Resonance Force Theory of Carotenoid Pigments[1]

BY WILLIAM T. SIMPSON

A theory of the electronic spectra of carotenoid pigments is presented in which the double bonds are treated as quasi-independent weakly interacting systems. The excited states are described as derived from an n-fold degenerate manifold, where n is the number of double bonds. The states split under a perturbation patterned after simple electrostatic resonance interaction. A number of experimentally observed phenomena are considered including the positions of energy levels, the ground state steric configuration, isomerization processes, intensities and intensity changes.

Introduction

In a previous paper[2] a model for polyenes was described in which exchange forces between the double bonds were omitted, only the electrostatic interaction being considered. It was shown that using such a model, formally at least, one could correctly describe the properties of short chain polyenes; and it was also shown that the limiting wave length of absorption for long chain polyenes appeared quite naturally. In the present paper the object is to go into detail for the long chain polyenes, making comparison with the carotenoid pigments and related compounds.

Briefly, what is found is that the present approach seems to be not merely a satisfactory alternative to the conventional treatments, but actually to offer a number of advantages. What we are concerned with is the discovery of a system of zero-order wave functions which in a perturbation calculation will give directly a set of electronic energy levels which match observation. Of course any approach in which a complete set of functions is used will ultimately prove to be satisfactory, so that the measure of advantage is essentially the rapidity of convergence, or the directness.

Resonance Force Model.—Before making the comparison with experiment, we shall develop the theory.[3] Given a system of weakly interacting individual parts, we can quite simply obtain accurate wave functions for the combined system. To do this we write products of wave functions referring to the individual parts, and take linear combinations of them as determined by the application of perturbation theory. The individual systems here are taken to be the double bonds, each electrically neutral, and to a first approximation for dipole–dipole interaction, one product function interacts with another only if adjacent double bonds are making an allowed transition. For example, consider the case of four bonds, as in all *trans*, all s-*trans* or all s-*cis*[4] octatetraene

s-*trans*   s-*cis*
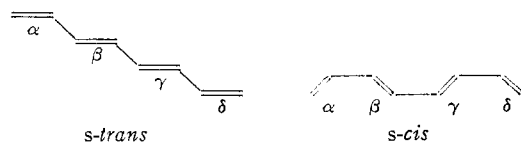
(1) This research was supported in part by the Air Research and Development Command, Contract No. AF 18(600)-375.

(2) W. T. Simpson, THIS JOURNAL, **73**, 5363 (1951).

(3) This will be largely a repetition of material in ref. 2 but with a different emphasis.

(4) s-*trans* or s-*cis* means about single bonds. For a discussion of this point see R. S. Mulliken, *Rev. Mod. Phys.*, **14**, 265 (1942).

Let the bonds be designated α, β, γ and δ as shown. Then if the numbered superscripts are quantum numbers for electronic excitation in the individual bonds considered as independent systems, a list of product functions ordered according to unperturbed energy would begin as

$$\psi_1 = \psi_\alpha^0\psi_\beta^0\psi_\gamma^0\psi_\delta^0$$
$$\psi_2 = \psi_\alpha^1\psi_\beta^0\psi_\gamma^0\psi_\delta^0$$
$$\psi_3 = \psi_\alpha^0\psi_\beta^1\psi_\gamma^0\psi_\delta^0$$
$$\psi_4 = \psi_\alpha^0\psi_\beta^0\psi_\gamma^1\psi_\delta^0$$
$$\psi_5 = \psi_\alpha^0\psi_\beta^0\psi_\gamma^0\psi_\delta^1$$
$$\psi_6 = \psi_\alpha^1\psi_\beta^1\psi_\gamma^0\psi_\delta^0$$

