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Joyce L. Beare-Rogers, Chairperson

Biological Effects Due to Changes in Fats during Heating

J.C. ALEXANDER, Department of Nutrition, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT

In deep-fat frying the fat is used over and over again, and moisture and air are mixed into the hot oil. Substantial quantities of the heated fat are absorbed into the fried foods. Many reports from experimental observations with animals fed these fats have shown biological effects ranging from a slight depression in growth, all the way to very poor growth, diminished feed efficiency, increased liver size, fatty necrosis of the liver, and various other organ lesions. Obviously, certain fat constituents may be changed by frying conditions, and the adverse biological effects are relative. We are at the stage in studying these heated fats where selected techniques including biochemical parameters, histopathological evaluations, and tissue culture in monolayers can be good indicators of some of the specific effects on biological tissues. Isolated fractions from heated fat samples, which contained concentrations of cyclic monomer and dimer derivatives, were used in animal studies. Incorporation of the above materials into rat diets produced distended flatulent stomachs and intestines, gastric ulcers, and multiple focal hemorrhages. Histological evaluation of heart, liver, and kidney tissue sections indicated extensive cellular damage. Livers and kidneys exhibited the most severe lesions. Neonatal heart cells established as monolayers on glass cover slips were exposed to fractions from heated or fresh fats. Cellular damage including pyknosis, vacuolization of the cytoplasm, and mitotic aberrations were observed. Uptake of ^{14}C-labeled fatty acid by the triglyceride fraction of the cells was increased with heated fats.

INTRODUCTION

Much of the fat consumed in our diet has been exposed to heat during processing and in the preparation of foods during cooking. For example, in deep-fat frying, often the fat is kept hot for long periods of time at ca. 180 C, and moisture and air are mixed into the hot oil. The fried foods absorb this heated fat and it becomes part of our diet.

Over the years there has been concern about changes taking place in heated fats, and what effects these derivatives might have on individuals consuming them. In view of this, many feeding studies with experimental animals have been carried out. Early reports were complicated by the possibility that some of the effects observed resulted from vitamin deficiencies brought on by oxidation of nutrients in the diet. Later it was recognized that other constituents of oxidized fats and related products could cause problems.

Some reports from feeding oils and fats after normal frying of foods have indicated little effect on the growth and life span of animals (1-3). In constrast with this, Simko et al. (4) concluded that nutritionally harmful substances may develop in fats under ordinary conditions of kitchen use. Guinea pigs fed the heated fats for 10 weeks developed fatty necrosis of the liver, and fatty calciferous lesions in the myocardium and aorta.

From feeding oxidized fatty materials, others also have shown significant adverse effects on animals. Morris et al. (5) found gastric adenomas in old rats fed rancid fats. Kaunitz and Johnson (6) studied ten different dietary fats, both nonaerated and aerated. Body weights and life span were influenced more by the kind of fat fed than by whether they were aerated. Histopathological examinations were done for several organs, and the most significant findings were nonspecific focal myocarditis and focal fibrosis. Rats fed autoxidized safflower oil (7) had enlarged livers with a low triglyceride content which was attributed to extra accumulation of protein. Yoshioka et al. (8) associated high carbonyl values of oxidized fats with their toxicity, because the rat liver enzymes thiokinase and

TABLE I

Estimates of Fat per Capita per Day in U.S. Diet[a]

Year	Total	Animal sources		Vegetable sources	
	g	g	%	g	%
1910	125	104	83.2	21	16.8
1948	140	104	74.3	36	25.7
1965	144	95	66.0	49	34.0
1972	159	96	60.3	63	39.6
1975	147	87	59.2	60	40.8

[a]Adapted from data in Nutrition Program News, U.S. Department of Agriculture, Washington, DC, May-August 1976.

succinicdehydrogenase had low activities when such fats were fed.

Much evidence indicates that secondary degradation products rather than peroxides are the principal factors in adverse effects seen with thermally oxidized fats. A recent study to substantiate this was reported by Paik et al. (9). Mice dosed with methyl linoleate hydroperoxides or autoxidized methyl linoleate containing secondary oxidation products had 50 and 100% mortality, respectively. Necrosis, fatty degeneration, and congestion in tissues were observed, and impairment was correlated with the type of sample fed. Histopathological observations confirmed these conclusions. Small molecular weight compounds containing carbonyl groups which are easily absorbed were suspected of being involved.

