tract are a mixture of the original compound and two or more labeled metabolites. The number of metabolites formed from piperonyl butoxide is at least eight.

In homogenates of housefly abdomens, the methylene-C^{14}-dioxyphenyl compounds are converted to water-soluble products always to a greater extent when $NADPH_2$ is added to the incubation mixtures than when it is not added (Table 2). In the cases of sulfoxide B and piperonyl butoxide, the microsomal fraction from the fly abdomens is active, when $NADPH_2$ is added, converting these compounds to water-soluble products.

The metabolism of the methylene-C^{14}-dioxyphenyl compounds proceeds as outlined in Fig. 1. The methylenedioxyphenyl compounds are substrates for the microsomal enzyme-$NADPH_2$ system which also metabolizes many drugs and insecticides. By serving as alternative substrates (and, therefore, as competitive inhibitors) for this system (*17*), the methylenedioxyphenyl compounds prolong the persistence of the drug or insecticide so that a lower initial dose is effective. It is not known whether any of the intermediates combine chemically with components of the microsomes, such as the active sites of the enzymes. This mechanism of hydroxylation accounts, in part, for the action of methylenedioxyphenyl compounds as insecticide synergists and as inhibitors of drug detoxification; it does not directly account for the retardation of growth (*18*), carcinogenic activity (*19*), or the production of fatty livers (*20*) by some methylenedioxyphenyl compounds.

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Occurrence of Isoprenoid Fatty Acids in the Green River Shale

Abstract. *The most abundant free fatty acids present in a sample of shale from the Green River Formation (Eocene, about 60 × 10^6 years) from Sulfur Creek are the acylic C_{19} and C_{20} isoprenoid acids. The dominance of these acids parallels the abundance of the corresponding hydrocarbons pristane and phytane.*

Four isoprenoid acids, including norphytanic (2,6,10,14-tetramethylpentadecanoic acid, $C_{19}H_{38}O_2$ and phytanic (3,7,11,15-tetramethylhexadecanoic acid, $C_{20}H_{40}O_2$), have recently been isolated from a California petroleum (*1*). The naphthenic acid fractions of crude petroleums are highly complex mixtures (*2*) and like all petroleum constituents are difficult to relate to the source rocks and hence to their biological origins. Further progress in organic geochemistry ideally necessitates study of geological situations where the extractable organic compounds are indigenous to the facies. Under such circumstances a chemical correlation with biological matter originally incorporated in the sediment is feasible. The Green River Formation (*3*) is of this type. This oil shale has been extensively studied both geologically (*4*) and chemically (*5*) and is the accumulated organic-rich sediment derived from large inland lakes of Eocene age.

A sample (200 g) of Green River Shale from the 334-m depth of a core taken from Sulfur Creek (*6*) was pulverized (to pass 200 mesh), digested with 40-percent hydrofluoric acid/hydrochloric acid (1 : 1) at room temperature for 24 hours. The residue was filtered off, washed with water, and dried. The repulverized residue (90 g) was extracted ultrasonically three times with benzene/methanol (1 : 1). The total free acids were isolated from the extract (5 g) according to the method of McCarthy and Duthie (*7*) and converted to their methyl esters (methanol/hydrochloric acid). Column and preparative thin layer chromatography afforded a pure methyl ester fraction (12 mg). Figure 1 represents a gas chromatogram of these esters on SE-30; the peaks labeled phytanic and norphytanic acid esters were enhanced when this mixture was coinjected with the authentic isoprenoid acid esters (*8*). All the labeled peaks have been identified by combined gas chromatography–mass spectrometry.

Fractions trapped from 3-percent Versamid on Gas Chrom Q were reinjected into the gas chromatography-mass spectrometry apparatus, equipped with a 6-percent SE-30 column (2 m by 4 mm; temperature, 190°C; scan time 2 seconds). Peaks which coincided on the Versamid column, that is, phytanic and n-C_{17} and norphytanic and n-C_{16} acid methyl esters, were well separated on the SE-30 column. The n-C_{14}→n-C_{18}, phytanic (Fig. 2) and norphytanic (Fig. 2) acid esters were identified by comparing the mass spectra so obtained with those of authentic compounds (*8*). In addition, the infrared spectra (film and in CCl_4 solution) of the isolated and the authentic

isoprenoid acid esters were identical. The n-$C_{19} \rightarrow n$-C_{26} acid esters were identified without prefractionation by recording the mass spectra of peaks which appeared in a programmed gas chromatogram using the combined gas chromatography–mass spectrometry instrument. Of course, the mass spectrometric identification, though adequate for the recognition of the carbon skeletons of these isoprenoid acids, does not provide any information about the configuration at carbon atoms 3, 7, and 11. The stereochemistry of phytanic acid derived from natural phytol has been defined as 7(R) and 11(R), the geometry at carbon 3 being determined by catalytic reduction (*9*).

