sponding to the average reflectivity is 11 ± 4, a moderately high value for a dry planetary surface at radar frequencies. For other parts of Venus where the reflectivity approaches 0.4, K calculated by assuming negligible conductivity and loss rises to 20.

How can these relatively high reflectivities be explained? From Eq. 2 we see that both the real and imaginary parts of ϵ contribute to the reflectivity. It seems extremely unlikely that dielectric losses (L) of naturally occurring dry rocks could be large enough to dominate the reflection mechanism; they normally control only the attenuation with depth (*9*). For the conductivity, S, to be important in reflection, not only must it exceed about 0.1 mho/m at our radar's operating frequency, but the conductive region must extend over an area with dimensions comparable to a wavelength or larger. If such large areas of conductive surface exist, it would seem likely that, at least occasionally, reflectivities near unity would be encountered, since occurrence of the precise frequency-dependent threshold value of S necessary to yield reflectivities consistently between 0.3 and 0.4 seems highly fortuitous.

We are left, then, with the necessity for explaining how the real part, K, of the dielectric constant can be raised to values between 11 and 20. The most likely mechanism is the presence in the rock of conducting inclusions much smaller than the observing wavelength. Meteorites containing relatively large amounts of free iron-nickel mixtures and sulfides display values of K ranging up to 100 or more (*9*). Free metals seem unlikely and in any case could not exist for long in any part of the Venus surface exposed to the atmosphere; highly conducting metallic sulfides would also be unstable to atmospheric exposure (*11*). But if overturning of the first few centimeters of surface proceeds slowly enough, or if material is being steadily stripped off the surface and blown elsewhere, atmospherically unstable minerals could be maintained sufficiently near the surface to be effective in raising the dielectric constant.

Nozette and Lewis (*12*) suggested that chemical erosion takes place at high elevations on Venus, where winds are comparatively strong and atmospheric densities and temperatures relatively low. The fine-grained eroded material is subsequently delivered to lower elevations, where it is chemically modified and possibly compacted. In the process, the putative conducting inclusions are transformed into gases and nonconducting minerals. If the "original" Venus surface contained on the order of 15 percent free metal—or, more likely, pyrite (FeS_2), which has been postulated independent of radar observations to explain the observed atmospheric chemical composition (*11*)—the high values of reflectivity seen at high elevations could be readily explained. Pyrite is one of very few minerals with the necessary high conductivity, having values between 1 and 10^5 mho/m (*8*).

The model that emerges from this discussion requires that the surface of Venus in the vicinity of the highly reflecting regions contain a significant amount of conducting mineral as inclusions in the rock. From a consideration of the present lower atmospheric composition, this material is likely to be pyrite and may be widespread in the original crustal rock, lying in radar view only at higher elevations, where new surface is constantly being exposed. It is also possible that pyrite is preferentially produced in sulfur-rich volcanic material.

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Cigarette Smoke Contains Anticoagulants Against Fibrin Aggregation and Factor XIIIa in Plasma

Abstract. *Gas-phase, water-soluble components of cigarette smoke cause delayed fibrin self-assembly and prevent fibrin cross-linking by inactivation of factor XIIIa (plasma transglutaminase). These anticoagulant properties of smoke are demonstrable in plasma, suggesting they play a role in the pathophysiology of smoking.*

There is little information on the effects of cigarette smoke on important biochemical interactions, though considerable information on its physiologic effects is available. Studies on interactions of cigarette smoke with certain proteins have provided biochemical evidence that specific functions of such proteins are inhibited (*1, 2*) or augmented (*3*) by smoke components. In view of the shortened half-life of radioactively labeled fibrinogen in dogs exposed to cigarette smoke (*4*) and of the reported increase in fibrinogen in human smokers (*5*), we examined the possible effect of smoke on certain fibrinogen functions in vitro. We found that water-soluble smoke components include two types of anticoagulants: one is directed against fibrin self-assembly and the other inactivates factor XIIIa, thereby preventing cross-link formation (that is, stabilization) in fibrin clots.