Poling et al. (10) reported that the severity of conditions (temperature, heating time, and aeration) plays an important role in the degree of deterioration of fats. Crampton et al. (11) and Friedman et al. (12) stated that fats containing a large proportion of polyunsaturated fatty acids yielded more toxic substances than fats containing a majority of saturated fatty acids. Perkins and Kummerow (13), and others (12,14) confirmed earlier observations of Crampton that the non-urea-adductable portion of heated fats had increased toxicity for animals. Several researchers (15-18) found that concentrates from thermally oxidized fats containing mostly monomeric and dimeric materials were absorbed more readily and were therefore more toxic than longer chain polymers.

Urea filtrate of heated fat caused a 30% reduction in the oxidation of 1-^{14}C-palmitic acid to CO_2 in rats (14), and also had an effect on the enzyme systems responsible for unsaturated fatty acid synthesis. Incorporation of ^{14}C-acetate into the unsaturated fatty acids of liver triglycerides was higher in animals fed oxidized fats (19). Animals given thermally oxidized fats and low levels of vitamins in their diet at a fixed protein concentration responded poorly compared to those which received fresh fats and the same amount of vitamins (20). An increase in the protein level gave a real positive response in the presence of the oxidized fat.

Toxic effects of thermally oxidized fats were observed by Gabriel et al. (21) when distilled fractions were fed to rats. Histopathological examinations of several tissues were carried out, and organ lipid compositions were determined. There were injurious effects on the heart, liver, and kidney, and these were tabulated in the form of histopathological scores.

TABLE III

Effect of Thermal Oxidation on Linoleic Acid Concentration

Fat	Level of linoleic acid[a]	
	Fresh	Oxidized
Corn oil	61.0	1.1
Olive oil	7.7	tr
LEAR[b]	21.7	1.1
Lard	10.7	1.4

[a]Expressed as % of total fatty acids.

[b]LEAR = low erucic acid rapeseed oil.

Functional changes associated with the hepatomegaly commonly observed from feeding thermally oxidized fats were investigated (22). Along with higher liver weights, there were increases in the concentrations of microsomal protein and endogenous malonaldehyde. The oxidized fat appeared to stimulate smooth endoplasmic reticulum proliferation and to induce a complex of microsomal enzymes. Miller and Landes (23) observed a reduced production of hemoglobin, serum proteins, transferrin, and ceruloplasmin, but an increase of cellular protein in the liver of rats fed heated oil. They postulated that the liver protein was being maintained to cope with the metabolic effects of the damaged fats, perhaps for the synthesis of hepatic enzymes to metabolize the unnatural dietary lipid to forms which could be expelled through the kidneys or lungs.

Over the past 65 years in the United States as well as in Canada, there has been an increase in the per capita consumption of fat per day (Table I). Most of this change had taken place by 1948. The decline in animal fat consumption has been more than compensated for by an increase in vegetable fat intake. So with fat levels up and a significant change in its nature, current studies with heated fats should consider these circumstances.

BIOCHEMICAL AND HISTOLOGICAL EFFECTS

In view of the controversy with respect to toxicity of oxidized fats of different types, four distinctly different fats were selected for study. These were corn oil, olive oil, LEAR (low erucic acid rapeseed oil), and lard. Heating conditions of the fats such as temperature, aeration, and time were selected to produce a product with similar toxicity to used fat samples obtained from commercial deep-fat frying operations. A relatively short feeding period of 28 days was chosen in order to determine some of the early effects of heated fat toxicity.

The fats were heated in stainless-steel beakers for 72 hr at a controlled temperature of 180 C. Each day they were stirred continuously for 12 hr with a mechanical stirrer and by hand every hour for the next 12 hr to ensure aeration and mixing (21).

TABLE II

Results of Chemical Analyses of Thermally Oxidized Fats[a]

Analyses	OCO	OOO	OLE	OLA
Carbonyl value (meq/kg)	170.00	190.5	192.0	189.5
Iodine value[b]	111 (120)	64 (85)	80 (121)	50 (70
Sap Value	190	192	189	200
Free fatty acid (%)	0.4	1.50	0.82	1.20

[a]OCO = oxidized corn oil; OOO = oxidized olive oil; OLE = oxidized LEAR (low erucic acid rapeseed Oil); OLA = oxidized lard.

[b]Values for fresh fats are in the parentheses.