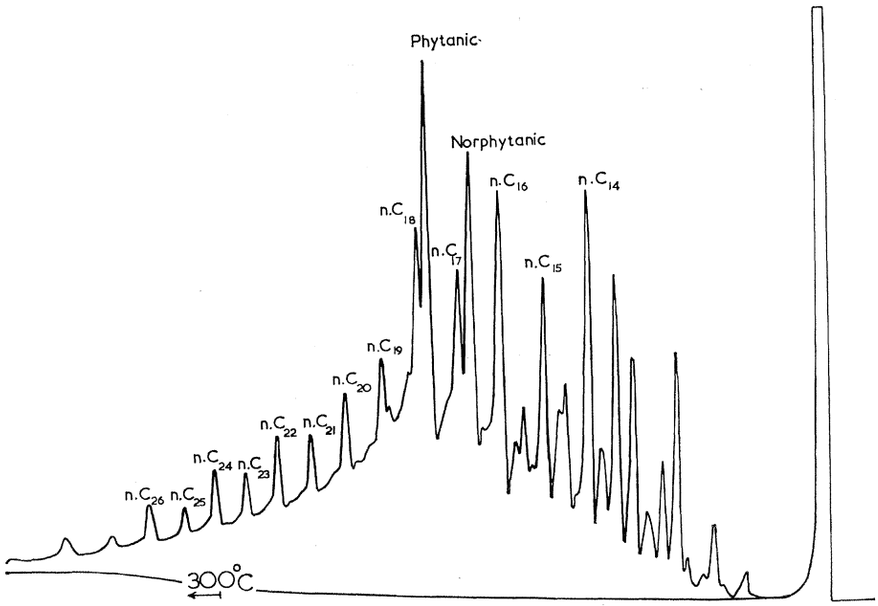

Fig. 1. Gas-liquid chromatogram of the free fatty acid fraction (as methyl esters) of a sample (*6*) from the Green River Shale. Conditions: 3 m by 3 mm column containing 1 percent SE-30 on Gas Chrom P, temperature programmed from 150° to 300°C at 8°/min. Flow rate, 20 ml/min. The base-line signal for injection of solvent alone is also shown.

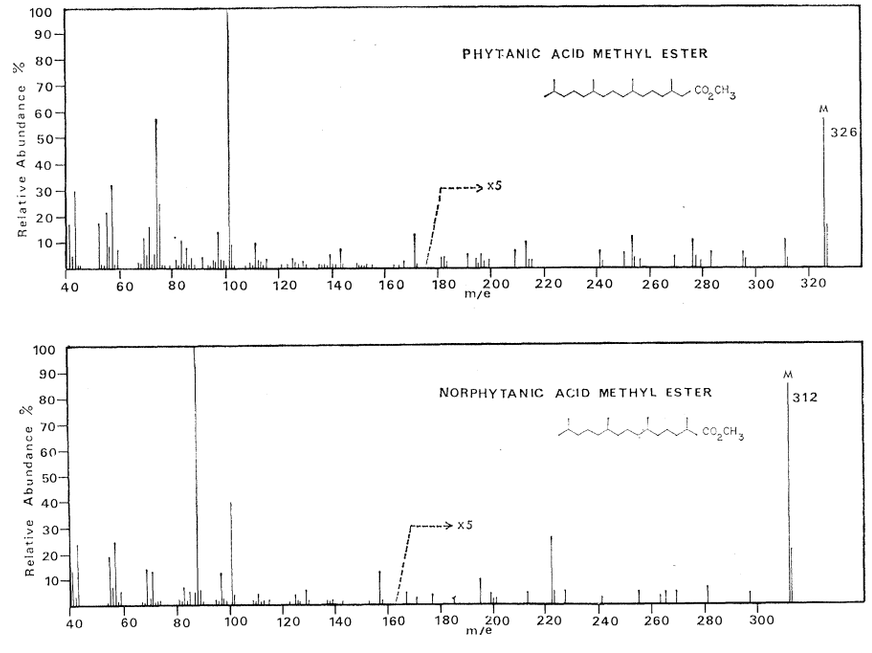

Fig. 2. Mass spectra of isoprenoid fatty acids (as methyl esters) isolated from the Green River Shale. Spectra obtained by combined gas chromatography–mass spectrometry (2 m by 4 mm column containing 6 percent SE-30 on Gas Chrom Q; temperature, 190°C., and scan time 2 seconds) on fractions trapped from a Versamid column (3 m by 6 mm column containing 3 percent Versamid on Gas Chrom Q).

There is a need to correlate the stereochemistry of the isoprenoid phytanic and norphytanic acids from geological (*1*) and biological sources (*10*), and laboratory synthesis (*11*). The same situation applies to the corresponding isoprenoid hydrocarbons (*12*). Stereochemical definition will afford a more rigorous correlation with presumed source materials. For some time it has been held that geological isoprenoids probably derive from the phytyl side chain of chlorophyll (see, *inter alia*, *5*, *12*), but Kates (*13*) has recently suggested that they may have arisen from the lipids of halophilic bacteria.

