much as a 120-fold difference between two subjects in one study[84]--advises caution in evaluating the data.

Most investigators have found fluorocarbon blood levels in humans well below those required for cardiac toxicity in epinephrine-sensitized dogs, (e.g., 20-25 ug/ml, Freon 11) after single or a few breaths of a bronchodilator. Draffan et al[31] found levels appraching that which, in the dog, would be toxic, if the subject inhaled Freon 11 on every breath up to two minutes; these 'dog' toxicity levels were not reached nor were any sign of cardiac or respiratory toxicity seen.

A study from Riker Research Laboratories,[102] which has developed and marketed several inhalation drugs containing fluorinated hydrocarbon propellents, found no toxicity in rats, mice, and dogs breathing large doses of fluorocarbons except occasional sedation, ataxia, or depression, which they attributed to the mild anesthetic properties of the propellents in such high doses. The studies varied from two weeks to 23 months of daily inhalation doses and hematology, blood chemistries, urine analysis, and EKG's (dogs only) were monitored. This opposes other animal studies which found significant acute cardiac toxicity at equal exposures.

German workers after thorough studies have classified all the commercially used propellent gases according to doses toxic for experimental animals.[64] Inhalation exposures of mice, rats, and guinea pigs

to concentrations of 0.5, 1.5, amd 5.0% by volume of mixtures of propellents 11 and 12, two hours a day for 100 days, showed no changes in weekly growth, lung histology, or blood parameters vs. control animals. Concentrations of Freons 12 or 114 required to produce anesthesia in rats and guinea pigs were extremely high--60% for 60 minutes--and no concentration resulted in death. Propellent 11 caused deep anesthesia at 10% concentration for 90 minutes and death at 20% for 5 minutes. These concentrations are not achieved when pressurized packs are sprayed; it is estimated that discharge of seven and one-half 18 oz. cans in a very small non-ventilated room, (176.5 cubic feet), could produce a Freon 11 concentration of 11%.

The health branch of Health and Welfare Canada designed a project in which 20 white female housewives used a number of household and personal aerosols over a four week period and were examined for changes in cardiac, respiratory, hematologic, and blood chemistry parameters.[70] No morbidity was found and no fluorocarbons discerned in the blood sampled at intervals during the study. Two major shortcomings of this modest study are fairly obvious; the study covered only four weeks of exposure as contrasted with potential years of use, and the women were carefully selected for excellent health: none had cardiac or respiratory abnormalities by history or function tests and only three smoked. Also, no description of space and ventilation characteristics of the home environments was given.

Although studies extending over years do not exist, on the basis of available information it is generally accepted that "normal" exposure to fluorocarbon propellents is not injurious to health.[10] Studies on

effects of these gases, adsorbed onto food and ingested, are underway at Dupont. On the other hand, the recent reports of the National Academy of Science[22] and the Interagency Task Force on Inadvertent Modification of the Stratosphere[60] have thrust the issue of direct fluorocarbon toxicity aside.

In 1974 Molina and Rowland[75] proposed that fluorocarbons, although chemically inert and stable at the lower atmospheric layers of Earth, are dissociated by ultraviolet light (190-220 nm wavelength) in the stratosphere and produce chlorine atoms which can catalyze the breakdown of ozone to oxygen. The ozone concentration at 20 to 25 kilometers up is maximal and acts as a shield against ultraviolet radiation. Excess uv radiation causes skin cancer, retards growth in crops, is possibly important in climate changes, and injures many forms of life, especially micro-organisms. According to Molina and Rowland, most fluorocarbons exist in the atmosphere for 40 to 150 years before reaching the stratosphere and therefore the quantity of these compounds already released into the atmosphere as of 1972 could deplete the ozone layer 7 to 18%, eventually.[96] With the increasing production of aerosols, this ozone depletion would become increasingly greater. The National Academy of Science estimated that a 5% decrease in ozone could produce 30,000 new cases of skin cancer per year in the United States, which represents and 10% increase.

The federal government commissioned NAS to study this urgent question; time is crucial when dealing with pollution measured in megatons per year. The report, issued September 1976,[22] agreed with the findings of the Interagency Task Force last June[60] in confirmation of

Molina and Rowland's thesis and calls for rule-making to restrict dissipative uses of fluorocarbons. The same month Congress passed the Toxic Substances Control Act, expanding regulatory authority of the Environmental Protection Agency to cover all fluorocarbon use, and a bill (H.R. 15759) was proposed in the House to amend theFederal Hazardous Substances Act to include aerosol containers which contain fluorocarbons.[23] This bill is before the Committee on Interstate and Foreign Commerce. Oregon has already banned the sale of fluorocarbon containing products; passed early last year, the law becomes effective June 1977.

Boyle-Midway may be packaging Pam with nitrous oxide, soon.

MATERIALS AND METHODS

I. Preliminary

Female Hartley short hair, smooth-coated, guinea pigs raised behind a "sterile barrier," (i. e., autoclaved materials and food, 10 ppm chlorinated water, strict personal hygiene, 15-20 room air changes per hour) were used in allexperiments except in II, where guinea pigs raised non-sterilely were used. The animals in both short term and long term experiments were housed in an all guinea pig room, segregated by experimental group, in stainless steel cages with removeable mesh bottoms. They were fed Purina guinea pig food and 10 ppm chlorinated water ad libitum. Temperature was maintained at 70-72^{o}F with 50% humidity, 12 hour day/12 hour night photoperiod, and 10-12 room air changes per hour. The animal papers beneath the cages were changed daily and the cages washed every two weeks.

Animals were selected for each group and for sacrifice at random. Sacrifice was performed with an intraperitoneal injection of 50 mg/kg body weight sodium nembutal. The trachea was immediately located and ligated to prevent atelectasis[48] and the lungs were gently freed and fixed for a minimum of 48 hours in 10% formalin solution. A section from each of four lobes was obtained for oil red O and hematoxylin/eosin staining. The oil red O reaction with Pam was checked by spraying Pam onto alcohol-cleaned slides; it stained orange-red with the dye.

Pam packaged in aerosol spray cans for consumers was used throughout.

The lack of previous inhalation studies with a commercial oil aerosol and also of well-described small animal intubation techniques

necessitated development of these in the first few months. Animals were sprayed briefly (15 seconds) with Pam, sacrificed immediately, and found to have oil-stained droplets in their lungs (see Figure 2). Using 400 gram female guinea pigs, it was discovered that (1) tracheostomy and spraying the aerosol into the tracheostomy needle, (2) intratracheal injection of fresh Pam with a high percentage of fluorocarbon propellent, and (3) intratracheal injection of the extremely viscous propellent-free Pam in any but minute amounts, all produced immediate death. In the first two methods, immediate absorption via the lung of a large amount of fluorocarbon coupled with anoxia most likely caused cardiac arrhythmia and arrest; in the third method, the viscous Pam was observed to remain in the trachea in sufficient amounts to cause nearly complete obstruction.

The length of spraying and exposure times in the Pam experiments was necessarily somewhat arbitrarily determined, but an attempt to simulate human experience was in mind. Pulmonary function measurements reveal that the tidal volume in guinea pigs weighing from 130 to 940 grams averages 1.7 mls with range 0.88 to 3.9 mls, the respirations per minute 80 (45 - 116), and the minute volume 145 (75 - 362) mls.[1] Thus in a 15 minute period an animal might inhale from 1135 to 5430 mls; the volume of the experimental box was greater by 10- to 50-fold. With a 3500 to 9000 mls minute respiratory volume, an adult human even in a small kitchen of 10 to 12 m^3 would inhale a smaller percentage of total room air; however, localized effects by the immediately surrounding air may be important here. In any case, most of the aerosolized Pam in terms of mass probably settles out quite rapidly; it is the submicronic,

respirable particles whose concentration decay with time would depend most upon room ventilation characteristics in a 15 minute exposure period.[40]

Amount of non-gaseous Pam delivered in a 15 second spray averaged 0.5324 mgm, in fifty 15 second sprays, indicating an average of 0.03% (by volume) concentration of fluorocarbons in the plasticene box per 15 second spray.

In light of these preliminary findings, and after numerous practice intubations in both deceased and alive animals, the following experiments were devised.

II. Pulmonary Function Tests

Four 600 gram female guinea pigs were given 15 and 30 second normal saline aerosol challenges following a 15 minute period of conditioning to surroundings in a body plethysmograph designed for guinea pigs. The animals then received two 15 second challenges of Pam aerosol spray. Flow rates and tidal volumes were recorded by the method of Amdur and Mead[1] and Dennis et al.[27]

III. Short Term Exposure Studies

Eighteen 250-350 gram female guinea pigs were used. Six were anesthetized with intraperitoneal sodium nembutal, 20 mg/kg body weight, and intubated every other day with a sterile #19 gauge, blunt-ended, curved steel needle with removable wire core through which 0.05 cc of viscous Pam (after propellents had been allowed to evaporate) was injected into the trachea. Six guinea pigs underwent intratracheal injections every other day with 0.05 cc sterile normal saline.

Six guinea pigs were sprayed simultaneously for 15 seconds with Pam four times a day, seven days a week, in a 35.5 x 35.5 x 44.4 cm (55,955 cc) plasticene box and allowed to breathe the resulting atmosphere for 15 minutes. Unrestrained, the animals' nostrils were not necessarily directed into the aerosol stream. The spray periods were divided over six hours. The plasticene box was cleaned weekly and its absorbent animal paper lining changed daily.

The guinea pigs were housed in the all guinea pig room three to a steel cage, transported to the laboratory in plastic cages with sawdust bedding, and handled by two people. One guinea pig from each group of six was killed 1, 24, and 72 hours and 7, 14, and 21 days after start of the experiment.

IV. Long Term Exposure Studies

A. Twenty-four 290-320 gram female guinea pigs were used after a two week quarantine period. They were housed by groups, originally four to a cage, and transported as above. Handling was by four people: two in the first half of the experiment and two in the latter half. Food, water, and weekly weights were monitored.

Two groups of four (A,B) were subjected twice a day to a 15 second spray in the plasticene box followed by a 15 minute inhalation of the resulting atmosphere in the box. The spray periods were three to four hours apart, five days a week.

One group of four (D) was anesthetized and injected intratracheally with 0.05 cc sterile normal saline, one dose, and another group (C) was anesthetized and injected intratracheally with 0.05 cc Pam after fluorocarbon evaporation, one dose. These two groups were not transported to

the lab after the initial intubation.

