constant yearly emissions from then on. It is likely that we are seeing this change of emission pattern in the time series of R for CCl_3F and CCl_2F_2.

For CH_3CCl_3, there is no such "leveling off" in the emissions, although in the past several years the rate of increase in emissions has declined considerably. The emissions patterns over the last 16 years have shown fluctuations that may translate into fluctuations of R because of the delays involved in the transport of CH_3CCl_3 from high northern latitudes to the far Southern Hemisphere (*7*). Because of the common standards maintained from 1976, the high precision of the data, and the use of ratios of concentrations, it is possible that the effects of changing emissions patterns on R have been observed in the antarctic and PNW data (Fig. 1). Further details of the data analysis are given in (*7*).

Our antarctic program has provided an ideal location for the measurement of trace gases in the Southern Hemisphere. The site is far removed from major industrial activity, and the availability of scientific support facilities makes it unique in the Southern Hemisphere. The data obtained so far have shown remarkable internal consistency and have been used in several theoretical studies reported in the environmental literature.

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Cross-Linked Fibrinogen Dimers Demonstrate a Feature of the Molecular Packing in Fibrin Fibers

Abstract. *A stable population of fibrinogen dimers cross-linked by Factor XIIIa has been prepared and examined in the electron microscope. The trinodular fibrinogen molecules are cross-linked through their outer nodules in an end-to-end, nonoverlapping fashion. These dimers form normal banded fibers after treatment with the clotting enzyme, thrombin.*

The trinodular structure of fibrinogen (*1*) has recently been confirmed by two different electron microscopic techniques (*2*). This 340,000-dalton protein molecule has an overall length of 45 nm and consists of two identical outer nodules about 6 nm in diameter linked to a central nodule 4 to 5 nm in diameter. During blood coagulation these molecules are proteolytically activated by thrombin to form fibrin monomers, which spontaneously polymerize to form fibrin fibers. Subsequently, the molecules within these fibers may be cross-linked intermolecularly (*3*) at specific sites by a plasma transglutaminase, Factor XIIIa. Fibrinogen molecules in solution can also be cross-linked by Factor XIIIa, presumably at the same sites. The positions of these cross-link sites within the amino acid sequence of fibrinogen are known and, in models relating the sequence to the trinodular structure, one of these sites (the γ-chain cross-link site) is placed in the outer nodules. Although the fibrin polymerization scheme in Fig. 1 is generally accepted, the precise location of the intermolecular contact sites and the orientation of the molecules has not been determined. We report here the isolation and electron microscopy of fibrinogen dimers cross-linked through the γ-chain sites by Factor XIIIa. This cross-link site is located in the outer nodules, and the structural arrangement of the two cross-linked fibrinogen molecules is that indicated in Fig. 1. This is the most direct evidence for the location of an intermolecular contact site on the trinodular molecule and the molecular packing within the fibrin fiber.

Fibrinogen contains two copies each of three different polypeptide chains designated Aα, Bβ, and γ, with all six chains covalently linked by disulfide bonds. Although Factor XIIIa is capable of intermolecularly cross-linking two γ chains and two or more Aα chains, sodium dodecyl sulfate–gel electrophoretic analysis shows that under our conditions only the γ chains are cross-linked. The γ-chain cross-link site consists of two closely spaced reciprocal groups, one donor and one acceptor, located near the carboxyl terminus of the chain. Since there are two γ chains in each fibrinogen molecule, incubation of a mixture of fibrinogen and Factor XIIIa can yield oligomers, as well as dimers, of fibrinogen (*4*). We found that the formation of oligomers is inhibited by including an excess of monodansyl cadaverine or glycine methyl ester in the mixture. These compounds are covalently linked by Factor XIIIa to the cross-link acceptor sites (*5*), blocking these sites and thereby inhibiting the cross-linking of fibrinogen. Whereas in the absence of inhibitor, dimers may continue to participate in the cross-linking reaction and grow to trimers and larger oligomers, in the presence of the inhibitor, the terminal cross-link acceptor sites (that is, those not participating in the formation of a dimer) may be blocked, thus making the dimer species-stable. Using this approach, we have succeeded in preparing stable dimers, which were separated from single fibrinogen molecules by gel filtration (Fig. 2).

We have studied the structure of the fibrinogen dimers by electron microscopy of negatively stained specimens prepared from the peak fraction of purified dimers. Images of representative molecules are shown in Fig. 3, a and b. The dimers are highly elongated with an average length of 89 ± 3 nm (*6*), twice that of the individual fibrinogen molecule (*2*). Such end-to-end dimers are virtually

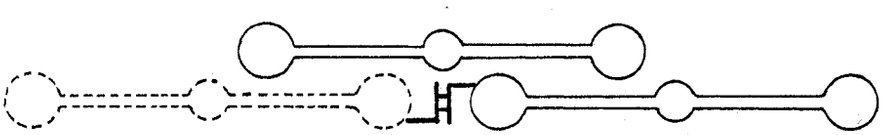

Fig. 1. In the accepted scheme for the polymerization of fibrin monomers, the first step is the lateral association of two monomers with an overlap of one half-molecule (solid lines). The next molecule adds in the position indicated by the dotted lines, forming the same type of lateral contacts and in addition a longitudinal contact. The position of the γ-chain cross-link, normally added after polymerization, is located between longitudinally oriented molecules, as indicated.