TABLE IV

Body and Organ Weights and Total Organ Lipids of Rats Fed Different Dietary Fats[a]

Dietary fat[b]	Body weights (g)	Heart		Liver		Kidney	
		Rel. wt. (%)	Lipid (%)	Rel. wt. (%)	Lipid (%)	Rel. wt. (%)	Lipid (%)
FCO	198	0.38	8.4	4.6	8.8	0.93	13.1
OCO	205	0.36	19.4	5.7	7.1	0.97	13.7
FOO	199	0.38	11.0	4.2	9.6	0.89	12.6
OOO	200	0.38	15.2	4.8	8.5	0.92	13.9
FLE	204	0.38	6.7	5.5	6.6	0.90	8.3
OLE	196	0.45	14.6	5.1	9.0	1.03	9.6
FLA	235	0.34	4.9	4.3	6.9	0.90	7.5
OLA	181	0.45	7.4	4.1	8.8	0.99	7.3

[a]Relative weights of organs expressed as % body weight with ten rats per group.

[b]FCO = fresh corn oil; OCO = oxidized corn oil; OO = olive oil; LE = LEAR (low erucic acid rapeseed oil); LA = lard.

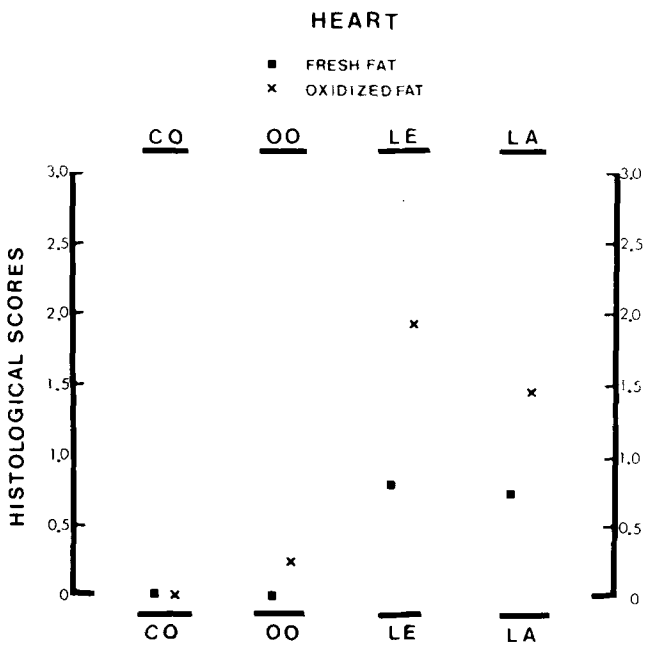

FIG. 1. Histological scores for the hearts of rats fed fresh or thermally oxidized fats.

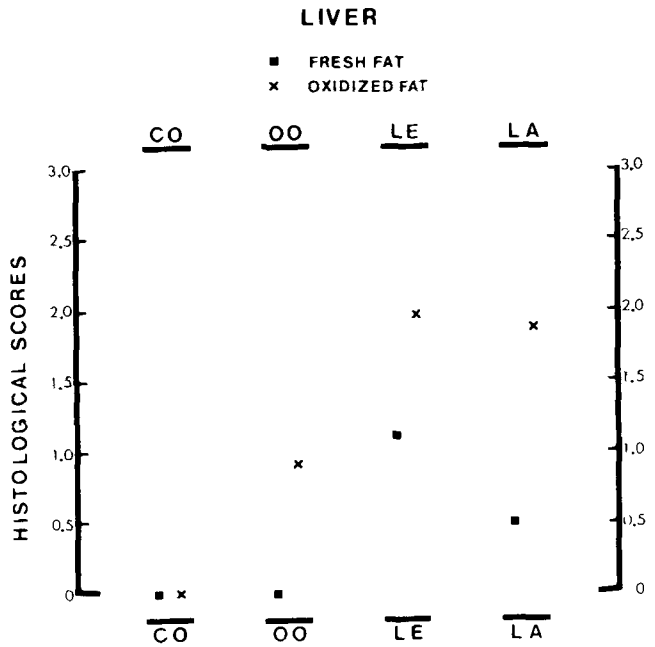

FIG. 2. Histological scores for the livers of rats fed fresh or thermally oxidized fats.

The saponification and free fatty acid values were essentially unchanged in the thermally oxidized fats (Table II), but the iodine values were reduced, and carbonyl values were greatly increased indicating the degree of degradation due to oxygen attack (24). The relative concentrations of the polyunsaturated fatty acids were reduced as the result of heating (Table III), but the amount of the monoenes was not affected.

The volatile fractions of the thermally oxidized and fresh fats were isolated by vacuum distillation at a pressure of 1 Torr and a head temperature between 150 and 180 C. These distillates were incorporated into diets at a level of 15% and fed to male weanling rats for 28 days with ten animals per group (21).