Illustrating the rule for interaction, if the 0 to 1 transition is the first allowed transition, ψ_1 interacts with ψ_6, ψ_2 with ψ_3, ψ_3 with ψ_4 and ψ_4 with ψ_5. The functions ψ_2, ψ_3, ψ_4 and ψ_5 have the same energy and mutually interact, so that perturbation theory for a degenerate level is required, and the energy corrections are of the first order. The name for the first-order forces is Resonance Forces. The ground state function does not interact with itself, so that there is no resonance force correction. The second-order correction involves mixing of ψ_1 with functions of the type of ψ_6 and the second-order forces are called Dispersion Forces. According to the resonance force approximation there is a spectrum in the long wave length region which consists of four transitions, as
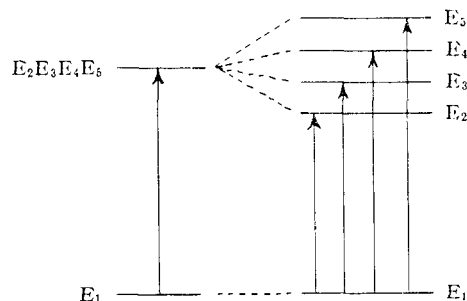
The splitting of the degenerate upper state centers about the unperturbed energy which, if the ground state energy is taken as the zero of energy, is the same as the first allowed transition energy for just one double bond. With n double bonds the degeneracy of the upper state is equal to n, and there will be $n/2$ transitions on the long wave length side of the center point, (n even) or $(n-1)/2$ transitions (n odd). Thus with five double bonds the pattern is similar to the one above except that there is an extra level on the right located exactly in the center of the system of excited levels.
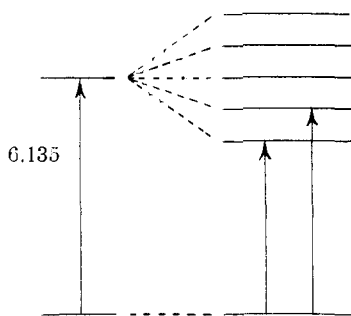
The isolated double bond transition energy is taken as 6.135 (in this paper one unit is 10^4 cm.$^{-1}$) corresponding to the N, V transition for ethylene at 1630 Å. The two transitions predicted to occur at longer wave lengths than 1630 Å. correspond to the arrows on the right side of the diagram.

The secular equation which gives the splitting of the n-fold degenerate upper state is the well known

$$\begin{vmatrix} -\lambda & 1 & 0 & 0 \\ 1 & -\lambda & 1 & 0 \\ 0 & 1 & -\lambda & 1 \\ & & & \ddots \end{vmatrix} = 0$$

with roots given by[5]

$$\lambda = 2 \cos \pi k/(n+1) \qquad k = 1, 2, \ldots, n$$

The units are the interaction integral, say

$$H' = \int (\psi_\alpha{}^1 \psi_\beta{}^0 \psi_\gamma{}^0 \ldots)^* \, H_{int} \psi_\alpha{}^0 \psi_\beta{}^1 \psi_\gamma{}^0 \ldots \, d\tau$$

and n is as previously defined. The present concern is mainly with long chain polyenes of which the carotenoid pigments are the best known examples. It is of interest, therefore, to work out the spectra for $n = 9$, 11 and 13 using the trigonometric formula. Whatever the value of n is, all the excited levels lie between $2H'$ and $-2H'$. For $n = 11$ the pattern of levels (lower half of pattern shown) is

6.135 + 0.000H'
+ 0.510H'
+ 1.000H'
+ 1.414H'
+ 1.732H'
+ 1.932H'

6.135

0
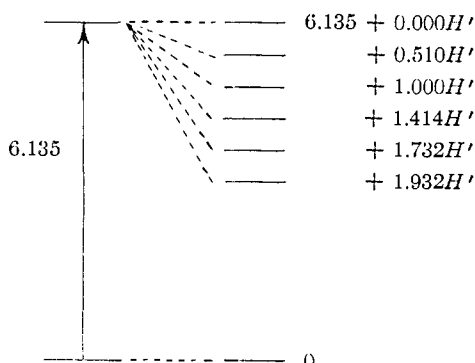
With regard to the value of H', we could choose it so that $6.135 + 1.932H'$ corresponds to the observed first transition energy for a compound with $n = 11$ (say, lycopene, with $\lambda_{max.}$ at ca. 4700 Å.). Or we could estimate a value from considerations in ref. 2. (This gives a calculated $\lambda_{max.}$ in the range 3000–5000 Å. depending on whether the molecule is assumed to be s-*trans* or s-*cis*.) Moreover we could reasonably use for the base value a

(5) C. A. Coulson, *Proc. Roy. Soc. (London)*, **A164**, 393 (1938).

transition energy somewhat smaller than 6.135 since the isolated double bonds are bound in an environment which includes methyl groups, etc. It does not matter what plan is followed because the agreement is in any event not perfect and would not be expected to be. It is the comparison of the actual number of transitions predicted and observed which is the real test of the model. We shall actually employ a best fit value $H' = -2.050$, which gives for the first transition energy, for $n = 11$

$$6.135 - 2.050 \times 1.932 = 2.174$$

corresponding to absorption at 4600 Å. The band systems for the three cases $n = 9$, 11 and 13 may now be determined, and are listed in Table I in italics.