Fibrinogen, a plasma glycoprotein, consists of three pairs of disulfide-bridged polypeptide chains termed Aα (molecular weight, ~ 70,000), Bβ (~ 60,000), and γ (~ 50,000). Cleavage by thrombin of arginyl-glycine peptide bonds at positions Aα16 and Bβ14 results in release of small polar peptides A and B from their respective chains: the resulting fibrin monomers polymerize noncovalently and form the fibrin gel.

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This gel (or clot) is stabilized by plasma factor XIIIa which catalyzes the formation of ε-(γ-glutamyl)lysine cross-links between adjacent fibrin molecules. Certain other proteins are also cross-linked in this way to fibrin, for example, fibronectin (*6*) and fast-reacting α_2-plasmin inhibitor (*7*).

In this work, the effect of cigarette smoke on fibrin aggregation was investigated by the use of water-soluble, gas-phase components of smoke, obtained (*8*) by bubbling the smoke produced from one cigarette through 3 ml of buffer or distilled water. This extract (SE) was incorporated in varying dilutions in the buffer to which fibrin monomer solution (in 0.25 percent acetic acid) was added in order to initiate fibrin aggregation, which was monitored turbidimetrically at 350 nm (*9*). A dose-dependent delay in fibrin aggregation was observed (Fig. 1A). Increasing the amount of smoke extract resulted in decreased absorbance of the clot (1:10 and 1:5 dilution) and (1:5 dilution) delayed the onset of fibrin aggregation. The decreased absorbance (indicating a less opaque or more transparent clot) remained undiminished for several hours. These effects were partly decreased in dialyzed smoke solutions and could not be corrected by incorporating calcium chloride (20 m*M*) in the buffer (not shown).

The fibrin aggregation inhibitor was also examined by use of two differing fibrin preparations with α chains lacking COOH-terminal segments. One fraction, termed I-6 and isolated from plasma, lacked approximately one-fifth of the native peptide from most of its α chains [that is, its α chains were of similar size (*10*)]. Another fraction, termed I-9D, was obtained from an 88 percent coagulable plasmic digest of fibrinogen and lacked longer segments, approximately two-thirds of the native peptide, from most of its α chains (*11*). Neither preparation displayed a delay in fibrin aggregation in the presence of smoke extract (Fig. 1, B and C). In addition, the clot absorbance of I-6 was decreased, suggesting that aggregation was partially inhibited or that this effect was distinct from that of the delay in fibrin aggregation (Fig. 1A). These results implied that the fibrin aggregation inhibitor required the presence of intact α chains to exert its effect. What is more, they suggested that the inhibitor interacted either directly with the α chains of fibrin or with another fibrin site which lost its capacity to interact with the inhibitor following conformational changes induced by the loss of intact α chains.

We also determined whether the smoke inhibitor was active in plasma. Smoke extract consistently prolonged the thrombin clotting times of plasma (Fig. 1D), but this effect could be abolished by decreasing the amount of extract added. In related analyses the clotting times of isolated fibrinogen fractions were examined in plasma containing the extract. To fresh plasma that had been depleted of its fibrinogen by heat precipitation (56°C) we added either fraction I-6 or band I fibrinogen and thrombin and determined clotting times. Consistent with the results on fibrin aggregation (Fig. 1A), the smoke extract prolonged clotting times in samples containing band I fibrinogen, and this defect was not corrected by the presence of calcium chloride (Fig. 1E). Samples containing fraction I-6 fibrinogen exhibited no

