Mechanisms of lipid peroxidation: Iron catalyzed decomposition of fatty acid hydroperoxides as the basis of hydrocarbon evolution in vivo

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Summary. Ethane and pentane are evolved during peroxidation of tissue lipids in vivo and are believed to be derived from ω3 and ω6 fatty acids respectively. We present evidence supporting a scission mechanism as the means of forming these hydrocarbons during hydroperoxide decomposition. A fatty acid ω6 hydroperoxide (methyl 13-hydroperoxy-6,9,11-octatrienoate), when incubated with a ferrous ion, yielded pentane as the major hydrocarbon (98%). Reaction with ferrous ion results in an alkoxy radical capable of undergoing scission to an aldehydic fatty acid and a hydrocarbon.

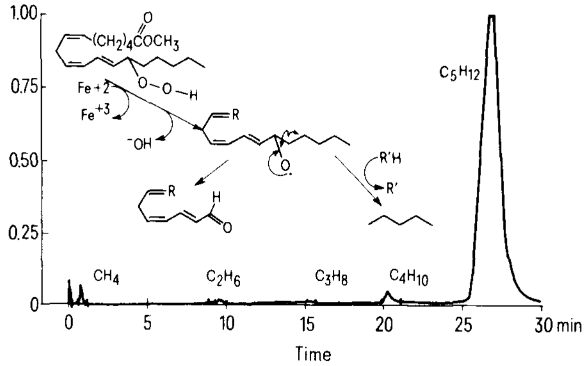
The evolution of volatile, saturated hydrocarbons from autoxidizing lipids has long been known[3,4] but only recently been appreciated as a means of monitoring peroxidation in vivo. The prooxidant stress of carbon tetrachloride (CCl_4) has been found to increase ethane production during both in vitro[5] and in vivo[6] studies. Deficiency of the dietary antioxidant, vitamin E, also increases the levels of ethane[7,8] and pentane[8] exhaled by rats. The inhibition of hydrocarbon production by vitamin E and other antioxidants suggests the termination of free radical-catalyzed fatty acid peroxidation which would account for the volatile hydrocarbons and presumably much of the underlying tissue damage.

A proposed mechanism for generating saturated hydrocarbons from oxidizing unsaturated fatty acids[4] involves homolytic cleavage of the hydroperoxide group followed by homolysis of the bond between the carbons α and β to the radical (figure). If the initial hydroperoxide group were proximal to the alkyl end of the fatty acid, a simple, short chain radical would result. With the abstraction of a hydrogen, and depending on the number of carbons from the alkyl end, saturated hydrocarbons of varying lengths would be formed. Pentane would be evolved by this mechanism from fatty acid derivatives having the hydroperoxide group at the ω6 carbon, numbered from the methyl end. For fatty acid derivatives having the hydroperoxide group at ω3, ethane would be evolved. We chose to investigate this reaction via the use of ferrous ion to produce an alkoxy radical from a pure fatty acid hydroperoxide.

Materials and methods. The ω6 hydroperoxide used in this experiment was methyl 13-hydroperoxy-6,9,11-octatrienoate, prepared by reaction with soybean lipoxygenase and purified by high pressure liquid chromatography according to the method of Funk et al.[9]. A chloroform solution containing 10 µmoles of hydroperoxide was added to a 2-ml vial and evaporated to dryness under N_2. A micro stirring bar and 0.5 ml solution of 0.1% tween-0.1 M acetate buffer (pH 5.0) were added; the solution was covered with O_2 and capped with a teflon-faced seal. A 10-µl aliquot of 0.5 mM ferrous ammonium sulfate (5 nmoles) was injected into the vial, which was then placed for 5 min on a Lab-Line Pyromagnestir heated to 85 °C. Gas samples of 0.3 ml were withdrawn for gas chromatographic analysis on a 183 × 0.32 cm stainless steel column packed with 45/60 mesh Carbosieve. The column conditions were: T_1, 50 °C for 4 min; temperature programming, 16 °C/min; T_2, 350 °C hold. A Hewlett-Packard 5700A gas chromatograph equipped with flame ionization detector and electronic integrator was used, and hydrocarbons were compared with known reference samples.