The organ areas selected for histopathological examination were the ventricular septum of the heart, right lateral lobe of the liver, and one-half of the left kidney. They were trimmed to a width of 3 mm, imbedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eoxin. The various types of lesions found in the tissue sections were graded as to incidence and severity on a scale from zero for normal to three for necrotic tissue. This involved four animals randomly selected per dietary group, and four separate histological sections per organ. The pertinent anatomical structures observed were:

Heart – myocardial nuclei
– nuclei of vascular media and endothelium
– interstitial tissue and myofibers
Liver – hepatocellular nuclei
– hepatocellular cytoplasm
– Kupffer and endothelial cells
Kidney – glomeruli
– tubules
– vessels and interstitial tissues

There were no significant differences in the average daily feed consumption or feed efficiency between groups that received the oxidized fats or their controls. Table IV shows the final average body weights, relative to organ weights and percent total organ lipids. Relative heart weights and the percent total heart lipids were increased significantly in both the oxidized low erucic acid rapeseed oil (OLE) and oxidized lard (OLA) groups. Rats in the oxidized corn oil (OCO) and oxidized olive oil (OOO) groups also showed substantial increases in the percent heart lipids. None of the test groups showed large changes in the total liver lipids

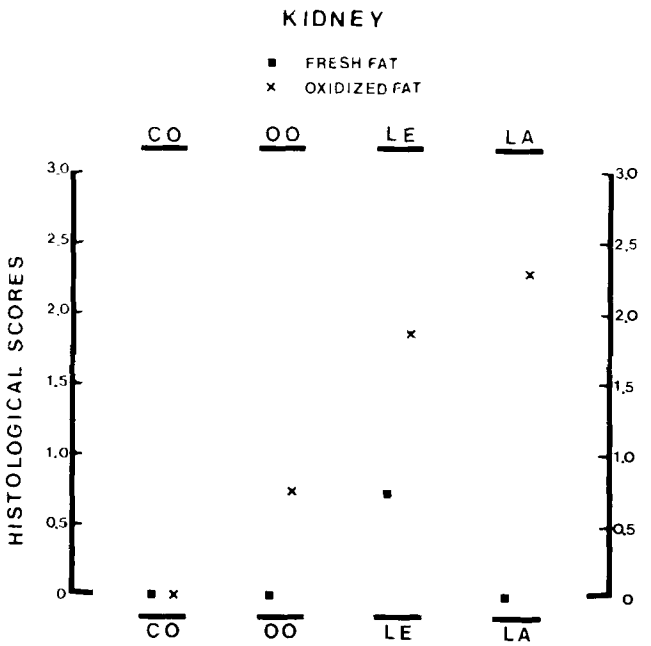

FIG. 3. Histological scores for the kidneys of rats fed fresh or thermally oxidized fats.

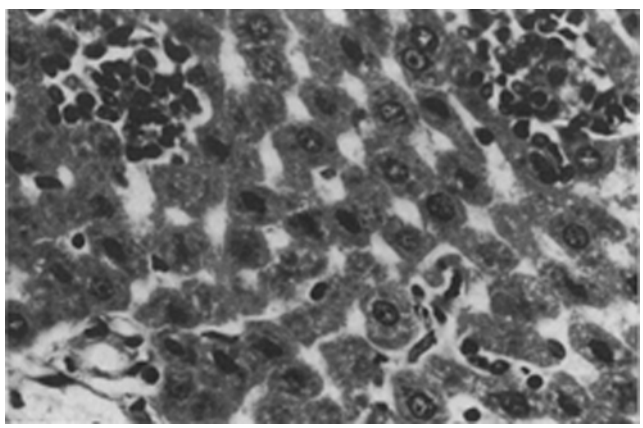

FIG. 4. Hepatic granulomas and numerous pyknotic nuclei indicate injured hepatocytes (X500, hematoxylin and eosin).

or kidney lipids as a result of heating the fats although there were some increases in the liver (OLE and OLA). Corn oil and olive, fresh or heated, produced significantly more lipid accumulation in the kidneys that did the other two fats.

There were several substantial changes in the liver fatty acid concentrations of animals that received OLE (21). For instance, the neutral liver fatty acids of these rats had a 190% increase in arachidonic acid and a 115% increase in stearic acid, while the oleic acid level dropped considerably. All of the animals exhibited rough greasy hair, possibly due to mild seborrhea as well as a noticeable decrease in grooming time. There was also evidence of diarrhea and polyuria, concomitant with increased water consumption.