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never seen in negatively stained fibrinogen preparations. The somewhat flexible fibrinogen molecules in the dimer always appear to be joined at the distal tips of the outer nodules and are most often seen oriented in a linear fashion, which we believe is the native configuration. The bending of some dimers, which is quite variable, has also been noted in similar preparations of single fibrinogen molecules (*2*) and may be due to forces in the attachment of molecules to the carbon film and to drying of the stain. Occasionally dimers appear to be composed of two straight molecules joined at a sharp angle (for example, see lower right corner of Fig. 3b). We suggest that these dimers may be cross-linked at only one of the two reciprocal groups of each γ chain, an arrangement that could be much less rigid than the double linkage.

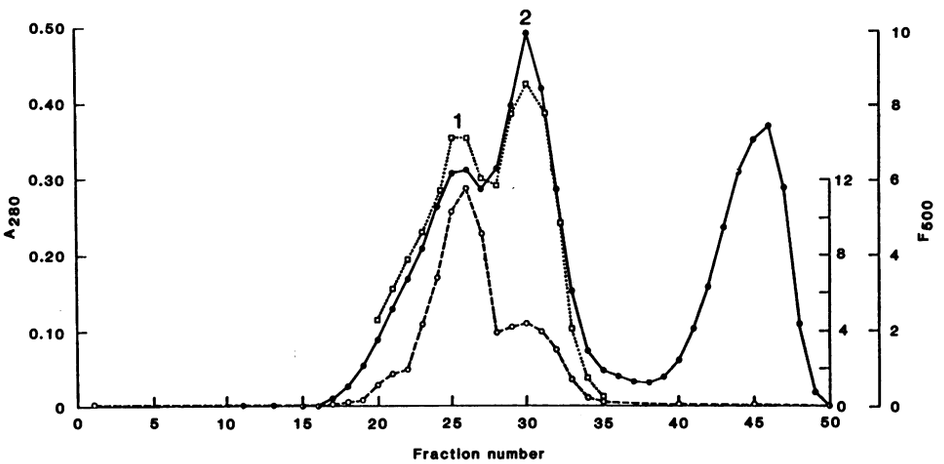

Fig. 2. Preparation of cross-linked fibrinogen dimers. Purified human Factor XIII (four times the concentration in normal plasma) was activated with thrombin ($6.2 \times 10^{-7}M$) for 30 minutes at room temperature in 0.5*M* NaCl, 0.05*M* tris, and 0.005*M* $CaCl_2$, *p*H 7.4; the thrombin was then irreversibly inhibited with a 160-fold molar excess of Val-Ile-Pro-Arg-CH_2Cl (Val, valine; Ile, isoleucine; Pro, proline, Arg, arginine) [supplied by C. Kettner and E. Shaw (*8*), Brookhaven National Laboratories]. This Factor XIIIa preparation was used to cross-link fibrinogen without removal of the fibrinopeptides. The cross-linking reaction was carried out for 180 minutes at room temperature in 0.5*M* NaCl, 0.05*M* tris, and 0.005*M* $CaCl_2$, *p*H 7.4, at a Factor XIIIa concentration 1.9 times that of normal plasma, $4.8 \times 10^{-6}M$ fibrinogen (Kabi, grade L), and $1.5 \times 10^{-3}M$ dansyl cadaverine. The reaction was terminated by lowering the *p*H to 6.6 and inhibiting the Factor XIIIa by alkylation with $10^{-3}M$ *N*-ethylmaleimide. The reaction mixture was concentrated fivefold in a Millipore immersible CX concentrator, centrifuged to remove small amounts of gel, applied to a Sepharose 4B column (1.6 by 83 cm), and eluted at 23 ml per hour with 0.1*M* NaCl and 0.05*M* tris, *p*H 7.4. Fractions (3.9 ml) were collected and the elution profile was determined by measuring the absorbance at 280 nm (closed circles, left ordinate) and the fluorescence at 500 nm (365-nm excitation; open squares, right outer ordinate). The peak tube of the dimeric fibrinogen fraction (1) was again chromatographed (same elution conditions), and the elution profile was determined by measuring the fluorescence at 500 nm on a scale 30 times more sensitive (open circles, right inner ordinate). Samples of the peak fraction of the chromatographed fibrinogen dimers were used for electron microscopy.