Four animals served as a control group (E) and travelled to and from the lab and were handled the same as groups A and B.

One guinea pig selected randomly from each group was killed at 30, 60, 130, and 180 days after starting. Blood was obtained for white blood cell count (Coulter 'S' Counter) and differential and the lungs were weighed. Alveolar macrophages were obtained by the method of Gee et al [45] using normal saline lavage fluid and then stained with Wrights and oil red O for the guinea pigs sacrificed at 180 days only.

B. Six guinea pigs were used. Two 570 gram animals were anesthetized and injected intratracheally with 0.1 cc normal saline daily for four days (H).

Two 570 gram animals were intubated and injected daily for three days with 0.1 cc of an ether-solubilized, water-dispersed solution of lecithin (J). This commercial lecithin coarsely grained powder was obtained from Central Soya Company, Chicago, which supplies Boyle-Midway with the ingredients for Pam, and is (soybean) oil-free, but otherwise equivalent to the phospholipids in Pam. It was soluble in few non-toxic liquids; after solubilization in ether and dispersion in water, the ether was evaporated leaving a watery emulsion of the lecithin, which was used for injection. Approximately 6 µg of commercial lecithin was injected per dose.

One 700 gram animal was injected intratracheally with 0.1 cc of the propellent-free Pam for four days (F).

These animals were sacrificed after 61 days and the lungs prepared

for histologic examination.

One 200 gram guinea pig was injected intratracheally with 0.05 cc of the propellent-free Pam every other day for a total of 10 doses (G); she was sacrificed 193 days after start of the experiment.

All guinea pigs appeared healthy at time of sacrifice.

For outline of guinea pig experiments see Table IV.

Blood agar and Sabaroud's with gentamycin plates, and Brain and Dubos broths were innoculated with small amounts of Pam sprayed freshly from the can. They showed no growth except for the blood agar plate which grew a few colonies of Bacillus species, a non-pathogen (for humans) and common contaminant.

RESULTS

I. Pulmonary Function Tests

There was no change in flow, (volume differentiated with respect to time), respiratory rate, or tidal volume after the normal saline aerosol challenge or the first Pam challenge in any of the guinea pigs. Following the second 15 second Pam challenge decreases in tidal volume and flow were observed in three of the four animals, but as there was no change in respiratory rate, no bronchoconstriction can be inferred. Bronchoconstriction in guinea pigs causes decreased tidal volume with increased rate and, if severe, decreased flow. The decreased tidal volume and flow seen is most likely due to differences in aerosol delivery rate.

II. Short Term Exposure Studies

Grossly the lungs of all the animals appeared normal. Histopathology of the lungs is summarized in Table V.

Oil droplets lining bronchioles and air spaces were seen in the sprayed guinea pigs sacrificed at 1 and 24 hours and 15 and 21 days. A few droplets were found at 3 and 7 days, but they were not greater in number than that seen in saline injected controls. Fat in the positive lungs was present in large particles measuring up to several microns and small, submicronic particles and was finely emulsified within cells, which were predominantly monocytes, in the 15 and 21 day animals. The heaviest concentrations of impacted particles were in

and around terminal bronchioles and alveolar ducts; many areas were devoid of droplets, while adjacent tissue showed significant concentrations. Although usually only one or two lobes per guinea pig contained stained material, upper and lower, left and right lobes were equally involved overall. The amount of oil in these sprayed animals was less than that seen in animals which had received Pam via intratracheal injection, but more than the artifactual material observed in saline injected individuals or controls. Artifactual fat droplets were large, very round, and above the tissue plane. In all groups fat droplets were observed within bronchial cartilage as has been described in guinea pigs by other workers,[6,83] and this oil was therefore ignored.

Pam injected animals had oil particles in several or all lobes, with distribution similar to that in the sprayed animals. The concentration of oil particles in these injected guinea pigs was the heaviest noted in any experimental group.

The lungs of the saline injected guinea pigs appeared normal upon hematoxylin/eosin staining, as compared with controls from the long term exposure study. In the animals sacrificed at 15 and 21 days after 10 intratracheal injections, mononuclear cells (MNC) were possibly increased on three and two sections, respectively, but this was not considered significant as it was non-focal and very minimal.

Animals which received Pam injections, however, demonstrated progressive pulmonary changes of focal MNC infiltrates, some near terminal bronchioles, atelectasis and extension of focal increased cellularity up to 0.5 mm in diameter, and appearance of foamy

histiocytic cells in these areas. Eosinophils were found in alveolar walls, but were also noted in the saline treated individuals. Many giant cells were seen in the guinea pig killed after three Pam injections (7 days); refractile material was observed in several of these upon polarized light examination (see Figure 3). A few foamy giant cells were observed in the animal receiving 10 Pam injections (21 days) in regions of atelectasis, foamy histiocytes, and possibly increased collagen (see Figure 4). Marked hyperplasia of peribronchial lymphoid tissue was also seen in this animal.

The lungs of animals sprayed with the aerosol after 7 days began to develop focal pneumonia with infiltration of macrophages and lymphocytes into the walls of alveoli and small bronchioles, and atelectatic areas (see Figure 5). Foamy histiocytic cells were seen after 21 days.

Signs of acute infection, including gross lymphadenopathy or polymorphonuclear leukocytes in the lungs, were not found in any of the animals.

III. Long Term Exposure Studies

A. Food and Weights

The monthly food and water intake per guinea pig and weekly weights are given in Tables VI and VII, and Figures 10-12. The weights of the sprayed animals were much lower than those of the controls or intubated animals; the numbers of animals were too small for statistical analysis. Food and water consumption increased overall as the animals grew, but the differences among groups A, B, and E (sprays and controls) and C and D (Pam and saline injected) were not significant, (t test for

comparison of means of independent groups). Control animals grew according to breeders' expectations.[81]

B. Peripheral Blood and Alveolar Macrophage Examinations

Peripheral white blood cell count and differential at time of sacrifice are listed in Table VIII. Wide variation among controls as well as experimental animals was observed and mild peripheral eosinophilia was noted in both sprayed and intubated guinea pigs, as compared with normal values for germ-free animals.[12]

Pulmonary lavage cells, (see Table IX), yielded equivocal fat staining results, with some cells in all groups positive for small amounts of fat. Eosinophils were impressive: 59% and 51% in the sprayed animals vs. 11% in saline and 14% in Pam injected animals. None were noted among the control animal's cells. Macrophage morphology was unremarkable.

C. Lungs, Gross

Grossly the lungs of groups C and D (Pam and normal saline single intratracheal injections) and E (controls) appeared normal. The lungs of the sprayed groups, A and B, appeared normal also except for mottling and scattered petechiae in the (B) 180 day animal, possibly related to trauma at time of sacrifice. Lung weights expressed as per cent of body weight, (see Table X), were comparable in all the animals and also consistent with data elsewhere.[116]

Of the animals intubated several times, group H (saline) and G (Pam) lungs were normal in appearance while the left upper lobe of F (Pam) exhibited several pale, raised 1-2 mm diameter areas. The lecithin injected guinea pigs' lungs (J) appeared dark and considerably

contracted.

D. Lungs, Microscopic

Histologically the lungs from the four control animals (E) stained only bronchiolar cartilage fat and rare artifact with the oil red 0 dye and were normal on hematoxylin/eosin stain except in the 180 day animal: here numerous scattered small foci of organized lymphoid tissue surrounded by otherwise normal lung were evident in all four sections. A few similar foci were noted in the left lung of the 130 day control. The saline injected lungs (D) contained no oil droplets in excess of control amounts; lung tissue was normal except for occasional very small areas of atelectasis in the 130 day specimen.

The lungs of the single dose Pam injected animals (C) showed bronchiolar and alveolar oil droplets at 30, 60, and 130 days, but not at 180 days,(see Figure 6). The amount of stainable oil decreased with time and the distribution appeared random with respect to lobes involved. The variety of size and location of droplets was similar to that seen in the short term studies: within terminal bronchi and alveoli and finely dispersed within large monocytic cells. The animals which had received several Pam injections (F,G) had more oil, in large and small particles, remaining after 61 and 193 days than the 130 day single dose animal (C), (see Figure 7). Oil was again observed within monocytic cells. On hematoxylin/eosin staining the lungs showed borderline to absent changes of focal atelectasis and MNC infiltrate in the single injected group (C) vs. noticeable changes of this type in the multiple dose animals (F,G). Foamy histiocytic cells and occasional small giant cells were found in the lungs of guinea pig F, (see Figure 8), and

specimens from both F and G contained occasional lipid droplets within eosinophic material, filling terminal bronchioles and alveolar ducts. Lungs from the 60 day single Pam injected guinea pig (C) contained hyperplastic peribronchial lymph nodes.

Lungs from the sprayed animals were normal or demonstrated these same changes to a milder degree. They contained very little fat staining material, although amounts greater than controls were seen at all sacrifice points. The fat was primarily located within small bronchioles and occasionally alveoli:fat lined these structures forming rims of red material. The lungs were essentially normal after 30 and 60 days with hyperplastic peribronchial lymph nodes in the 30 day (A) animal,(see Figure 9). At 130 days both guinea pigs demonstrated in all lobes minimal focal MNC bronchopneumonia with atelectasis. In addition to these changes, a few foamy histiocytic cells were also present in one of the animals at 180 days. The other animal sacrificed at this time had very minimal focal MNC collections, probably within normal limits.

Lungs from the two lecithin injected animals (J) showed minimal focal atelectasis and acute changes of pulmonary edema and hemorrhage in the lower lobes, but findings to account for the marked lung contraction observed grossly were not apparent. Fat stains were omitted in this group.

DISCUSSION

These experiments were designed to test for the presence of and tissue response to Pam aerosolized cooking oil in guinea pig lungs after moderate "normal" use of the aerosol as well as following excessive amounts of the oil introduced into the lungs artificially in an attempt to visualize the extreme tissue reaction.

I. Intubated Animals

Significant amounts of oil were deposited in these animals, where histologic findings of atelectasis and focal MNC infiltrations, and foamy histiocytic cells in the animals with greatest exposure to the oil were consistently observed as compared with saline injected controls. The oil persisted in small but significant amounts up to 57, 130, and 172 days (guinea pigs F, C and G), after intratracheal instillation of 4, 1, or 10 doses, (see Figures 6 and 7). These histological findings and persistence of the fat are in close agreement with the results of Gowar and Gilmour[48] using iodized poppy seed oil in rabbits and Fried and Whitaker[41] using the same oil in cats. Gowar and Gilmour did not note cellular oil or foamy macrophages, as the latter authors did and as was seen here.