Histological examinations of heart, liver, and kidney tissue sections are represented by histological scores (Fig. 1-3). Neither the corn oil (CO), fresh or oxidized, nor the fresh olive oil (OO) produced any adverse effects in the heart (Fig. 1). There was, however, very evident heart injury due to fresh and oxidized LEAR (LE) and lard (LA). Animals fed OLE sustained the highest degree of injury to the heart. In the case of the liver (Fig. 2) CO was without effect. Fresh LE and LA showed lesions. Thermal oxidation

FIG. 5. Large vesicular nuclei with chromatin rimming in vascular media and myofibers (X1300, hematoxylin and eosin).

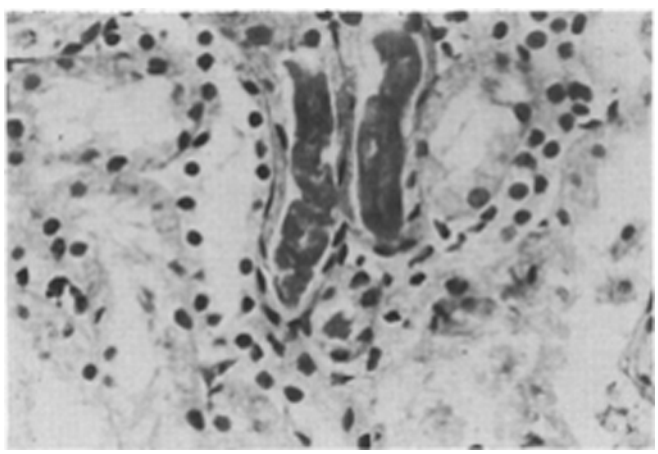

FIG. 6. Coarse granular casts blocking renal tubules (X500, hematoxylin and eosin).

of OO, LE, and LA significantly increased liver damage for these three fats. Heated LA was most damaging to the kidney (Fig. 3) followed by heated and fresh LE and heated OO.

Necrotic foci, manifested by focal granulomas, were numerous in the livers of rats fed OLE (Fig. 4) or OLA. They represented an inflammatory lesion characterized by an invasion of histiocytes and lymphocytes. This reaction occurred near vascular structures often associated with hepatocellular dissociation. Rats fed OOO had no distinct necrotic foci, but severe pyknosis of the hepatic nuclei and cytoplasmic atrophy leading to cell death. The livers of these animals were undergoing extensive regeneration, as indicated by many mitotic hepatocytes. These changes were not present in control groups.

Cross sections of hearts from animals fed OLE showed extensive fatty vacuolation of the myocardial fibers as reported by other workers [Kramer et al. (25)]. Figure 5 shows large vescicular nuclei with chromatin rimming in vascular media and myofibers of rats fed OLA.

The action of OLE and OLA on the kidneys was very severe and most probably responsible for producing polyuria. Cellular degeneration and necrosis of the tubular epithelium was seen (Fig. 6), and there were coarse granular casts obstructing tubular lumina. In the group fed OOO, damage consisted mainly of activated nuclei of the epithelial cells of the tubules.

The heating of fats in air is known to cause the formation of volatile scission products, less volatile nonoxi-

TABLE V

Mitotic Index and Percent Pyknotic Cells of Cultured Heart Cells[a] Treated with Fresh or Thermally Oxidized Olive Oil[b]

Treatment[c] μg/ml of the medium	Mitotic index Incubation time (hr)			Percent pyknotic cells Incubation time (hr)		
	24	48	96	24	48	96
FOO (60)	0.42	0.69	0.62	0.26	0.38	0.50
OOO (60)	0.46	0.63	0.50	0.52	0.70	0.86
FOO (100)	0.21	0.39	0.35	0.27	2.69	2.40
OOO (100)	0.20	0	0.01	1.35	5.47	7.70

[a]A combination of endothelial, muscle, and fibroblast-like cells.
[b]Each value represents an average of 20 determinations.
[c]FOO = fresh olive oil; OOO = oxidized olive oil.

dized derivatives such as cyclic monomers and dimers, as well as nonvolatile polymers [Artman and Alexander (2), Paulose and Chang (26), Waltking (27), Waltking et al. (28), Perkins and Wantland (29)]. However, the nature and extent of these degradation products depend on the kind of fat and the way it is heated. Thermal oxidation of fats used in deep frying results in high carbonyl values which are indicative of considerable oxidation due to aeration and heating.