Although in the present study the n-alkanoic acids have not been separated from the branched and cylic acids, there is an obvious predominance of the even-numbered normal acids, as has been reported by Lawlor and Robinson (*14*) for the total fatty acids extracted from a sample from the Mahogany Zone of the same formation. These authors have pointed out the close similarity in the distribution of the n-alkanes and n-fatty acids and argue that this indicated a geogenetic relationship between the two groups of compounds, the acids giving rise to the hydrocarbons. Our results indicate a futher parallelism between the distribution of the isoprenoid acids and alkanes, though here the dominant C_{20} isoprenoid acid is paralleled by the dominance of the isoprenoid alkane with the same carbon number.

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Plasma Kinins and Cortisol: A Possible Explanation of the Anti-Inflammatory Action of Cortisol

Abstract. *Kinins are naturally occurring vasoactive polypeptides thought to be mediators of acute inflammatory responses. Kinins are released from a plasma protein substrate by glass-activated plasma enzymes (kallikreins) or by isolated intact granulocytes. Cortisol in concentrations of* 2.5×10^{-6} *to* 2.5×10^{-5}M *prevented the release of active kinin from substrate by granulocytes or contact with glass. Deoxycorticosterone, progesterone, and etiocholanolone in comparable concentrations were significantly less effective in preventing kinin release. Plasma obtained from patients receiving prednisone released no kinin after activation by glass and less kinin than control plasma when exposed to granulocytes. Cortisol also partially inhibited the release of kinin by purified urinary kallikrein. Certain adrenocorticosteroids may exert their anti-inflammatory effect by inhibiting the release of plasma kinins. Steroids may act in part by preventing interaction between the activated kallikrein and its substrate.*

Kinins are small naturally occurring polypeptides of known structure. Their pharmacologic activity is extraordinarily high; in concentrations of 0.1 to 1 mμg/ml the kinins cause vasodilatation, produce edema by increasing capillary permeability, stimulate some smooth muscle, provoke pain, and are leukotactic (*1*). Since these pharmacologic effects resemble the manifestations of acute inflammation, kinins are believed to be a hormonal mediator of the response to tissue injury (*2*). This concept is supported by considerable experimental evidence of two general types: kinin concentrations are increased at the sites of inflammation, and when a synthetic kinin such as bradykinin is applied to certain tissues it mimics an inflammatory response (*3*).

Kinins are released from a plasma protein substrate by either plasma enzymes (kallikreins) or by granulocytes. The agent activating the plasma enzyme(s) that liberate kinins from biologically inactive substrate (kininogen) appears to be blood clotting factor XII (Hageman factor) in its activated configuration. Thus, release of kinin and initiation of the clotting mechanism are closely linked processes. Hageman factor can be activated in vitro by contact of plasma with a glass surface. The release of free kinin from plasma substrates or from purified kininogen by exposure to granulocytes of rabbits and man was recently described in preliminary reports (*4, 5*). This phenomenon is dependent on the concentration of mature granulocytes (neutrophils, metamyelocytes, and myelocytes) and is not produced by normal or neoplastic lymphocytes.

The present studies were designed to examine the interaction between the kinin system and the anti-inflammatory adrenocorticosteroids, cortisol, and prednisone.

Leukocytes and plasma were obtained from normal subjects and patients with hematologic diseases or arteriosclerosis (control subjects), and from a group of patients with nonbacterial inflammatory diseases or with comparable hematologic diseases who were receiving 60 to 80 mg of prednisone per day (experimental subjects). The isolation of leukocytes and the in vitro incubation conditions have been described in detail (*6*); the only modification was the use of Eagle's minimal essential medium (*7*) containing human albumin (25 mg/ml) as the suspending medium.

Plasma kinins were prepared for assay by the method of Diniz and Carvalho (*8*) and were measured by bioassay on the estrus rat uterus (*9*) with synthetic bradykinin (Sandoz, Inc.) as the standard. Assays were usually performed in duplicate, and the results differed from the mean by no more than 7 percent. These peptides were characterized by their insensitivity to trypsin, their inactivation by chymotrypsin, and their hypotensive effect in the rat. Substrate (kininogen) concentration was determined by the difference between the total kinin released from a sample by trypsin digestion and the kinin concentration of the undigested sample. Since the known individual kinins, which are nona-, deca-, and unadecapeptides, have an approximately equal effect in the bioassay system, no effort was made to differentiate them, and their combined activity was expressed as bradykinin concentration.

Plasma substrates (kininogen) were prepared by a minor modification of the method of Webster and Pierce (*10*) and shown to be free of kinin-destroying (kininase) and kinin-generating activity. Various preparations of substrate contained 0.05 to 0.2 μg of bradykinin per milliliter as contaminant; this amount was not sufficient to interfere with the interpretation of the results. Urinary kallikrein was prepared by the method of Moriya, Pierce, and Webster (*11*) and had a specific activity of 5.7 Frey units per milligram.