lymphocytes, polymorphonuclear granulocytes (including eosinophils) and fibroblast were also seen. The cells aggregated around muscle fragments (figures 1b and 3a).

Electron microscopy revealed that the apparently well preserved myofibres were in reality in various stages of degeneration. However, where muscle was alone, the degree of disruption was much less marked than at the corresponding period of mixed culture. In the mixed 6-day-cultures, the myofilaments and sarcotubules were matted together into fibrillary or amorphous sarcoplasmic 'sap'. The mitochondria were enlarged, dark, arranged in rows or clumped together. Myonuclei, where recognized, were small, pale with coarse chromatin and a 'blistered' envelope. Within myofibre basement membranes as well as outside them. Frank myoblasts rich in polyribosomes, small pale mitochondria and recognizable thick and thin myofilaments were also seen (figure 3b, c). In many cells, phagocytosis and myofilament synthesis occurred side by side.

In chambers where gold treated bone marrow was mixed with muscle fragments, no myoblastic activity was seen at 6 days of culture, and it was still slight at 12 and 18 days. The gold precipitated in large macrophage-like cells containing multivesicular and heterogeneous bodies[9,10]. These cells were always well separated from the myofibres.

In this study we were not concerned with quantitative factors known to influence proliferation and differentiation[11], but with the effect of distally arising cells on early changes in explanted myofibres. No particular attention was paid to the changes in blood vessels or nerves. Our results indicate that a remarkable acceleration of degenerative and regenerative processes takes place when bone marrow cells are mixed with muscle fragments (table).

The bone marrow derived cells (including polymorphs which soon degenerate and mix with the general pool of debris) are probably responsible for most of the rapid myofibre degradation, although young myoblasts, themselves phagocytic[12], may contribute to a 'mop up' operation. The contractile protein decrease acts as a powerful stimulus to biosynthesis[2] and myoblast formation in the generally accepted ways. After gold treatment, there are not only fewer bone marrow cells but their phagocytosing and metabolic activities are much decreased[7]. Thus, without rapid degeneration no accelerated regeneration occurs.

Another possible, even if far-fetched, interpretation could be that cells originating from bone marrow are themselves induced to transform into myoblasts. This could be brought about by incorporation of material from the degenerating muscle or by fusion with myogenic cells. The paucity of degenerating mononuclear cells, the scarce mitoses and other morphological features suggest that a transition from relatively simple mobile mononuclear cells into large complex forms actively engaged in myogenesis may indeed be taking place. Theoretically, such an interpretation is feasible as macrophages are known to engage in reutilization of ingested products and they apparently can transform into other, even unrelated, forms such as melanoma cells in culture[13].

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Free radicals produced in a nitrosofluorene-unsaturated lipid reaction[1]

Robert A. Floyd

Biomembrane Research Laboratory, Oklahoma Medical Research Foundation, 825 Northeast 13th Street, Oklahoma City (Oklahoma 73104, USA), 13 July 1976

Summary. We report here the first demonstration that the carcinogen 2-nitrosofluorene reacts directly with lipid molecules containing carbon-carbon double bonds to yield free radicals which appear to be the nitroxyl free radical of the carcinogen covalently bound to the lipid.

2-Nitrosofluorene (NOF) is an activated form of the carcinogen 2-acetylaminofluorene (AAF) and produces tumors in mammary gland as well as at the site of subcutaneous injection[2]. NOF can be formed either by deacylation of N-hydroxy-N-acetyl-2-aminofluorene (N-OH-AAF), itself an activated form of AAF, and then subsequent non-enzymatic oxidation[3]; or by the peroxidase or free radical route of N-OH-AAF activation[4–8]. We report here the first demonstration that NOF when exposed to lipid molecules containing a carbon-carbon double bond reacts readily to form a free radical which we postulate is the nitroxyl free radical of the NOF-lipid addition product.

NOF was synthesized as described previously[7]. The synthesized compound was pure by thin layer chromatography and had the same melting point and UV spectrum as reported by Lotlikar et al.[9]. Linoleic acid, oleic acid, octanoic acid and squalene were purchased in their pure forms from Sigma Chemical Co. These were diluted with deoxygenated methanol to a concentration of 2% and stored under nitrogen at −20 °C. Optical surveillance at 233 nm indicated the absence of hydroperoxides. All reactions were carried out in 0.05 M pH 7.4 potassium

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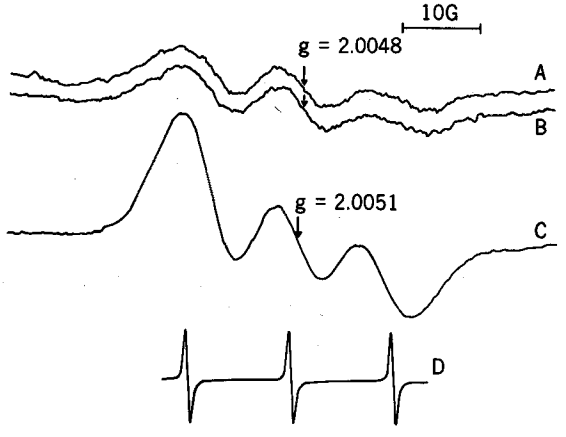
phosphate buffer. Electron Spin Resonance (ESR) spectra were obtained at room temperature using a Varian E-9 X-band system operating at 100 kHz.