The accumulation of lipids in the hearts of animals fed oxidized fats may have resulted from impaired mobilization or metabolism of these lipids. Landes (30) and Landes and Miller (31) found an increased serum lipoprotein lipase activity in rats that received oxidized fat. From our data it can be concluded that as a result of this activity the heart would take up additional lipids from the bloodstream leading to a fatty buildup.

Fatty livers resulting from the feeding of oxidized fats have been reported in the literature [Gabriel et al. (21), Poling et al. (18), and Hemans et al. (20)]. Our animals fed OLE or OLA showed this effect, but OOO and OCO did not. Hemans et al. (20) suggested that an inadequate protein or vitamin level may have contributed to the accumulation of liver lipids. Since these conditions did not apply to this study, it is probable that the damaged fats were assimilated by the liver but not mobilized adequately for use in normal body growth. The dramatic increases of stearic and arachidonic acids of the neutral liver lipids provide evidence in favor of this concept.

Histological evaluation of the tissue sections from the test animals by Gabriel and co-workers (21) indicated that the majority of lesions occurred at sites of transport of the toxic fat degradation products across blood vascular membranes in the heart and liver, and renal tubules. Once these had entered the cell, they inhibited normal metabolic pathways with resulting fatty accumulation (heart and liver) or cellular necrosis (kidney).

It is obvious that the thermally oxidized fats were more damaging to the organs of the rats than the fresh fats. The diffuse liver damage from feeding fresh LEAR was unexpected and not easy to explain, but fatty acid imbalances might be considered. The fact that OOO and OLA were more deleterious than OCO as indicated by the histological scores is contradictory to reports in the literature that heated highly unsaturated fats are more detrimental than the relatively more saturated fats [Johnson et al. (32), Reddy et al. (33)]. It has been shown that the polyunsaturated fatty acids, being more susceptible to oxygen attack during thermal oxidation, form scission products quite readily, which are then converted to relatively inert polymers in Diels-Alder type addition reactions.

Gabriel et al. (21) have stated that methyl oleate or fats containing a large concentration of oleic acid, were more toxic when thermally oxidized and fed to rats than fats with a relatively low oleic acid concentration.

TABLE VI

Protein Content of Heart Cells Treated with Fresh or Thermally Oxidized Olive Oil

Treatment[a]	Concentration μg/ml of the medium	Total protein μg/tube[b] Time in hours			
		0	24	42	96
FOO	60	55	108[f]	112[f]	139[e]
OOO	60	55	161[d]	129[e]	123[e]

[a]Fresh or oxidized olive oil free fatty acids were added to the medium in the form of an emulsion with bovine serum albumin (fraction V, poor in unesterified free fatty acid). The ratio of albumin to lipid was 60:1 (w/w). FOO = fresh olive oil; OOO = oxidized olive oil.
[b]Each value is an average of five determinations. Values with different superscripts are significantly different from each other ($P<0.05$).

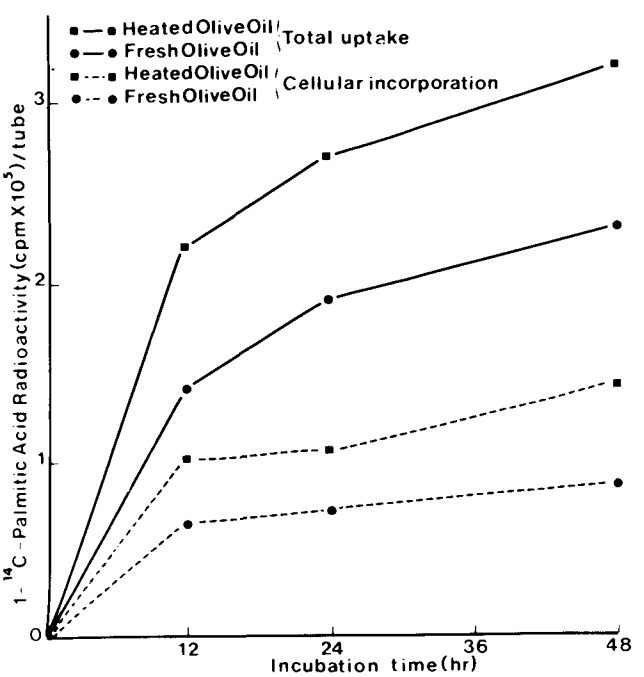

FIG. 7. Heart endothelial cells.