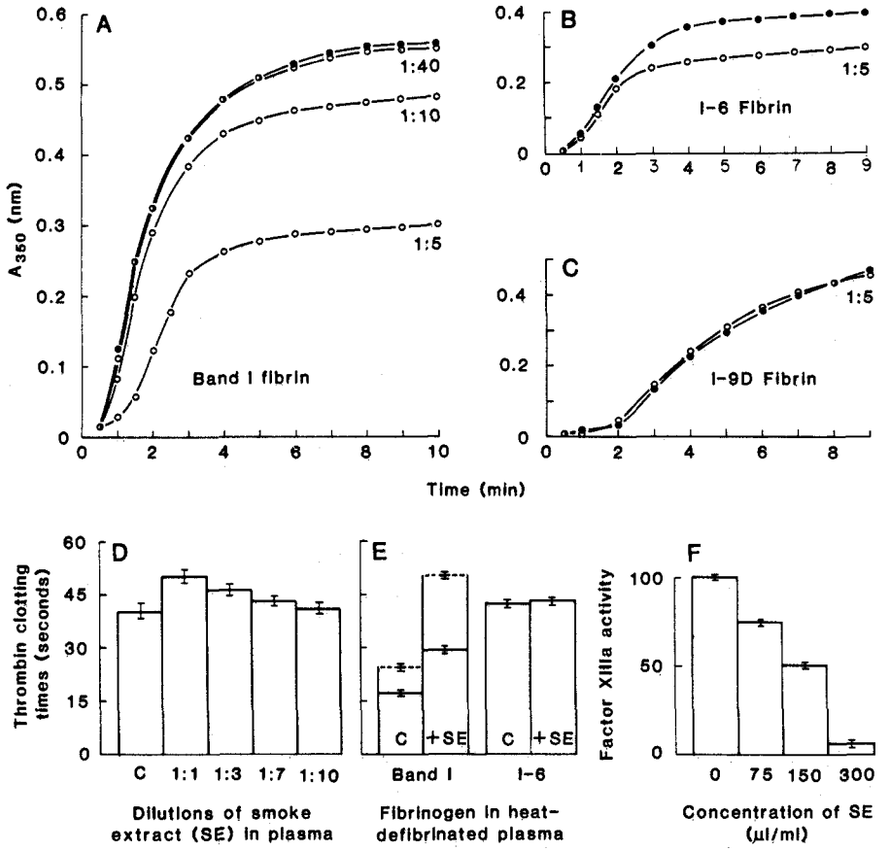

Fig. 1. Anticoagulant effects of smoke extract (*SE*) on fibrin and factor XIIIa. (A) Effect of different dilutions (open circles) on the time course of aggregation of (band I) fibrin which has intact α chains (*10*); closed circles indicate buffer control. After dilution of the fibrin solution with 20 volumes of buffer (0.025*M* tris-hydrochloride, *p*H 7.4, 0.135*M* NaCl) (*9*) containing SE, the final concentration of fibrin was 0.5 mg/ml. The *p*H remained constant during the experiments; in certain control experiments buffer containing SE alone or SE plus human albumin (0.5 mg/ml) in buffer was monitored for several hours and displayed no increase in absorbance (not shown). (B and C) Effect of SE on the aggregation of fibrin which lacks COOH-terminal segments from its α chains: fraction I-6 lacks approximately one-fifth (*10*) and fraction I-9D lacks approximately two-thirds (*11*) from their α chains. The final concentration was 0.4 mg/ml; the buffer was as in (A) and the SE dilution was 1:5. (D) The effects of SE on the clotting times of fresh human plasma (*9*). The height of each column reflects the mean and the brackets the range of at least three determinations in the presence of different dilutions of SE as shown; control plasma is designated *C*. Human thrombin (in 0.01*M* tris-hydrochloride, *p*H 7.4, and 0.15*M* sodium chloride) was added to citrated plasma (to 0.4 U/ml) and the clotting times were determined. (E) Histograms of clotting times for two differing fibrinogen preparations, showing that the inhibitory effect of SE on band I is not present when I-6 fibrinogen is clotted. The experiments were carried out in plasma that had previously been heat-treated (56°C, 10 minutes) to remove its own fibrinogen [which is heterogeneous (*10*)]. To this treated plasma, fibrinogen (either band I or I-6) was added (to 2 mg/ml) and clotting times were determined. Final thrombin concentration was 0.5 U/ml and SE dilution was 1:3 in all experiments (*C*, control). Columns with solid or interrupted lines indicate the presence and absence of calcium chloride (20 m*M*), respectively. (F) Effect of SE on the activity of isolated factor XIIIa, showing inhibition of [^{14}C]putrescine incorporation into casein. The conditions of the assay (*16*) as applied here were as follows: To 0.5 ml of 0.01*M* tris-acetate buffer, *p*H 7.4, containing calcium chloride (20 m*M*), dithiothreitol (5 m*M*), Hammerstein casein (1 percent), different concentrations of SE (as shown), and [^{14}C]putrescine (0.1 μCi/μmole), we added 5 μg of XIIIa. The mixture was incubated for 60 minutes at 37°C, and the reaction was stopped by adding 4 ml of 7 percent cold trichloroacetic acid. The precipitate was assayed by liquid scintillation (*16*).

change in their clotting times in the presence of the extract (Fig. 1E), again in agreement with the fibrin aggregation results. These findings indicated that smoke extract inhibits fibrin aggregation in the plasma environment. Moreover, the similarity of its dose-response whether plasma or isolated fibrin was used implied that competitive interaction between the smoke inhibitor and nonfibrinogen plasma proteins was minimal.