Results and discussion. Pentane was the principal hydrocarbon evolved under these conditions, accounting for greater than 98% of the evolved gases (figure). Small amounts of Cl-4 gases were also detected, in agreement with findings for the volatiles of autoxidizing methyl linoleate. Under the conditions of our experiments, however, the levels of these gases were minimal. Only trace levels of hydrocarbon gases were found in room air and in samples taken from a control vial.

Linoleic acid (9,11-octadienoic acid) has been studied most extensively as the source for the volatile hydrocarbons of fat autoxidation. The generation of volatiles occurs slowly at room temperature. At elevated temperatures (200 °C) thermolytic decomposition is rapid, and the ω6 hydroperoxide of linoleic acid (13-hydroperoxy-9,11-octadienoic acid) has been found to yield pentane as the principal hydrocarbon under these conditions[10]. Ferrous ion accelerates the homolytic cleavage of hydroperoxides in an oxidation-reduction reaction yielding ferric ion, OH^-, and an alkoxy radical[11]. Utilizing ferrous ion, decomposition of our ω6 hydroperoxide occurred rapidly at a lower temperature than that required for thermolytic decomposition. To account for the observed formation of pentane from the ω6 hydroperoxide, the most likely mechanism is the classical homolytic cleavage of β scission. While pentane and ethane evolution in vivo may be specific for the peroxidation of the essential and non-essential series of fatty acids respectively, the quantitative relationships between the release of these hydrocarbons and peroxidation of the parent fatty acid remain obscure. If peroxidation of fatty acids in vivo follows the conventional autoxidation of alkenes, formation of ω3 and ω6 hydroperoxides will occur only as no more than $\frac{1}{3}$ or $\frac{1}{4}$ of the total fatty acid oxidized, assuming that trienoic and tetraenoic fatty acids are those most readily oxidized[12]. The availability of ferrous and other transition metals in biological systems would make the metal catalyzed decomposition likely. The ferrous ion decomposition of ω6 hydroperoxides to pentane is essentially quantitative.



Gas chromatograph of hydrocarbons evolved during the ferrous ion catalyzed decomposition of an ω6 hydroperoxide (methyl-13-hydroperoxy-6,9,11-octatrienoate). The proposed mechanism for the generation of the major product, pentane, is shown. R represents $(CH_2)_4COOCH_3$, and R'H is any suitable hydrogen donor.

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Streptostyly and muscle function in lizards

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Summary. Retraction of the quadrate during jaw closure in lizards increases the moment arm and thereby the mechanical advantage at which the jaw adductors work on the lower jaw.

Cranial kinesis in lizards has attracted the attention of many workers already, the classic contributions being those of Bradley[2] and Versluys[3]. More recently, Frazzetta[4] reviewed the problems involved and found that most Lacertilia of present times are characterized by amphikinesis. Not only is the braincase movably suspended within the dermatocranium, but also the upper jaw and with it the whole muzzle unit can be rotated upwards and downwards around the mesokinetic joint dorsally and the hypokinetic joint ventrally (figure). Upwards rotation of the muzzle unit involves a protraction of the basal unit (pterygoid); the lower end of the movably suspended (streptostylic) quadrate swings forwards. Depression of the upper jaw results from a retraction of the basal unit; the lower end of the quadrate swings backwards. Frazzetta[4] and Iordansky[5] discussed previous theories and put forward new ones concerning the adaptive significance of amphikinesis. These will be critically evaluated elsewhere.