HEART CELLS IN CULTURE

As a result of their metabolism, lipids are concerned with cellular structure and function, especially that of the membrane. Rat heart cells in culture grown in a lipid-deficient medium lost their ability to beat and also developed changes in the level of enzymes related to specific heart functions [Fujimoto and Harary (34), Kuramitsu and

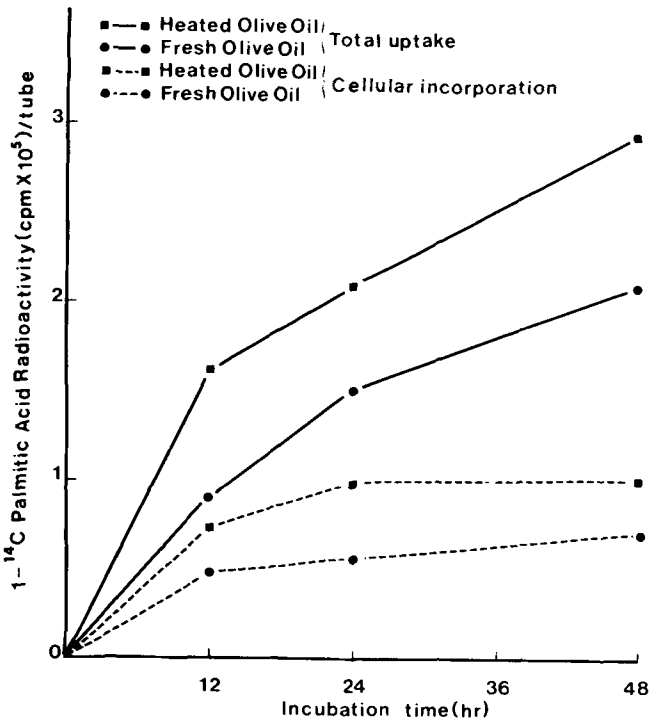

FIG. 8. Heart muscle cells.

TABLE VII

Percent Incorporation of 1-^{14}C-Palmitic Acid into Cellular Lipids of Heart Cells[a]

Incubation time (hr)	Lipid class	Endothelial FOO	Endothelial OOO	Muscle FOO	Muscle OOO
12	Phospholipid	49.3	48.4	46.2	38.6
24		52.4	39.1	46.0	38.4
48		42.0	44.2	48.8	38.0
12	Cholesterol	3.5	4.1	3.8	4.3
24	and	3.6	4.4	3.7	4.5
48	Diglyceride	4.5	4.5	4.3	4.8
12	Free fatty	10.5	8.4	16.1	11.9
24	acid	13.5	13.8	16.0	11.9
48		12.9	10.1	16.0	11.7
12	Triglyceride	34.9	37.4	31.7	42.7
24		27.5	41.6	31.4	42.8
48		38.3	38.9	27.3	43.8
12	Cholesterol	1.5	1.4	1.8	2.0
24	ester	2.3	1.6	2.9	2.0
48		2.1	2.0	3.5	1.6

[a]FOO = fresh olive oil; OOO = oxidized olive oil.

Harary (35)]. Addition of serum lipids to the medium restored the beating activity [Harary et al. (36)].

The myocardia of experimental animals fed thermally oxidized fats show lipidosis, necrosis, and other pathological changes as indicated by Simko et al. (4) and Gabriel et al. (21). There is, however, little information on effects of oxidized fat components on the function of the myocardium.

Cells grown in culture medium in the form of a monolayer are an important biological living model, which can be examined continuously to reveal physiological, morphological, or metabolic changes in the presence of compounds added to the medium. While the cells are dividing by mitoses to increase their population, a harmful substance will affect the growth rate as well as other metabolic activities. In this way chemical compounds can be screened with in vitro techniques.

Various studies have shown that the components of heated fats which may be toxic to animals can be concentrated in that portion of the fat which does not adduct with urea [Crampton et al. (11), Artman and Alexander (2), Shue et al. (14)].

Prasad et al. (37) reported on effects of thermally oxidized olive oil on in vitro heart cells. The distillable non-urea-adductable fraction (DNUA) from fat thermally oxidized as described earlier was isolated as indicated by Artman and Alexander (2). The ethyl esters then were hydrolyzed to recover the free fatty acid portion. Free fatty acids from fresh olive oil were used as a control. Bovine serum albumin V (unesterified fatty acid poor) was found to be an efficient carrier of lipid fractions in the tissue culture medium. Fatty acid fractions were added to the culture medium containing 5% fetal calf serum at graded levels from 20 to 100 μg per ml. Monolayers derived from heart cells of 3-day-old rats were obtained by the method of Wenzel et al. (38), as modified by Rogers (39). The hearts were excised aseptically and transferred to a petri dish containing phosphate-buffered saline. The tissue was chopped finely and separated into single cells by trypsinization using a 0.25% trypsin solution. Leighton tubes were seeded with 1 x 10^5 cells/ml of medium, and these were grown for 4 days.