We then examined factor XIIIa–catalyzed fibrin cross-linking in the presence of the smoke extract. Fresh whole blood or plasma fibrin clots are cross-linked and therefore insoluble in 5*M* urea. When such clots were obtained in the presence of smoke extract (1:3 dilution) they were soluble in 5*M* urea (not shown). Similarly soluble were clots obtained from isolated fibrinogen and factor XIIIa in the presence of the extract. Cross-linking inhibition was also demonstrated by subjecting reduced fibrin samples to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (*12*). In this analysis cross-linking can be shown by the characteristic depletion of α and γ chains and the concurrent appearance of more cathodal electrophoretic bands, termed α-polymers and γ-γ dimer, respectively (Fig. 2, gel 1). The presence of smoke extract resulted in a concentration-dependent inhibition of α-polymer and γ-γ dimer formation (Fig. 2, gels 2 and 3). Moreover, higher concentrations of the extract were required to inhibit the [more rapid (*13*)] formation of γ-γ dimer than that of α-polymers.

The possibility that this inhibitor was directed against (or binding to) fibrin cross-linking sites was examined. Noncross-linked fibrin clots (*14*) exposed to smoke extract, followed by extensive washing and subsequent exposure to XIIIa, resulted in unimpaired fibrin cross-linking, indicating that the inhibitor did not bind to fibrin. In addition, dialysis removed the cross-linking inhibitor from the smoke extract (Fig. 2, gel 4), and its activity remained in freeze-dried samples.

Factor XIIIa–catalyzed incorporation of monodansyl cadaverine (fluorescence) (*15*) and of ^{14}C-labeled putrescine (*16*) into casein was used to assess the smoke inhibitor effect on XIIIa. These amines compete with the ε-amino group of peptide-bound lysine and prevent its cross-linking to peptide-bound γ-glutamine; thus measurement of casein-bound fluorescence or radioactivity serves to assay XIIIa by use of substrates other than fibrin. Both assays disclosed irreversible inhibition of XIIIa which depended on the concentration of smoke extract and required calcium chloride. Fig. 1F illustrates the results of the experiments with [^{14}C]putrescine. Direct inactivation of XIIIa was demonstrated after it was incubated with smoke extract. That is, the loss of XIIIa activity was proportional to the concentration of the smoke extract, and it could not be reversed by removal of the smoke inhibitor by dialysis or by gel filtration (G-25 Sephadex). By contrast, when the inactive zymogen (XIII) was subjected to the same treatment, it displayed no loss of activity following dialysis and activation to XIIIa.

Thus cigarette smoke contains two distinct coagulation inhibitors: one prolongs the clotting times of plasma by inducing delayed fibrin aggregation and requires the COOH-terminal region of fibrin α chains to exert its effect; the other inactivates XIIIa, preventing the cross-linking of fibrin polymers. By extension, this second smoke inhibitor may similarly affect other physiologically important proteins (*6*, *7*) also known to be cross-linked to fibrin by XIIIa.

We believe that these results permit speculation on potential pathophysiologic effects that may result from the exposure of lungs or other tissues to cigarette smoke. Diminished fibrin cross-linking could result in premature removal of fibrin, by proteolytic enzymes, thereby impairing the role of fibrin in initiating the provisional or temporary extracellular matrix during normal tissue repair. Besides protective fibrin-to-fibrin cross-links, other proteins [such as fibronectin (*6*) and α_2-plasmin inhibitor (*7*)] are cross-linked by XIIIa to fibrin, the latter conferring additional resistance to proteolysis (*7*). The presence of the smoke inhibitor is therefore likely to result in an initial wound matrix lacking the protective effect of such cross-links and consequently susceptible to early degradation. Blood complement abnormalities, suggesting ongoing low-grade inflammation in cigarette smokers (*17*), are consistent with this possibility, and the elevated fibrinogen levels (*5*) as well as the shortened half-life of radioactively labeled fibrogen (*4*) associated with smoking can be explained as a response to such an inflammatory process. What is more, that fibrin(ogen) degradation fragments induce a leukocyte-mediated increase in fibrinogen synthesis in cultured hepatocytes (*18*) is also consistent with this speculation.