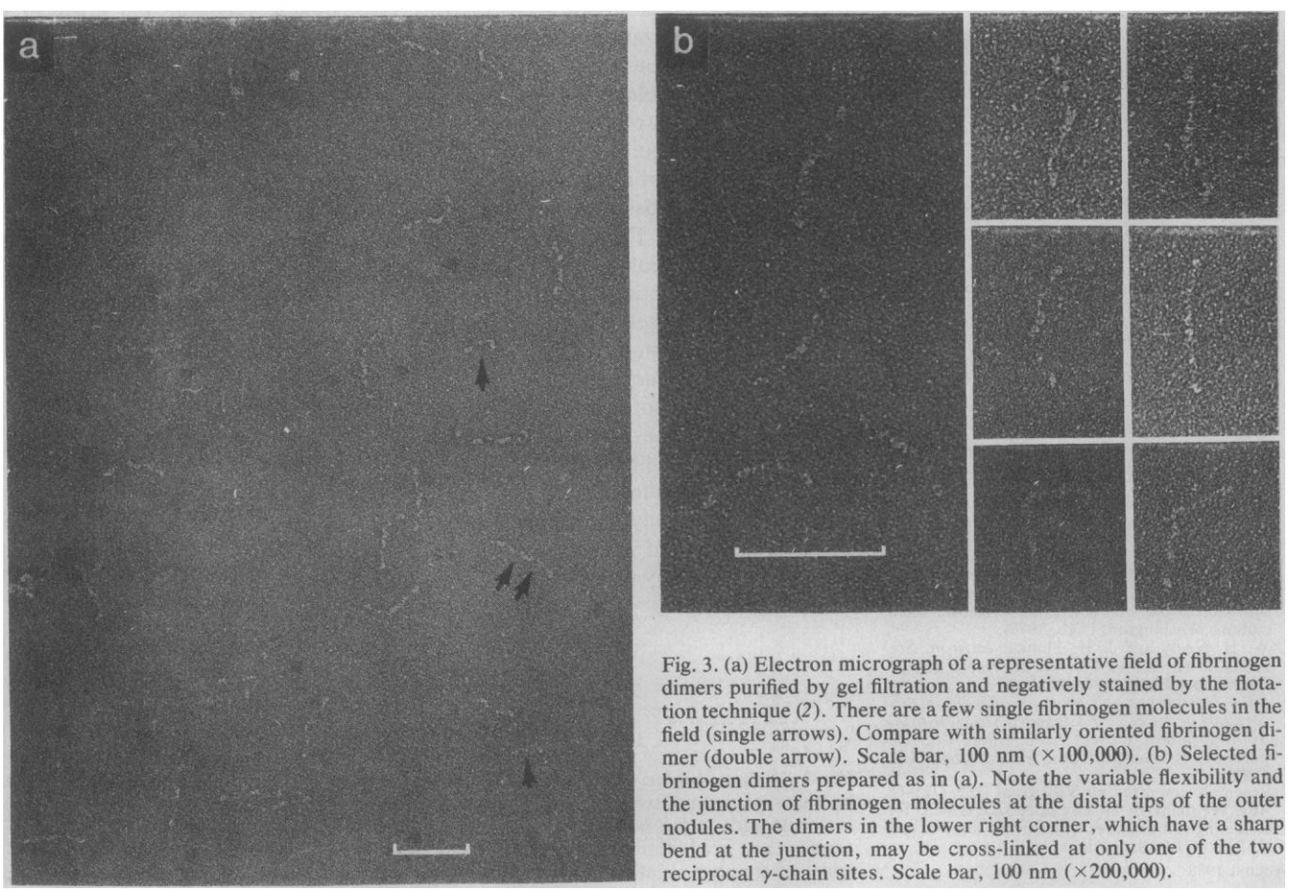

Fig. 3. (a) Electron micrograph of a representative field of fibrinogen dimers purified by gel filtration and negatively stained by the flotation technique (*2*). There are a few single fibrinogen molecules in the field (single arrows). Compare with similarly oriented fibrinogen dimer (double arrow). Scale bar, 100 nm (×100,000). (b) Selected fibrinogen dimers prepared as in (a). Note the variable flexibility and the junction of fibrinogen molecules at the distal tips of the outer nodules. The dimers in the lower right corner, which have a sharp bend at the junction, may be cross-linked at only one of the two reciprocal γ-chain sites. Scale bar, 100 nm (×200,000).

These observations confirm that the γ-chain cross-link site is in the outer nodules of the trinodular fibrinogen molecule and that the mode of cross-linking is that diagrammed in Fig. 1. They also confirm that the outer nodule contains Fragment D, a large, globular proteolytic fragment of fibrinogen, since this fragment is known to include the γ-chain cross-link site (*7*). Finally, the images show that the molecules in the dimer are joined at the distal tips of the outer nodules and that there is little, if any, intrinsic bend at this junction.