These results are in agreement with the general observation that vegetable oils injected into the lungs of experimental animals produce a subacute or chronic response, rather than an acute or fulminant reaction as with animal oils.[58,65,83,104] The marked peribronchial cellular response of polymorphonuclear leukocytes described by

Patterson[83] after chemical lecithin injection into rat lungs was not seen in Pam or commercial lecithin injected groups, although some peribronchial lymphoid hyperplasia, (short term 21 days, long term (C) 60 days), as well as numerous oil-laden macrophages were noted, in accordance with his findings.

It is difficult to correlate precisely the degree of MNC infiltrate or to determine the time course of the changes, as both variables were present simultaneously in the experiments. Also, the methods used do not lend themselves to easy quantification: it is impossible to say at this stage how much Pam reached the alveoli or bronchi in injected or sprayed animals. The amount of Pam eventually reaching the parenchyma in the intratracheally injected animals probably varied considerably from guinea pig to guinea pig. The greatest changes, in terms of extent of infiltrate, were seen in the animals injected every other day over a period of 21 days; Pam injected animals (C) in the long term study received only one dose of Pam but were all sacrificed later than 21 days. Was the oil effectively cleared by 30 days in these single dose individuals, or was a single very minute dose of viscous Pam insufficient to produce the MNC infiltrate? The latter may very well be true, as the guinea pigs injected several times with Pam and sacrificed at 61 and 193 days had noticeable histological changes, unlike the single injected guinea pigs after 60 days.

In the short term injected animals changes increased markedly with time, but so did the total quantity of Pam introduced into the lungs. The theory that vegetable oils maintain their relatively non-toxic status by efficient pulmonary clearance before problems of

obstruction, foreign body reaction, or degradation to more harmful components, i.e., unsaturated FFA, can occur suggests that changes observed in the short term animals reflect their attempts to handle repetitive small doses delivered over an exposure period too brief for complete clearance. In patients with chronic bronchitis, COPD, heavy smoking history, or other conditions associated with altered mucociliary clearance, frequent exposure to an amount of oil handled adequately in a single dose could swing the delicate balance in these individuals from chronic to acute disease.

Even in these guinea pigs, exposed artificially to a relatively large quantity of Pam -- although most of the oil did not descend past the trachea -- no fibrosis or granuloma formation was seen. However, these were healthy animals, apparently able to mobilize the foreign oil: the amount of oil in the lungs of single dose guinea pigs decreased from 3^+ (30 and 60 days) to 1^+ and absent (130 and 180 days).

II. Sprayed Animals

Changes found in the animals sprayed four times a day, (short term), and two times a day, (long term), for 15 seconds were similar and milder in degree than the changes described above in intratracheally injected animals. The sprayed guinea pigs' lungs demonstrated focal MNC infiltrates and atelectasis, (3 short term, 5 long term animals), and vacuolated histiocytic cells, (1 short term, 1 long term animal). Increased changes followed increased daily exposure: the findings were greater in the animals sprayed four times a day in the short term study than in the long term study with two daily sprays.

Possibly the animals chronically exposed to the oil for months adapted with improved clearance mechanisms, or perhaps two sprays daily of Pam could be handled adequately whereas twice this exposure could not.

Shoshkes et al,[100] studying oil deposition in mice after short and long term inhalation of aerosolized mineral, corn, peanut, and motor oils with mean particle size 2.5 μm, found considerable alveolar and terminal bronchiolar oil after 48 to 108 hours of exposure over 14 to 30 days. The spray periods were much longer -- hours of exposure at a time -- than the 15 second sprays used here, so that the animals' usual pulmonary clearance mechanisms were probably overwhelmed. They observed the corn oil series of lungs to retain the least amount of oil; except for a slight increase diffusely in the number of macrophages, no inflammatory changes were attributed to the oil. They believe that this lack of pulmonary reaction is due to rapid clearance; in the Pam experiments, short exposure periods of 15 seconds probably allowed the oil to be effectively cleared by time of sacrifice.

Pam was observed within small bronchioles immediately after exposure; the oil was found within alveoli and cells on later examinations. The particle size distribution of aerosolized Pam is not known, according to the company,[13] nor would it be easy to determine. Measurement techniques are complex[38,95] and as the can empties not only does the percentage of oil in fluorocarbon increase, but oil coating the discharge apparatus changes the physical characteristics of the aerosol generator. Also Pam, being hygroscopic, absorbs water in the upper respiratory tract and therefore its site of deposition will depend on its growth in size.

"Aerodynamic filtration"[14] is an important defense mechanism of the lung whereby particles larger than 10 μm and smaller than 0.5 μm are effectively removed. Most large particles, greater than 10 μm in diameter, deposit in the nasal cavity or impact at the carina or within the first two bronchial divisions. Particles in the range of 0.2 to 0.5 μm sediment in later bronchial divisions and the alveoli and are capable of producing a tissue reaction.[93] Still smaller particles settle by diffusion in the alveoli and are cleared systemically. However, even large particles of oil, depositing in the mucous-covered ciliated epithelium of the nose, pharynx, and bronchioles, may be important.[121] Experiments with rabbit and human ciliated sinus epithelium have shown that oil mixtures, (mineral oil), interfere with ciliary streaming and are propelled very little, if at all.[88] Other agents, such as carbon dioxide, cigarette smoke, alcohol, and possibly sulfur dioxide, are ciliotoxic[79] and may further inhibit transport of a substance out of the lung. Oily nose drops probably reach the lung via retrograde pooling due to ineffective ciliary function, as may oils that have been introduced into the mouth.

The tiny cellular oil droplets seen in the Pam experiments are consistent with macrophage ingestion and clearance from the alveoli and terminal bronchioles. This is the accepted theory for the fate of all fat not expectorated and is in agreement with Patterson's observations of "finely," as opposed to coarsely emulsified oil in cells following phosphatidyl choline and vegetable oil injections into rabbit lungs, which he attributed to easier emulsification of the unsaturated oils.[83]

III. Poor Growth of Sprayed Guinea Pigs

In absence of apparent illness, growth of the long term sprayed guinea pigs vs. controls and breeders' normal values was remarkably poor despite nonsignificantly different food and water ingestion and identical handling. Growth in guinea pigs is affected by many nutritional factors and depressed in acute or chronic infection[12] and cigarette smoke inhalation exposure.[57] Evidence for acute infection was absent as judged by WBC count and differential, absent lymphadenopathy, and lung histology. Guinea pigs grow well on diets with a wide range of fat intake and in fact require small amounts of unsaturated fat, (e.g., 7.3% corn oil), for best growth.[12] Therefore ingestion of Pam licked from the fur would not be expected to affect weight gain adversely.

Chronic exposures to considerably higher concentrations of fluorocarbons has not produced poor growth in a number of laboratory animals, including guinea pigs.[64,102] It is possible that the daily psychological trauma of being "sprayed" may have interfered with the animals' digestion although signs of malabsorption in feces were not noted and the loud noise of the aerosol discharging was heard essentially equally by the parallel control animals.

Weights of the guinea pigs injected with normal saline (D) were somewhat greater than those of Pam injected animals (C), but the difference was not consistent and may not have been related to pulmonary presence or absence of Pam in the lungs, particularly as exposure to the oil was a single dose at the start of the experiment and histological

changes were minimal.

IV. Possible Pathogeneses

The described results may reflect guinea pig response to aspirated phospholipid/soybean oil or a coincidental reaction to undetermined stimuli. Fluorocarbons are very unlikely to be important here in light of extensive chronic inhalation literature documenting their lack of pulmonary toxicity in many laboratory animals. Acute or chronic "spontaneous pneumonitis," not directly associated with an etiologic agent, usually localized to one or rarely two lobes and containing predominantly intralveolar polymorphonuclear leukocytes or closely packed monocytic cells, is a problem frequently encountered in colonies of guinea pigs.[92] According to Richardson,[92] this mandates consistent, widespread abnormalities compared with simultaneous controls if reliable conclusions are to be drawn. The controls here did not exhibit spontaneous pneumonitis; the individual at 180 days demonstrated a phenomenon unique to guinea pigs termed pulmonary perivascular lymphoid nodules, reported in 14 to 85% of animals including one "germ-free" guinea pig, analogous to the animals used here.[12] Whether this finding is normal or a response to chronic low-grade infection is not known. White blood cell counts and differentials in the long term study were within normal limits for germ-free animals, who tend to have lower counts and more polymorphonuclear leukocytes than conventional animals.[12] Streptococcus infection in guinea pigs is the only common infection in which the animals continue to appear healthy; however, this infection usually produces prominent cervical lymphadenopathy,[12] not noted here.

It is difficult to determine with the small numbers of animals used whether the mononuclear bronchopneumonia and focal atelectasis, which were the most common findings in Pam sprayed and intubated groups, are examples of this frequently encountered pneumonitis or a reaction to inspired Pam. The observed changes were not confined to one or two lobes and the animals referred to by Richardson were not raised "sterilely" as they were here,which may be significant. In addition, the changes appeared to be progressive, which lends greater weight to their presence. Therefore, despite Richardson's caution, it would seem that the histological differences between control and experimented groups are real.

The changes most likely represent pulmonary response to a relatively non-irritating oil: emulsification, engulfment by macrophages, saponification and digestion within cells, and lymphatic or tracheobronchial clearance with ingestion or expectoration. Greater quantities of fat in the parenchyma seemed to produce more intense MNC inflammatory response. The giant cells noted in three intratracheally injected guinea pigs raises the question whether granuloma formation and fibrosis might have resulted with use of larger doses or less healthy animals. Giant cells in one of these animals contained refractile material, possibly a result of contamination during intubation; neutral fat was observed to contain anisotropic crystals, (refractile with polarized light), in one study of pulmonary response to oils.[48] MNC inflammatory process in the guinea pigs did not progress after exposure to the oil ceased, in agreement with other vegetable oil studies.

The relationship of chronic lung disease to the effects of normally easily handled foreign substances in the lung is important. If giant cells, granulomas, or fibrosis are seen only when vegetable oils

remain in the lungs for a long time, as experimental workers using iodized vegetable oil have suggested,[39,103] then conditions that predispose to poor clearance, such as chronic bronchitis or cigarette smoking, may be necessary for these more impressive histological findings to appear. This hypothesis cannot be tested in young, healthy, germ-free animals.