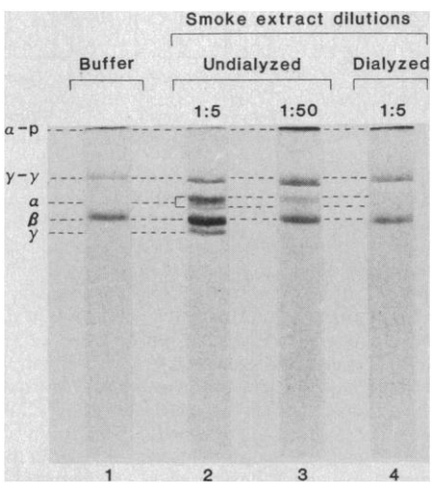

Fig. 2. Polyacrylamide gels (9 percent, Coomassie stained) of cross-linked fibrin clots (*9*) which had been washed and reduced prior to electrophoresis (*12*), showing non-cross-linked α-polymer (gels 2 and 3) and γ chains (gel 2) in clots obtained in the presence of smoke extract (SE). The absence of γ chains in gel 3 reflects the higher rate of γ-γ dimer (than α-polymer) formation (*13*) and indicates that the rate of XIIIa inactivation by the 1:50 SE dilution was lower than that of γ-γ dimer formation. The cross-linking mixture (*9*) contained dialyzed (gel 4) or undialyzed (gels 2 and 3) SE, isolated (band 1) fibrinogen, and factor XIII.

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Metabolism of 2,4',5-Trichlorobiphenyl by the Mercapturic Acid Pathway

Abstract. *Carbon-14–labeled 2,4',5-trichlorobiphenyl was found to be metabolized by the mercapturic acid pathway to metabolites that are excreted in bile. About 57 percent of the carbon-14 was excreted in the bile; 30 to 35 percent was present as mercapturic acid pathway metabolites. Mercapturic acid was also isolated from the urine (0.3 percent of the dose).*

Although polychlorinated biphenyls (PCB's) have not been shown to be metabolized by the mercapturic acid pathway (MAP), there is evidence that the MAP may be involved. Biphenyl and 2,2',5,5'-tetrachlorobiphenyl are metabolized to dihydrodiols (*1*, *2*), and the NIH shift occurs in the metabolism of 4-chloro- and 4,4'-dichlorobiphenyl (*3*, *4*). Both of these metabolic routes usually indicate that an arene oxide precursor was formed, and compounds that form arene oxides are often metabolized in part by conjugation with glutathione, that is, by the MAP. Also, biphenyl is known to be metabolized by the MAP (*5*).

The most common indication that a xenobiotic was metabolized by the MAP is the isolation of the appropriate mercapturic acid from the excreta; however, this may also be indicated by formation of metabolites that contain metabolically introduced thiol, *S*-glucuronyl, methylthio, methylsulfinyl, or methylsulfonyl groups (*6*–*8*). Several chlorinated biphenyls were found to be excreted by mice as metabolites containing methylthio and methylsulfonyl groups (*9*), and chlorinated biphenyl methyl sulfones were also isolated from various tissues (*10*–*12*) and from milk from a lactating female (*13*). The radioactivity from intraperitoneally administered [^{35}S]cysteine was incorporated into 2,4',5-trichlorobiphenyl (triCB) methyl sulfones that accumulated in the lungs of mice given oral doses of triCB (*14*).