Our novel results are presented in the figure. NOF reacted with squalene, oleic acid and linoleic acid to produce free radicals as indicated by the ESR spectra. The 3-line-spectrum in each case and the g values suggest that the free radicals produced are nitroxyl free radicals (also see below) with nitrogen splitting constants of 11.85 gauss for both linoleic and oleic acid and 11.35 gauss for squalene. All hydrocarbons containing carbon-carbon double bonds that we have tested thusfar including purified egg lecithin suspended as liposome vesicles react with NOF to yield ESR spectra very similar to those shown in the figure. However, all hydrocarbons lacking a carbon-carbon double bond that we have tested do not yield a free radical spectrum. We have found that the amount of free radical formed tends to follow the number of double bonds in the lipid molecule. This is not a strict relationship, however, and we believe the positioning of the double bonds in relation to each other is also very important. A more detailed treatment of our observations will be published; but for the present communication, it is important to point out that EDTA (ethylenediamine tetraacetic acid) did not influence the amount of free radicals formed or the reaction rate. Therefore we believe the reaction occurs directly without a catalyst (such as a trace of metal ions) being necessary.



ESR spectra obtained after 2-nitrosofluorene (NOF) was allowed to react with various unsaturated lipids. NOF (0.25 μmoles) was added to 2 ml of potassium phosphate buffer containing: a) 3.57 μmoles of linoleic acid, b) 3.57 μmoles of oleic acid and c) 2.44 μmoles of squalene. The ESR sweep was started 20 sec after the reaction was initiated by addition of the lipid in a small amount of methanol. Under these conditions, the amount of free radical reached a maximum after about 15 min and stayed relatively constant in amount for the next hour. The ESR conditions were: sweep time, 100 gauss in 30 min with a filter constant of 10 sec, frequency 9.5305 GHz at 15 mW incident power, modulation 8 gauss at 100 kHz, temperature 25 °C. Spectrum D is that of the Fremy's salt standard. No free radical signal was obtained with either NOF only in buffer or with any one of the lipids alone in buffer.

Our results were very surprising but a thorough search of the literature revealed that Sullivan[10] had observed a somewhat similar reaction in 1966 in that nitrosobenzene reacted directly with 2,3-dimethylbutene to yield stable nitroxyl free radicals having g values and nitrogen splitting constants similar to the ones we report here. He postulated that the reaction occurred via a 'novel pseudo Dield-Alder' mechanism in which the hydroxylamine was an intermediate and that oxygen and/or nitrosobenzene oxidized the hydroxylamine to the nitroxyl free radical form. Knight succeeded in isolating the hydroxylamine intermediate in good yield 4 years later[11]. We have found that oxygen is not required in the reaction described here and that under these conditions N-hydroxy-2-aminofluorene (i.e. reduced NOF) is formed in the reaction. Therefore, because of these and many other observations to be described in detail later we believe NOF adds directly to the carbon-carbon double bond of a hydrocarbon producing the hydroxylamine intermediate which is then oxidized to the nitroxyl free radical.

It is clear from the results presented here that NOF when formed in vivo will react with lipids containing double bonds to produce a novel form of the carcinogen. The true significance of this reaction to the understanding of AAF carcinogenesis must await further investigation. It should, however, be noted that Stier et al.[12] observed nitroxyl free radicals having properties somewhat similar to the free radical observed here in the chloroform-methanol extract of rabbit liver microsomes metabolizing AAF. All of these observations tend to indicate that the reaction reported here does occur in vivo and point to a need to understand the nature of this reaction in greater detail as well as its significance to AAF carcinogenesis.

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Nitrothiophenes with schistosomicidal activity

R. M. Lee, M. W. Mills and G. S. Sach

Research Institute, Smith Kline and French Laboratories Ltd., Welwyn Garden City, Herts (England), 6 July 1976

Summary. A series of nitrothiophene compounds, with activity against Schistosoma mansoni in mice, is described and shown to be more effective than the corresponding nitrofurans.

Investigations carried out to determine the structural requirements of nitroheterocyclic antischistosome compounds have so far indicated that activity can be found in nitroimidazoles[1], nitrothiazoles[2], nitrofurans[3] and nitrothiophenes[4]. Of these, the nitroimidazoles have produced oogram changes only; within the nitrothiazoles and nitrofurans, the range of active compounds has been limited to certain specific structural features[3–5] and the only nitrothiophenes to exhibit even weak activity have been the analogues of active nitrofurans[4].

The majority of nitroheterocyclic compounds tested do not possess any significant antischistosome action, and this lack of effect has been attributed by Bueding and his co-workers[3–5] to the absence of such structural necessities