The eosinophilia discovered upon pulmonary lavage of the 180 day animals suggests a hypersensitivity reaction. The percentage of eosinophils observed in the sprayed guinea pigs was four times that of the Pam and saline injected individuals, possessing normal lung histology; no eosinophils at all were noted among the control animal's cells. Hypersensitivity reactions to injected and inhaled antigens are well-studied in the guinea pig.[33,46,72,73,92,118] Eosinophilia is a prominent response to antigenic stimulation via injection into the foot pads or peritoneal cavity of these animals.[47,66,67] Eosinophilic response in nearby lymph nodes is specific for injection of antigen into footpads.[67] However, injection of normal saline into the peritoneal cavity yields fluids rich in eosinophils, although the total numbers are less than when antigen is injected.[47] Increased numbers of eosinophils are found in guinea pig lungs following parenteral sensitization and subsequent aerosol challenge with a variety of antigens. Thus it is not absolutely clear in guinea pigs whether the appearance of eosinophils signifies an immunologic process or non-specific irritation.

The majority of lipids known by serologic tests to be antigenic are incomplete antigens, or haptens; they induce antibody production only if injected in combination with a heterologous carrier protein.[90]

Cardiolipin, the reagin responsible for the Wasserman serological test for syphilis, and Forssman haptens are examples of lipid antigens. Only phosphatides and glycosphingolipids are known to function as haptens. After repeated intravenous injection of phosphatidyl inositol and carrier protein methylated bovine serum albumen (BSA), rabbits produce antibodies. This hapten is immunogenic only if the injected lipid suspension contains lecithin or lecithin and cholesterol in addition to the carrier protein; resulting antibody is directed against phosphatidyl inositol alone. This requirement for "auxiliary lipid" is seen with many other lipid antigen systems.[90] An antibody response can be elicited in rabbits by phosphatidyl inositol oligomannosides even without carrier protein if administered with incomplete adjuvant.[78]

As regards cellular, or delayed type hypersensitivity (DTH), Coon and Hunter[24] discovered in guinea pigs that BSA conjugated with dodecanoic acid, a saturated straight chain fatty acid present normally in mammalian tissues, without adjuvant stimulated sustained DTH but no detectable antibody production, whereas large amounts of antibody, but no detectable DTH, resulted from injection of BSA alone without adjuvant. Dodecanoic acid alone is not antigenic, nor does it serve as a hapten when conjugated with other materials, or as an adjuvant when mixed with protein antigens.

One could speculate that oils would be excellent media for the transport of dust particles, food, micro-organisms, toxic gases, and radioactive substances into the respiratory tract. The commercial lecithin in Pam contains phosphatidyl inositol and ethanolamine as well as lecithin; if heterologous protein were carried with these

phospholipids into guinea pig lungs, a hypersensitivity reaction could result. Pam contains minute impurities, including 26.2 ppm aluminum, 67 ppm iron, 10.0 ppm boron, 0.5 ppm copper, 12.2 ppm zinc, and 1.0 ppm manganese, due to processing and is packed in an aluminum can.[111] Soybean oil when containing phospholipids is known to absorb significant amounts of metal cations, (e.g., Zn^{+2}, Fe^{+3}), from ordinary water.[69] Although both zinc and aluminum are associated with granulomatous lesions in the lungs,[114] it is doubtful that these elements are present in sufficient quantity in Pam to produce hypersensitivity.

DTH in guinea pigs in response to aerosolized PPD,[74,92] asobenzene-arsonate-N-acetyltyrosine,[92] Micropolyspora faeni,[118] potassium dichromate, and Candida albicans[73] has been studied. Richardson has carefully examined Types I, III, and IV immunologic reactions in guinea pigs following inhalation of aerosolized antigen with previous parenteral sensitization.[92] He found acute asthma and eosinophilia of bronchioles in Type I (anaphylactic) reactions, hemorrhagic pneumonia and vasculitis in Type III (immune complex) reactions, and mononuclear infiltration, primarily by macrophages and lymphocytes, of alveolar septi and air spaces in Type IV (DTH) reactions. In addition to these MNC changes, parenchymal eosinophilia and neutrophilia characterize DTH following inhalation of Micropolyspora antigens.[118]

Skin test results with Pam as well as serologic evidence of antigenicity would be helpful in establishing whether the phospholipid/soybean oil stimulated a hypersensitivity response. The relative lack of findings in the two groups with the greatest exposure frequency, A and B, with 255, 15 second Pam sprays and fewer pulmonary changes than

in the animals sprayed twice as frequently for only three weeks, is against this theory unless a mechanism for tolerance is postulated also. Despite these concerns of an immunologic reaction to foreign proteins, simple chemicals, metal ions, and lipids, it seems more likely that the histological findings, if not the remarkably poor weight gain, of the Pam exposed guinea pigs are attributable to foreign body reaction and gradual mobilization of the oil from the lungs.

V. Problems with the Animal Model; Future Studies

Guinea pigs were chosen for this study because of their low cost and easy care and the availability of germ-free strains, a device to accurately calculate pulmonary function measurements, and literature on previous inhalation studies. Nevertheless, certain problems arise when using these animals. The dimensions of alveoli and terminal bronchioles in guinea pigs and man are comparable upon light microscopic examination, but whether cellular responses to various stimuli are also alike is not known. Guinea pigs as with all rodents are obligate nose breathers; in addition, their airway dimensions are naturally much smaller than those in man, which may alter the favored deposition sites for particles. In mice, fates for each particle size resemble that in humans.[100] As mentioned before, young, healthy guinea pigs are not analogous to humans compromised by chronic lung disease or other problems. Although an attempt to simulate human exposure was made, particularly in the long term sprayed groups, analogy with human "normal" use remains qualitative rather than quantitative.

Interpretation of the results is primarily limited by the small

numbers of experimental animals, with only one or two to a group. Within these limits, chronic exposure of up to 6 months to brief sprays of the aerosolized lipid Pam did not cause overt disease in guinea pigs, but was associated with poor weight gain and histologic changes in the lungs. Fat droplets penetrated in small amounts to alveoli following inhalation of the aerosol and in somewhat larger quantities after injection of oil into the trachea. Both methods produced focal MNC infiltrates, atelectasis, and foamy histiocytic cells, with the greatest changes associated with repetitive exposure over a two to three week period.

These results indicate the need for further examination of the fate of this aerosol product. Inhalation experiments, both chronic and acute, with dogs or primates, possessing a tracheobronchial tree more like that of humans, perhaps employing a radiopaque iodized form or radioactively-labelled form of the oil for more precise localization, could better approximate the problem of whether aerosolized oil reaches the alveoli in humans and if so, where it goes. Long term inhalation studies with larger numbers of laboratory animals and careful monitoring of food, weight, and blood parameters is needed to disprove or confirm and explain the disparity in growth between exposed and control animals seen here. In addition, skin tests for DTH to the sprayed oil, repeat pulmonary function measurements after chronic exposures to the aerosol, and pulmonary lavage for cells at numerous points during exposure studies should be performed.

It is important to pursue the possibilities of pulmonary toxicity

with Pam as well as with other cooking oil sprays and household aerosols of this too rapidly proliferating market. Aside from fluorocarbon propellents, manufacturing companies and FDA have ignored the potential inhalation toxicity of aerosol ingredients and have instead concentrated on cutaneous, ocular, and gastrointestinal reactions. This could be a serious oversight: the ubiquitous aerosol spray can must be considered a device for spraying potentially dangerous substances into our air and into our lungs.

SUMMARY

Two cases of possible lipoid pneumonia associated with use of Pam cooking oil aerosol are presented and the product's components, soybean oil, phospholipids, and fluorocarbon propellents, discussed.

Literature is reviewed concerning the effects of various oils on the lungs, in animal experiments and human cases; vegetable oils are observed to produce a milder reaction than animal or mineral oils. Vegetable oils appear to be important clinically in rare granulomatous reactions following bronchography with iodized oil. Exogenous and endogenous lipoid pneumonia, surfactant phospholipids, and commercial aerosol known pulmonary complications are also discussed.

Guinea pigs were sprayed (15 seconds) and injected intratracheally (0.05 or 0.1 cc) with commercial Pam in short and long term exposures up to six months. An attempt to simulate chronic human "normal" exposure was made in the long term sprayed animals. Growth of these animals was much slower than that of controls, with nonsignificantly different food and water ingestion. Lung weights per body weight were comparable.

Fat droplets were demonstrated in the lungs in widely varying amounts and sizes, from several microns to submicronic in diameter. Focal MNC infiltrate, foamy histiocytic cells, and occasional giant cells were noted; these changes correlated with increased exposure in sprayed and injected animals. Signs of acute infection were absent. The chronology of the lesions after exposure to the oil was not determined, but it appeared to resemble the chronic processes reported by other

researchers working with vegetable oils: several days were required before significant cellular infiltrate was evident.

Marked eosinophilia of pulmonary lavage cells was noted in animals sprayed for six months vs. controls. The potential for lipid aerosol induced pulmonary hypersentivity reaction is discussed. The histological findings are attributed, rather, to foreign body reaction and pulmonary clearance of the oil.

Future studies to confirm or disprove these findings, limited by the small numbers of animals and choice of guinea pig for model, and to determine their significance for humans are suggested.