A living monolayer of heart cells in culture was observed with an inverted phase contrast microscope. A combination of May Gruenwald and Giemsa stains was used to characterize the nucleus and cytoplasm of the cells [Paul (40)]. Intracellular lipidosis was observed under the contrast microscope, and in severe cases most of the extracellular space was occupied with lipid droplets.

To determine the mitotic index and the number of cells per field, a stained coverslip culture was observed under a standard light microscope (10 x 45). From 20 different random fields, the number of cells in each field, the number of cells undergoing mitosis, and the number of pyknotic nuclei were observed. Cellular protein was quantitated by the method of Oyama and Eagle (41), and expressed in terms of bovine serum albumin equivalent.

Both endothelial and muscle cells (containing a small amount of endothelial cells) were studied in the primary cultures of heart cells. Most cytoplasmic and nuclear changes were associated with intracellular lipid accumulation. Severe lipidosis was observed in many cells exposed to the OOO samples. Some were abnormal and spherical in shape and had a constricted nucleus in the center. Most of these cells left the cover slip during staining indicating cell death. Vacuolization of the cytoplasm and a network appearance were pronounced with the heated fat treatment indicating cytoplasmic degranulation. Endothelial cells were more damaged than muscle cells.

The mitotic index and the percent pyknotic cells are shown in Table V. Prolonged treatment of the cells at 100 μg level of sample from OOO suppressed the rate of mitosis, and at both treatment levels significantly increased the percent pyknotic cells. The total number of cells per microscopic field also was reduced greatly with strong evidence for cell necrosis (lifting from the cover slip). Table VI shows that there was an increase in protein content of cultures exposed to the heated fat sample for 24 and 48 hr. The decrease at 96 hr reflects inhibition of cell growth.

The uptake and utilization of 1-^{14}C-palmitic acid by heart muscle and endothelial cells in culture were studied with 100 μg of added free fatty acid fractions in the media (2 ml per culture tube). These cultures grown for 24 hr were rinsed with phosphate-buffered saline and exposed to a medium containing 0.25 μCi of 1-^{14}C-palmitic acid and a total of 2.5 μg of palmitic acid per tube. Both portions of the fatty acid were added to the culture medium as potassium salts bound to delipidized serum proteins. Cellular

lipid was extracted by the Folch procedure [Folch et al. (42)].

Radioactivity of the medium was measured at 12, 24, and 48 hr (total uptake). Cellular incorporation of ^{14}C (retention) was determined in the cell portion only, and the difference in values indicated loss due to oxidation of palmitic acid and its derivatives. The total cellular lipid was fractionated by thin layer chromatography (TLC) using glass plates coated with Silica Gel G of 0.5 mm, and a solvent system of heptane, isopropyl ether, and acetic acid in a volume ratio of 60:40:3.

The uptake of 1-^{14}C-palmitic acid by cultured myocardial cells was greater for both endothelial and muscle cells treated with oxidized fat (Fig. 7 and 8). Cellular incorporation (retention) also was increased.

Heart muscle cells treated with the OOO sample showed a high level of ^{14}C in the triglyceride fraction and a reduced level in the phospholipids (Table VII). Heart endothelial cells did not react in this way.

Thermally oxidized fat components have been shown to be absorbed, metabolized, and toxic to experimental animals when fed as part of their diet. They also induced biological changes in cultured heart cells. Included in these were increased mitotic index, more pyknotic nuclei, and extensive vacuolation of the cytoplasm. Intracellular lipid accumulation also was observed, and this excess fatty material could reflect an accelerated uptake of these components from oxidized fat, or inhibition of their intracellular mobilization for other cellular frunctions.

Cells treated with oxidized fat components differed metabolically in that they had a significantly higher uptake of exogenous 1-^{14}C-palmitic acid. Although the radioactivity was incorporated into all the lipid classes, heated fat caused a severe shift into the triglycerides of heart muscle cells. Consequently there must have been some effect at the cell membrane level, with a change in permeability allowing a rapid influx of the fatty acids into the cells. If these disturbances continue and are great enough, cell death occurs.

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