The evidence cited above indicated that some chlorinated biphenyls are metabolized by the MAP and that the common products of this pathway (the corresponding mercapturic acid and its precursors) are metabolized further before excretion. The mechanism was thought to be similar to that described for pentachlorothioanisole, where the biliary MAP metabolites were excreted mainly in the feces as bis-(methylthio)tetrachlorobenzene and nonextractable residues (*15*) and about 1 percent of the dose was present in the urine as *N*-acetyl-*S*-(methylthiotetrachlorophenyl)cysteine. The excretion of triCB methyl sulfide and methyl sulfone in feces from mice given triCB (*9*) prompted a search for MAP metabolites in bile from rats given ^{14}C-labeled triCB. In addition, triCB is a significant component of technical PCB containing 42 to 48 percent chlorine.

Bile collected from four bile duct–cannulated rats given single oral doses of ^{14}C-labeled triCB (*16*) (4 mg, 2.94 μCi per rat) contained 52.7 ± 19.2 percent of the dose after 48 hours, and 84 to 90 percent of the radioactivity was extracted from the bile (*17*). The radioactivity in the extract was separated into six fractions by reversed-phase high-performance liquid chromatography (HPLC) (*18*). The fractions were examined for possible MAP metabolites by converting the xenobiotic moieties to the corresponding triCB-*S*-acetates (*19*). Fractions 4 and 5, which contained 4.5 and 33.5 percent of the biliary ^{14}C, respectively, yielded significant quantities of triCB-*S*-acetates. Small quantities were obtained from fractions 1, 2, and 3. Two isomeric triCB-*S*-acetates were separated by gas chromatography (*20*) and found to have retention times and mass spectra identical with those of authentic triCB-3-*S*-acetate and triCB-4-*S*-acetate (*21*). After derivatization (*22*) of fraction 4, the derivatized triCB-*S*-cysteinylglycine and -cysteine conjugates were isolated by HPLC. After derivatization of fraction 5, the methyl ester of triCB-*S*-(*N*-acetyl)cysteine was isolated by HPLC. From the mass spectral data (*23*), structures were assigned to these derivatives and to the underivatized mercapturic acid as outlined in Fig. 1 (*21*).

About 30 to 35 percent of the radioactivity in the bile was present as MAP metabolites, showing that the MAP is a major metabolic pathway for this chlorinated biphenyl and that significant quantities of the metabolites are available for further metabolism by intestinal enzyme systems.

The fate of biliary triCB MAP metabolites in the intestine could not be deduced from the identities of the metabolites reported previously (*24*); therefore, the metabolic fate of ^{14}C-labeled triCB in

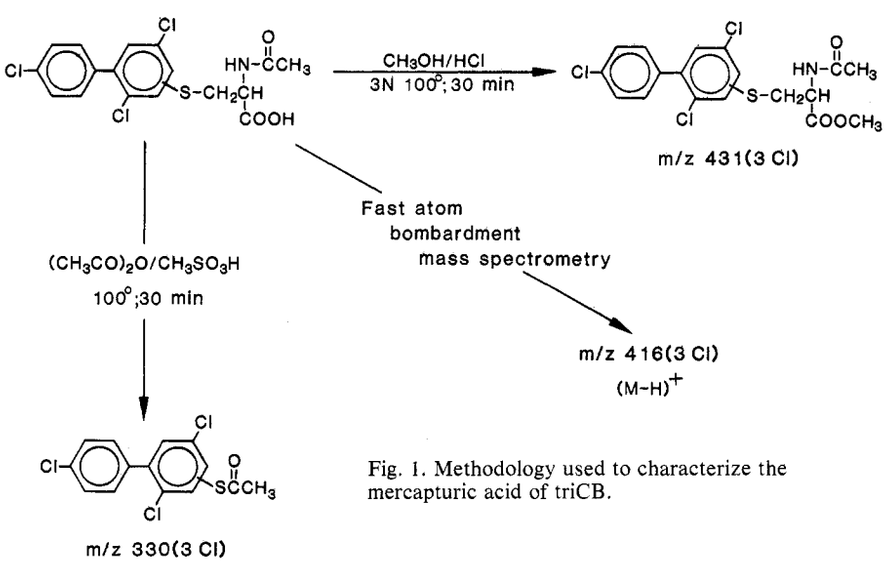

Fig. 1. Methodology used to characterize the mercapturic acid of triCB.

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