In our preparation, the fibrinogen molecules are cross-linked in solution, in contrast to the cross-linking in situ of fibrin monomers in the fibrin fiber. It is therefore important to demonstrate that these fibrinogen dimers are cross-linked in the same arrangement as the molecules in the fibrin fiber. We find that after treatment with thrombin the fibrinogen dimers polymerize to form banded fibrin fibers indistinguishable from those formed from thrombin-treated fibrinogen molecules. We therefore conclude that the arrangement of the molecules in the fibrinogen dimers is the same as in the fibrin fiber.

Since the fibrin fiber is an extended three-dimensional polymer, there must be lateral contacts, probably of the staggered overlap type shown in Fig. 1, in addition to the end-to-end contact we have shown here. These lateral contacts are probably the ones activated by thrombin and may play the most important role in the initial stages of polymerization. The important conclusions of our work are that the end-to-end contact is the site of the γ-chain cross-link and that the linear arrangement of molecules connected through these contacts must be a feature of the molecular packing in fibrin fibers.

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Deoxyribonucleic Acid Structure: A New Model

Abstract. *Models of deoxyribonucleic acid (DNA) having chain directions opposite to those of the Watson and Crick model offer strikingly different alternatives for DNA structures. Satisfactory models of the B and C forms of DNA have been built. Left-handed models readily form by twisting right-handed ones, and models can be bent into tight supercoils.*

There are two specific features of the widely accepted, generalized Watson and Crick model for DNA structures (*1*, *2*) which have not been well characterized. These are (i) the direction of each deoxyribophosphate chain and (ii) the handedness of the several double-helical forms of DNA. Consider two right-handed, intertwining helical chains asymmetrically disposed about a common helical axis as shown in Fig. 1. If each arrow represents the 5′ → 3′ direction in the deoxyribofuranose (sugar) residues in a DNA chain, two possible antiparallel chain configurations can be defined. As viewed with the helical axis held vertically, the chain on the left side of the minor groove can have the direction 5′ → 3′ either upward (configuration I, Fig. 1a) or downward (configuration II, Fig. 1b), while the chain on the right has the opposite direction. Although there is no mention of the justification, Watson and Crick used chain configuration I [see figure 7 in (*2*)]. In this initial report it is proposed that the family of model structures based on configuration II, which includes both right- and left-handed double helices, is more appropriate for representing DNA, in general.

Twenty-seven years ago, the structure of the high humidity B form of DNA (B-DNA) was elucidated (*1*, *2*) as a sodium salt. Some modifications to this right-handed, double-helical model have been published (*3*, *4*), but its more salient features have remained unchanged. X-ray diffraction analyses of crystalline or semicrystalline fibers of natural DNA offer evidence of other forms such as the A form (A-DNA) at lower humidity (*5*, *6*) for which a right-handed structure has been proposed (*7*), and the C form of the lithium salt (C-DNA) at still lower humidity, which is also postulated to be a right-handed double helix (*8*).

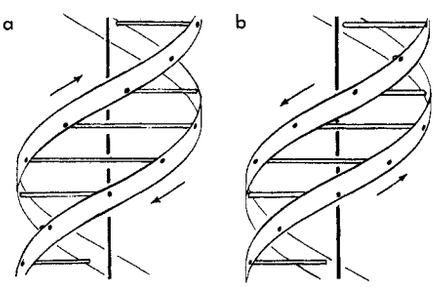

Fig. 1. (a) Configuration I and (b) configuration II of intertwining double-helical chains asymmetrically disposed about their common helical axis. The arrows represent the 5′ → 3′ deoxyribofuranose directions in DNA models.

These x-ray diffraction studies provide firm evidence for the double-stranded helical character of the original model, with the deoxyribophosphate chains on the outside of the helix. Other compelling features of the Watson-Crick model have been supported subsequently as follows. (i) The complementary purine and pyrimidine bases on the two strands being held in pairs by the specific hydrogen bonding scheme originally proposed is consistent with more recent x-ray studies (*9*) and (ii) the two chains being antiparallel has been confirmed by research on DNA replication (*10*).

Despite its major explanatory strengths, however, the original Watson-Crick B-DNA model was found to be consistent with neither density measurements nor x-ray intensity data (*5*), both of which strongly favor a more compact helical structure. In reaffirming these inconsistencies, x-ray data were interpreted using shortened bond lengths resulting from a postulated ionic compression (*3*). Other models (*4*, *7*, *8*) have required dihedral angles of 8° to 16° between the planes of the paired, hydrogen-bonded nucleotide bases. Although plausible, there has been a vague, lingering skepticism about the details of such DNA structures (*11*). Still other attempts

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