Table I. Aerosolized Cooking Oil Products

brand	company	ingredients
Pam	Boyle-Midway	soybean oil, lecithin, fluorocarbon propellents
Golden Touch	Boyle-Midway	corn oil (6%), lecithin, artificial flavor, isopropyl citrate, methyl silicone, artificial color, nitrous oxide propellent
Cooking Ease	Chlorox	vegetable oils (30%), lecithin,butter flavor, -carotene, propellent
Mazola No Stick	Best Foods	mazola corn oil, lecithin, artificial flavor, propellent

Table II. Pam Fatty Acid Composition[111]

	soybean oil	commercial lecithin
$C_{16:0}$	10.3%	16.4%
$C_{18:0}$	4.4	5.9
Total saturates	14.7	22.3
$C_{18:1}$	24.5	16.9
$C_{18:2}$	53.8	54.1
$C_{18:3}$	7.0	6.8
Total unsaturates	85.3	77.8
Unsaturated:saturated	5.8:1	3.5:1

pH 6.7 - 7.1 (1% in distilled water)

Table III. Fluorocarbons in Pam[7]

			boiling pt.
Low pressure propellents			
Trichlorofluoromethane	Freon 11	$C Cl_3F$	23.8°C
Dichlorotetrafluoroethane	Freon 114	$C Cl F_2-C Cl F_2$	3.8
High pressure propellents			
Dichlorocifluoromethane	Freon 12	$C Cl_2F_2$	-29.8

Table IV. Outline of Guinea Pig Experiments

Short Term Exposure Studies

Sprayed: four 15 second sprays Pam daily (6)

Pam: 0.05 cc propellent-free Pam injected intratracheally every other day (6)

Saline: 0.05 cc normal saline injected intratracheally every other day (6)

Animals sacrificed after 1, 24, 72 hours; 7, 15, 21 days

Long Term Exposure Studies

Group E: controls (4)

Group A: sprayed with Pam 2 times daily, 5 times per week, for 15 seconds (4)

Group B: animals treated as group A (4)

Group C: intratracheally injected 1 time with 0.05 cc propellent-free Pam (4)

Group D: intratracheally injected 1 time with 0.05 cc normal saline (4)

Animals sacrificed after 30, 60, 130, 180 days

Group H: intratracheally injected daily with 0.1 cc normal saline for 4 days (2)

Group J: intratracheally injected daily with 0.1 cc "watery" solution of chemical lecithin for 3 days (2)

Guinea Pig F: intratracheally injected daily with 0.1 cc propellent-free Pam for 4 days

Animals sacrificed after 61 days

Guinea Pig G: intratracheally injected every other day with 0.05 cc propellent-free Pam to total 10 doses

Animal sacrificed after 193 days

Table V. Histopathology

SHORT TERM #Doses/Sacrificed		Oil Droplets (+ to ++++)	Hematoxylin/Eosin
Saline Injected			
1	1 hour	-	WNL
1	24	-	WNL
2	72	-	WNL
3	7 days	-	borderline increase MNC, diffuse, probably WNL
7	14	+/-	WNL
10	21	-	borderline increase MNC, diffuse; probably WNL
Pam Injected			
1	1 hour	+++	slightly increased MNC,diffuse; probably WNL
1	24	+++	few areas focal MNC infiltrate, lower lobes; lipid vacuoles in bronchioles
2	72	++	few areas focal MNC infiltrate, all lobes
3	7 days	++	more areas MNC infiltrate, large FB type giant cells, all lobes
7	14	+++	focal MNC infiltrate, vacuolated histiocytic cells, all lobes
10	21	++++	exacerbation of 14 d. with a few small foamy giant cells; hyperplasia of peribronchial lymphoid tissue
Sprays			
1	1 hour	+++	WNL
4	24	+	WNL
12	72	+	WNL
28	7 days	+	focal MNC infiltrate, atelectasis, vacuolated histiocytic cells, all lobes
56	14	++	few areas focal MNC infiltrate
76	21	+++	focal MNC infiltrate, greater than 7 d.; some atelectasis
LONG TERM			
Controls E			
	30 days	-	WNL
	60	-	WNL
	130	+/-	occasional perivascular lymphoid nodules, left lung
	180	-	numerous perivascular lymphoid nodules, showing organization, all lobes
Sprays A			
43	30 days	+	few areas small focal MNC infiltrate; hyperplastic peribronchial lymph nodes
86	60	-	WNL
195	130	+/-	focal MNC infiltrate and atelectasis, all lobes
255	180	+	focal MNC infiltrate, atelectasis, vacuolated histiocytic cells, all lobes

Table V. Histopathology, continued

Sprays B			
43	30 days	+/-	WNL
86	60	+/-	few areas atelectasis LUL, RLL; probably WNL
195	130	+	few areas focal MNC infiltrate, atelectasis, all lobes
255	180	++	occasional focal MNC infiltrate
Pam Injected C			
1	30 days	+++	diffuse increase MNC; probably WNL
1	60	+++	hyperplasia peribronchial lymph nodes
1	130	+	few areas atelectasis right lung; WNL
1	180	-	few areas focal MNC infiltrate, atelectasis LUL; probably WNL
Saline Injected D			
1	30 days	-	WNL
1	60	-	WNL
1	130	-	few areas atelectasis lower lobes; probably WNL
1	180	-	WNL
Saline Injected H			
4	61 days	-	areas of atelectasis
4	61	-	WNL
Pam Injected			
4	(F) 61 days	++	focal MNC infiltrate left lung, few small foamy giant cells; lipid vacuoles within alveoli
10	(G) 193	++	few areas focal MNC infiltrate, atelectasis left lung
Lecithin J			
3	61 days	(not done)	pulmonary edema and hemorrhage, loss of alveoli; lungs congested
3	61	"	congestion, rare areas of pulmonary edema; most of lung WNL

Table VI. Long Term Exposure: Food and Water Intake

FOOD (mls/month/guinea pig)

MONTHS		1	2	3	4	5	6
Controls	E	1380	1258	1425	1625	2800	3100
Sprays	A	1144	1241	1460	1538	2583	2300
"	B	1325	1271	1513	1363	2000	2500
Pam Injected	C	912	1050	1520	1355	1567	2400
Saline Injected	D	745	966	1570	1795	1783	2800

WATER (mls/month/guinea pig)

Controls	E	4605	4850	4900	3825	9643	10250
Sprays	A	4375	5167	5450	4363	9950	9650
"	B	4808	5134	5000	4350	7515	6150
Pam Injected	C	3516	3467	4775	4270	5040	4100
Saline Injected	D	3444	3226	4540	4363	4342	4500

Table VII. Long Term Exposure: Weekly Guinea Pig Weights

Wk.	Controls E				Mean	Sprays A				Mean	Sprays B				Mean
1	305	312	302	291	303	324	334	290	274	314	338	320	327	308	314
2	377	381	388	374	380	329	361	303	299	342	408	359	338	341	342
3	395	393	404	372	391	348	382	311	317	356	418	373	349	348	356
4	472	260	485	437	464	397	420	351	352	397	472	385	392	403	397
5	504	507	531		514	424	452	372		434	502		415	437	434
6	548	526	563		546	456	465	408		463	529		449	469	463
7	569	549	587		568	488	509	419		497	577		483	508	497
8	576	580	609		588	512	534	442		526	608		525	534	526
9															
10		612	662		637	488		523		521	588			484	521
11		635	692		664	534		565		580	639			583	580
12		654	732		693	496		538		570	649			598	570
13		698	758		728	556		553		597	665			612	597
14		710	675		693	548		538		599	687			623	599
15		650	708		679	567		562		586	648			566	586
16		673	728		701	557		560		605	727			575	605
17															
18		688	816		752	603		565		624	675			654	624
19		740	832		786	630		652		672	730			676	672
20			865		865	684				693				701	693
21			854		854	670				688				705	688
22			895		895	670				682				691	682
23			887		887	675				694				712	694
24			875		875	706				699				691	699
25			899		899	700				709				717	709
26			910		910	707				703				698	703

Table VII. Weekly Guinea Pig Weights, continued

Wk.	Saline Injected D				Mean	Pam Injected C				Mean
1	302	343	299	305	312	323	341	304	335	326
2										
3	390	419	396	388	398	419	461	419	454	438
4										
5	466	460	460	430	454	474	428	489	504	474
6	467	489	477	426	465	472	441	494	575	496
7	503	523	513	457	499	525	436	545	557	516
8	542	561	554		552	563		588	599	583
9										
10	485	429	448		487	572		605	615	597
11	533	550	585		556	596		601	613	603
12	610		583		597	583			610	597
13	703		648		676	581			526	554
14	693		666		680	593			543	568
15	686		673		680	590			540	565
16	701		643		672	621			586	604
17										
18	704		783		744	661			579	620
19	819		830		825	710			651	681
20	814		850		832	722			680	701
21	819		790		805	743			717	730
22			791			761				
23			766			760				
24			793			759				
25			812			780				
26			846			785				

Table VIII. Long Term Exposure: WBC and Differential

		WBC	segs	bands	lymphs	monos	eos	basos	metas
30 Days									
Control	E	3,700	37	3	55	3	2		
Sprays	A	2,600	32	2	53	4	9		
"	B	5,900	46	3	49	0	4		
Pam Injected	C	2,100	32	1	60	2	4		1
Saline Injected	D	5,000	43	2	51	2	2		
130 Days									
Control	E	2,200	39	2	53	4	2		
Sprays	A	1,400	64	1	31	1	3		
"	B	2,300	43	2	50	5	0		
Pam Injected	C	5,800	34	4	57	3	1		2
Saline Injected	D	3,400	30	2	53	5	10		
180 Days									
Control	E	2,600	38	3	56	2	1		
Sprays	A	2,200	25	3	65	1	4		2
"	B	3,700	43	0	48	1	3	2	
Pam Injected	C	4,000	28	1	62	4	4		1
Saline Injected	D	3,100	63	1	30	3	0		3

Table IX. Long Term Exposure: Pulmonary Lavage for Cells in 180 Days Guinea Pigs

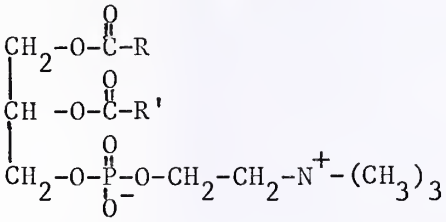
Wright's Stain		monos	eos	basos	lymphs	segs
Control	E	99			1	
Sprays	A	35	59	1	2	3
"	B	38	51	2	9	
Pam Injected	C	78	14		8	
Saline Injected	D	83	11		5	1

Oil Red O Stain		Fat Droplets (+ to ++++)
Control	E	+
Sprays	A	+
"	B	++
Pam Injected	C	+
Saline Injected	D	+

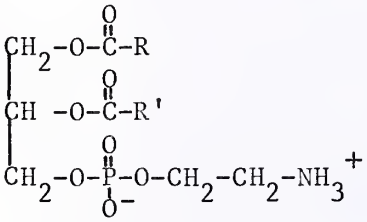
Table X. Long Term Exposure: Lung Weights

	body weight (grams)	lung weight	% body weight
30 Days			
Control E	470	3.3370	0.71
Sprays A	373	2.4991	0.67
" B	363	2.3595	0.65
Pam Injected C	436	2.7468	0.63
Saline Injected D	457	3.1076	0.68
60 Days			
Control E	627	4.5144	0.72
Sprays A	453	2.8086	0.62
" B	538	3.6584	0.68
Pam Injected C	601	4.8080	0.80
Saline Injected D	550	3.1900	0.58
130 Days			
Control E	740	5.1800	0.70
Sprays A	652	5.2812	0.81
" B	730	5.6940	0.78
Pam Injected C	717	4.9473	0.69
Saline Injected D	819	5.7330	0.70
180 Days			
Control E	910	6.0060	0.66
Sprays A	707	5.5853	0.79
" B	698	4.9558	0.71
Pam Injected C	785	5.7305	0.73
Saline Injected D	846	5.8374	0.69