During the transition from fossil amphibians (or rhipidistians) to reptiles, a reorientation of the jaw musculature took place leading to the change from a 'kinetic inertial system' to the 'static pressure system' of captorhinomorphs[6,7]. Jaw adductors no longer exerted maximal force on the lower jaw ramus with the jaws maximally depressed, as in rhipidistians or anthracosaurs, which resulted in a snapping bite. Instead, maximal force is exerted on the lower jaws near to closure, an important adaptation of the early reptiles to an insectivorous habit[7].

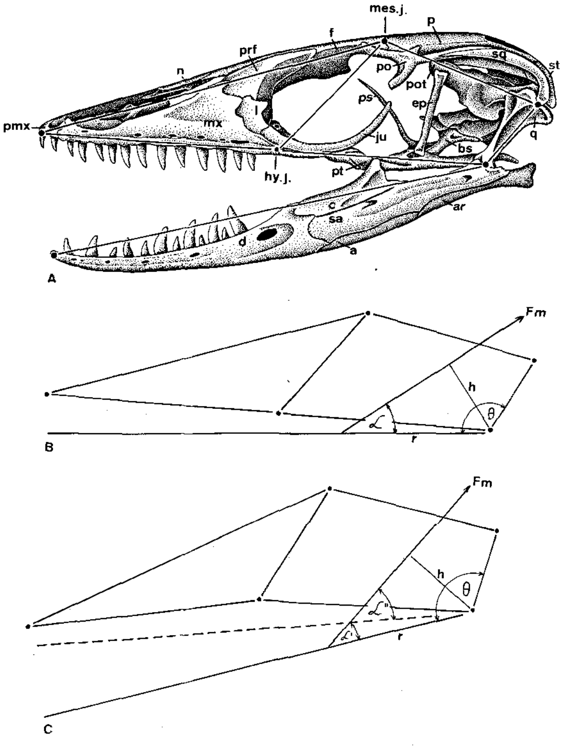
The first lizards evolving during the Triassic, the Eolacertilia, showed a streptostylic quadrate but no hinge-joint between the frontals and the parietals. They lacked mesokinesis[8].

During the Jurassic and Early Cretaceous, modern lacertilian infraorders became established. Amphikinesis appears as a characteristic feature of modern lizards[4].

Recent X-ray cinematography on a feeding *Varanus bengalensis* showed quadrate protraction and corresponding upwards rotation of the muzzle unit to occur during jaw opening. Assuming a constant angle θ between the lower jaw ramus and quadrate, protraction of the lower end of the quadrate will decrease the gape during mouth opening, as has been pointed out by Frazzetta[4] and Throckmorton[9]. Cranial kinesis therefore does not increase the gape.

Retraction of the lower end of the quadrate and corresponding depression of the muzzle unit occurs during jaw closure, as it has also been reported for *Gerrhonotus*[4] and *Uromastyx*[9]. It will be noted from the figure, C, that, assuming a constant angle θ between the lower jaw ramus and the quadrate, the latter's retraction will cause an increase in gape.

The most massive jaw adductor muscle in *Varanus* is the posterodorsally sloping m. adductor mandibulae externus, the greatest bulk of which originates from the parietal unit, e.g. from the upper temporal arch and from the parietal.



The application of the quadric crank model[4] to the skull of *Varanus salvator* to illustrate quadrate retraction during jaw closure. The main muscle force Fm is assumed to work along the direction indicated by the bodenaponeurosis and associated tendons. For further explanation see text. Abbreviations: a, angular; ar, articular; bs, basisphenoid; c, coronoid; d, dentary; ep, epipterygoid; f, frontal; h, moment arm; hy.j, hypokinetic joint; ju, jugal; l, lacrimal; mes.j, mesokinetic joint; mx, maxilla; n, nasal; p, parietal; pmx, premaxilla; po, postorbitofrontal; pot, prootic; prf, prefrontal; ps, parasphenoid; pt, pterygoid; q, quadrate; sq, squamosal; st, supratemporal; Fm, main muscle force exerted by the external adductor.