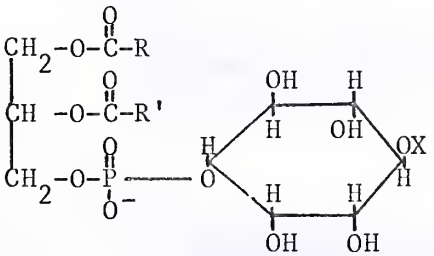
Figure 1. Composition of Commercial Lecithin[111]



Phosphatidyl choline (chemical lecithin)



Phosphatidyl ethanolamine (cephalin)



X = one or more phosphatide groups linked to one or more sugar molecules

Phosphatidyl inositol

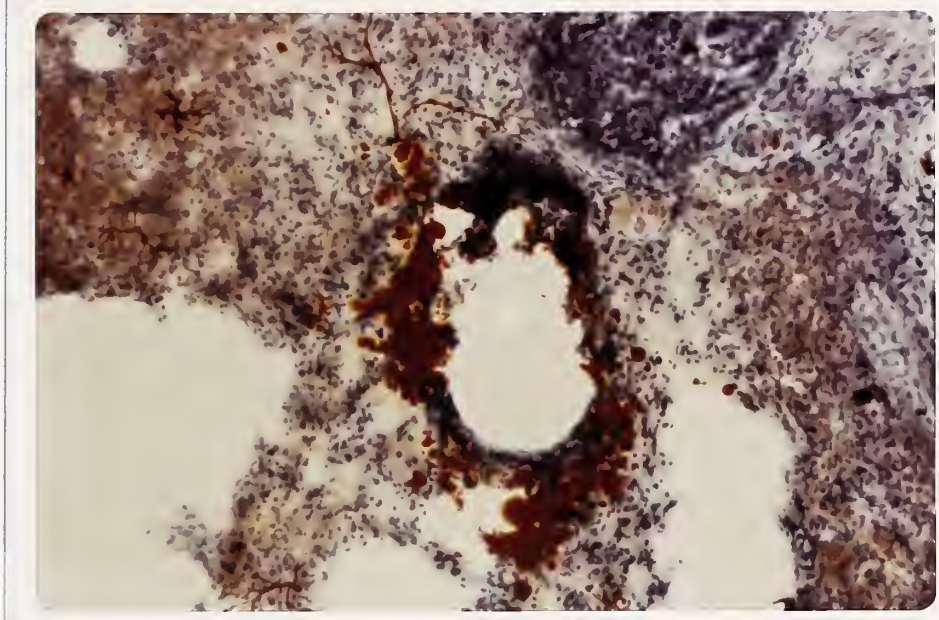


Figure 2. Oil within bronchiole. This preliminary guinea pig was sacrificed immediately following an intratracheal injection of 0.05 cc Pam. (Oil red O, x 100)

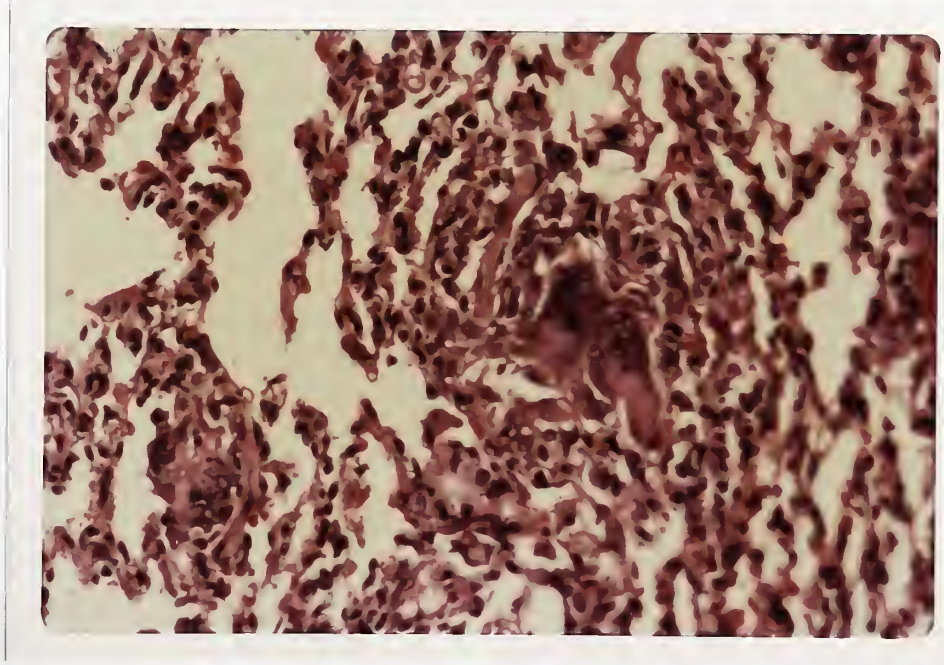


Figure 3. Giant cells containing refractile material, Pam injected 7 day animal. (Hematoxylin/eosin, x 250)

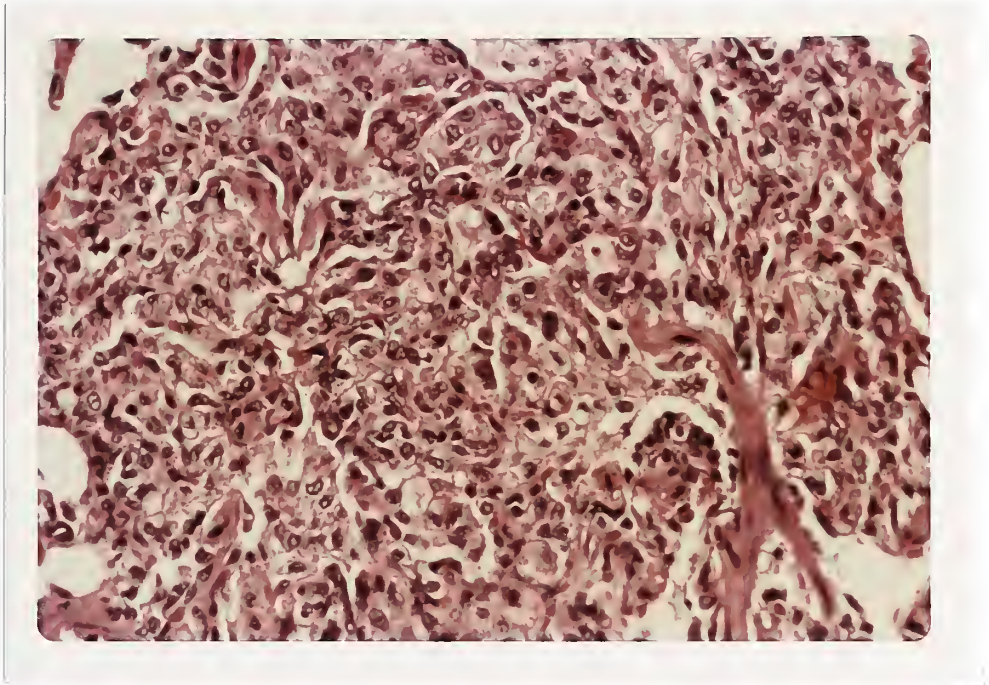


Figure 4. Foamy histiocytes within a large area of focal MNC infiltrate, Pam injected 21 day animal. (Hematoxylin/eosin, x 250)

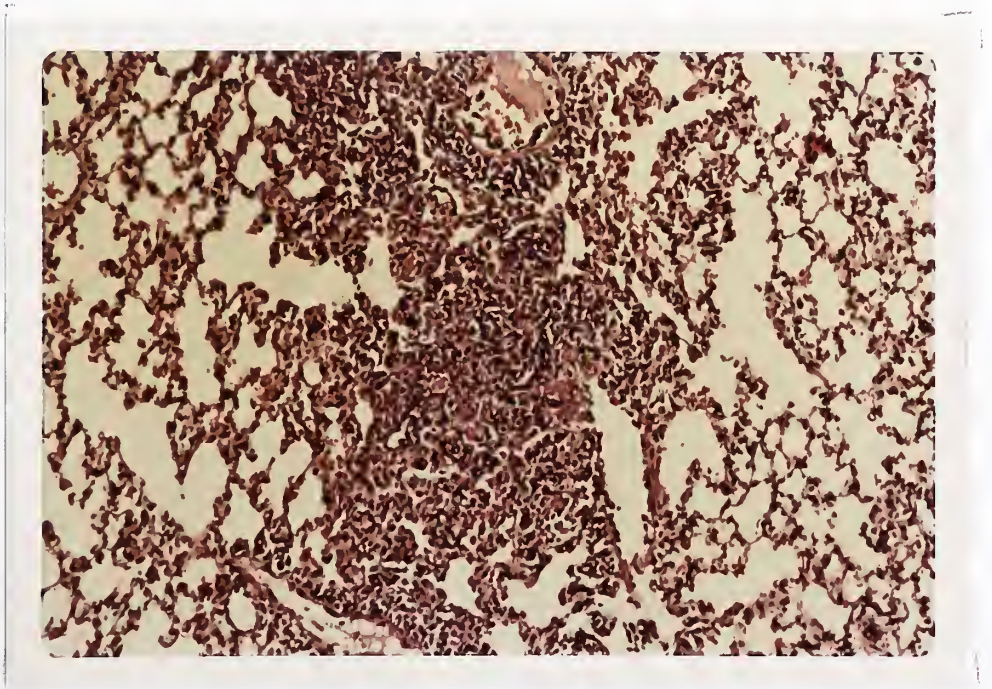


Figure 5. Focal MNC infiltrate, sprays 21 day animal. This demonstrates the most common histological finding in Pam exposed animals. (Hematoxylin/eosin, x 100)

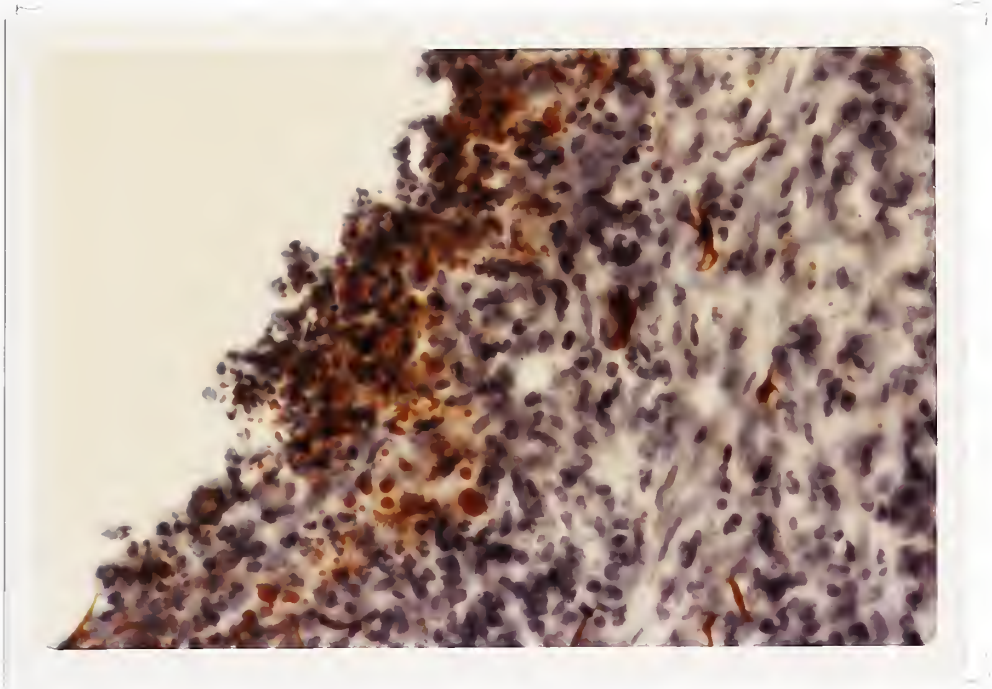


Figure 6. Oil lining a large bronchiole, within the surround-cellular infiltrate, and within monocytic cells 60 days after intratracheal injection of 0.05 cc Pam, (group C). Only a small portion of the bronchiole is shown; the remaining portion was similar. (Oil red O, x 250)

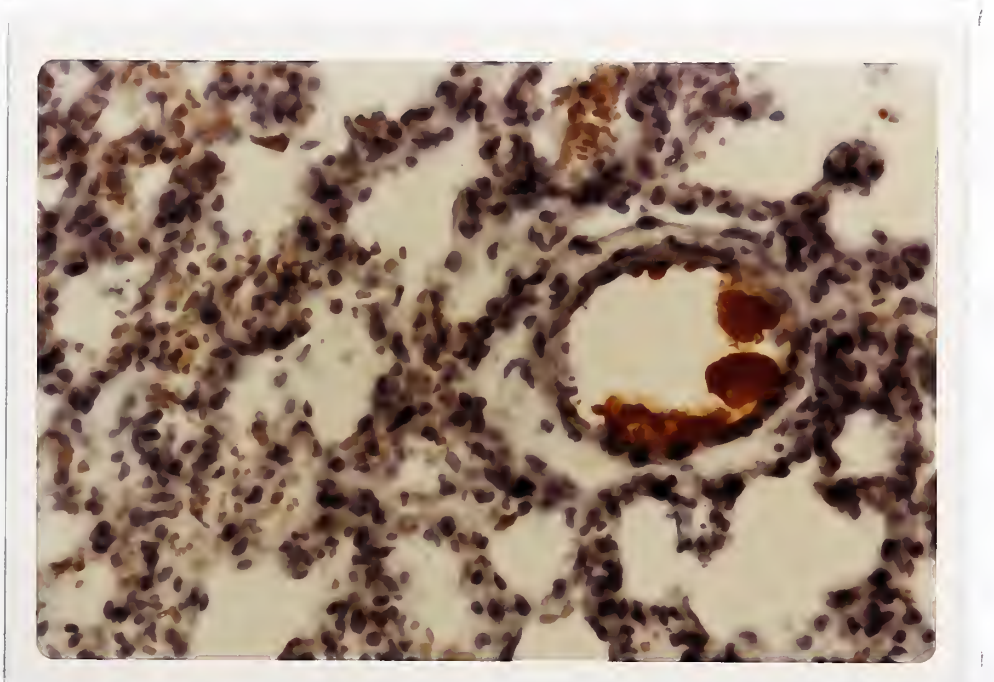


Figure 7. Oil within a bronchiole 172 days after 10 intratra-tracheal injections of 0.05 cc Pam, guinea pig G. Elsewhere tiny oil droplets were noted withir monocytic cells. (Oil red O, x 250)

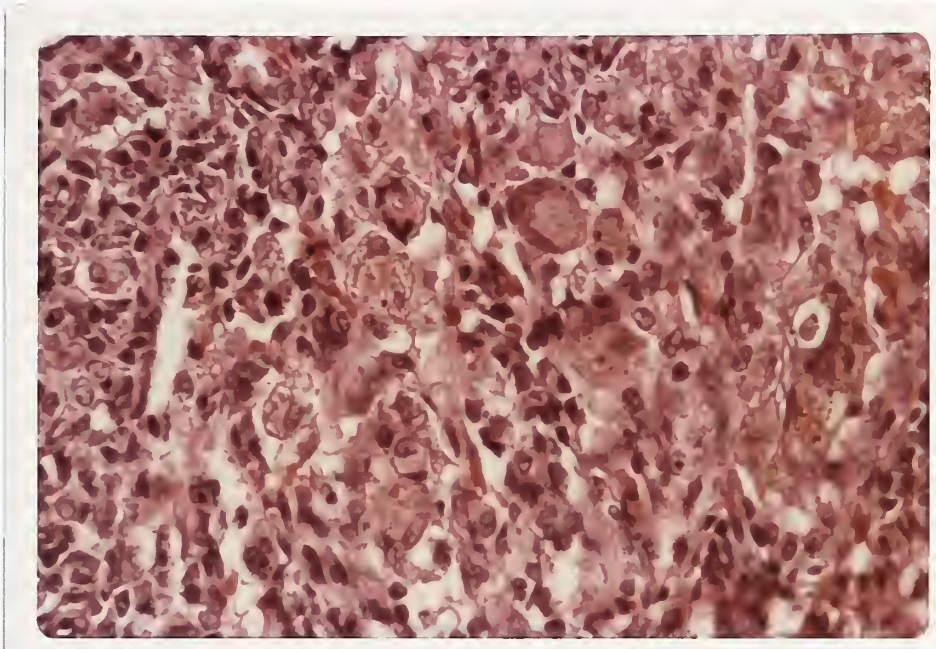


Figure 8. Small giant cell and foamy histiocytes within a large area of MNC infiltrate, guinea pig F. (Hematoxylin/eosin, x 400)

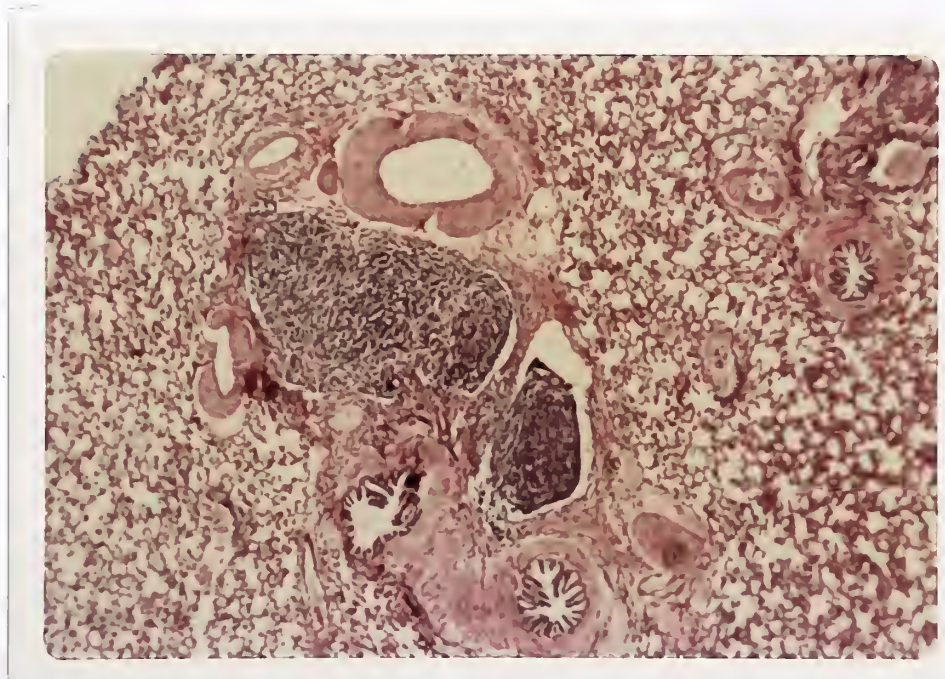
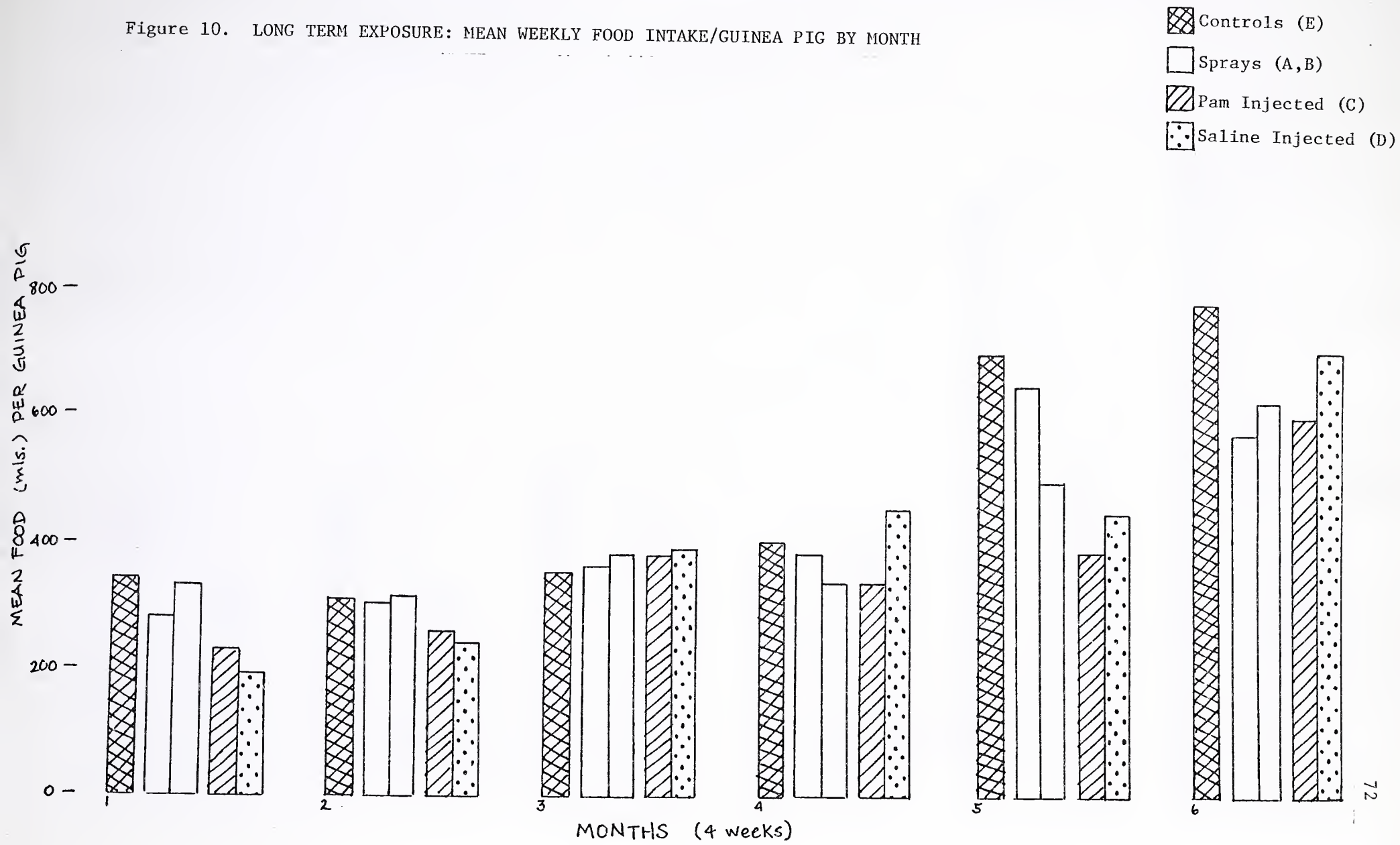


Figure 9. Peribronchial lymphoid hyperplasia, 30 days sprayed (A) animal. (Hematoxylin/eosin, x 40)

Figure 10. LONG TERM EXPOSURE: MEAN WEEKLY FOOD INTAKE/GUINEA PIG BY MONTH



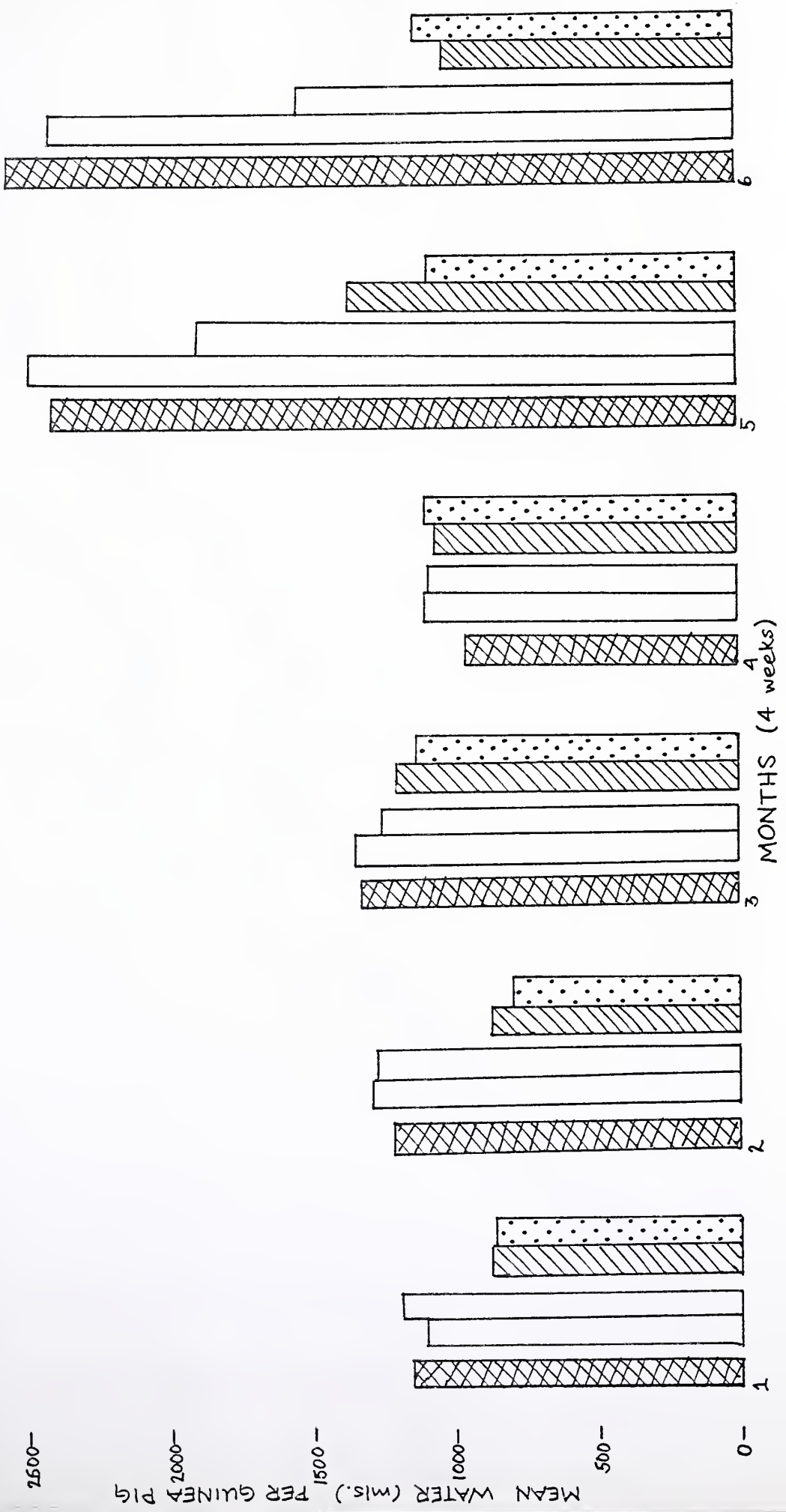
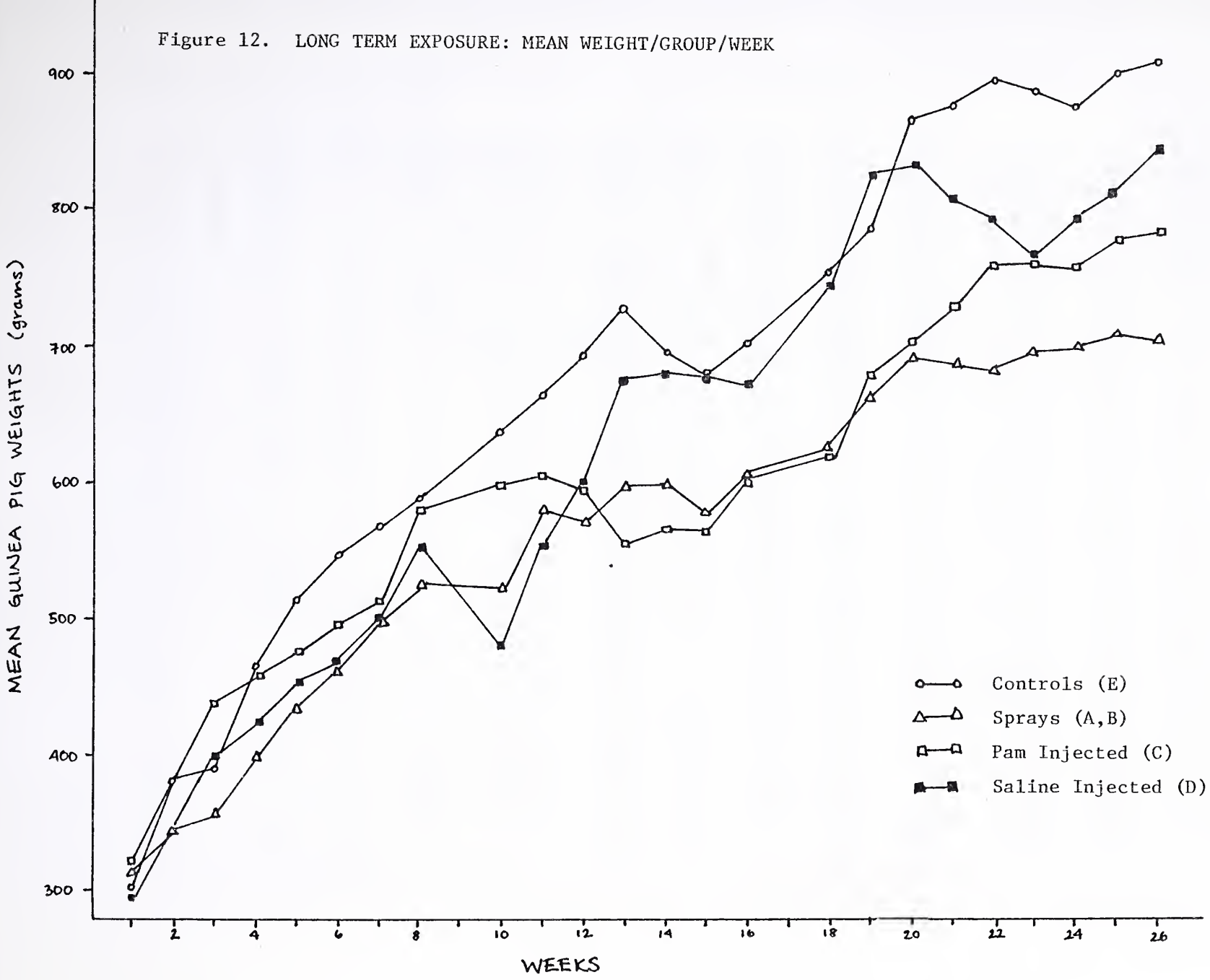


Figure 11. LONG TERM EXPOSURE: MEAN WEEKLY WATER INTAKE/GUINEA PIG BY MONTH

Figure 12. LONG TERM EXPOSURE: MEAN WEIGHT/GROUP/WEEK



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