TABLE I

CALCULATED (ITALICS) AND OBSERVED TRANSITION ENERGIES FOR POLYENES, EXPRESSED AS WAVE LENGTHS (Å.) OF ABSORPTION MAXIMA

	2050	2680		3550	4460
$n = 9$	2300?	2650		3280	4390[a]
				3340	4400[b]
$n = 11$	1970	2450	3090	3870	(4600)
	2280	2580	2910	3610	4700[c]
$n = 13$	1910	2290	2790	3400	4080	4670
				3330	4080	4880[d]
	2250	2600	2900	3200	3720	4730[e]
						4910

$n = \infty$

[a] A. Polgar and L. Zechmeister, THIS JOURNAL, **65**, 1528 (1943), 5,6-dihydro-α-carotene. [b] L. Zechmeister and B. Kenneth Koe, *ibid.*, **76**, 2923 (1954), neurosporene. [c] L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgar and L. Pauling, *ibid.*, **65**, 1940 (1943), lycopene. [d] L. Zechmeister and L. Wallcave, *ibid.*, **75**, 4493 (1953), bis-dehydro-β-carotene. [e] G. Karmakar and L. Zechmeister, *ibid.*, **77**, 55 (1955) (on dehydrogenation products of α- and β-carotene, Fig. 3).

Theory and Experiment

A review article by Zechmeister[6] and work by the same writer and co-workers have formed the basis for the comparison with experiment given here. The emphasis in Zechmeister's work is to a considerable degree on the relation between steric configuration and spectral changes in energy and intensity. It is believed that much of this material can be given a satisfactory interpretation in terms of the present model; however, in this section the emphasis will be on a different aspect of the experimental findings.
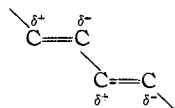
The cardinal point now is that just as predicted there are present in the near ultraviolet and visible, $n/2$ or $(n - 1)/2$ independent electronic transitions, where n is the number of double bonds. To be sure the data do require careful evaluation. For example, sometimes one needs to look at a stereo-isomer of a particular substance to find a transition (the intensity may be too weak in the parent substance). The experimental values listed in Table I give an indication of what is found, but the agreement between the pattern of energy levels predicted and observed goes beyond the specific comparisons listed in the table. In fact it has not been possible

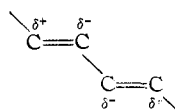(6) L. Zechmeister, *Chem. Revs.*, **34**, 267 (1944).

in an examination of carotenoid spectra to find any indications of disagreement between predicted and observed numbers of transitions in the energy range examined.

Further support for the resonance force model may be obtained from an examination of the first two transition energies. According to the models which have been employed previously, whether molecular orbital, or Lewis and Calvin, there is given the prediction that the second transition energy is in the nature of a harmonic with respect to the first. As we have seen in the resonance force model all the excited states must lie between $+2H'$ and $-2H'$ so that adjacent states, in particular the first and second, must be quite close together for large n. Looking at experiment we find the first and second transitions for bis-dehydro-β-carotene (see ref. d, Table I) are at 4880 and 4080 Å. giving a transition energy ratio of 1.20. The calculated ratio is 1.15 according to the resonance force model. What may be thought of as a tremendously large observed anharmonicity is thus accounted for by the theory.
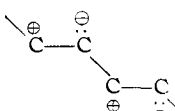
The resonance force model was constructed by postulating that it is possible completely to neglect exchange of electrons between ethylenic double bonds. This assumption leads to the adoption of a particular set of trial functions, which then interact to give states substantially as found experimentally. This does not mean that the underlying assumption itself is exactly true.[7] In fact there is a definite reason for expecting exchange to take part in the interaction. To understand this we look at the probability density, ψ^2, for the lowest energy excited state, for which the interaction integral ($H' < 0$) makes the largest positive contribution to the energy. As expected there are enhanced contributions from favorably lined-up pairs of dipoles, as for example

and correspondingly diminished contributions from unfavorably lined-up dipoles, as
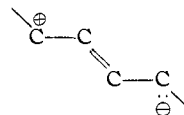

What makes the dipoles appear at the double bonds is a displacement of π electrons, which can be represented as involving the contribution of ionic structures. In consequence, there are enhanced contributions from structures like

and diminished contributions from the other kind. The last pictured structure is made further stable by movement of electrons from one double bond region to another, namely, through a generalized acid base neutralization, to give

The result is that the *effective* interaction integral is larger than would be anticipated on the basis of pure electrostatic interaction between the double bonds.

Ground State Effects.—Zechmeister has concluded that in many cases the carotenoids as obtained from natural products are all *trans*. The configuration about the single bonds[4] is not considered. The existence of conventional appearing electronic spectra in all probability requires that there be no free rotation about the single bonds.

We now adopt the view that a particular configuration about the single bonds is the low energy configuration, and then go on to consider some of the effects observed by Zechmeister and co-workers. First, the strange blurring out of the envelope of the absorption band corresponding to the second transition in the solvent, carbon disulfide[3] can be understood. It is suggested that the cause is a lowering of the potential barrier against nonplanarity owing to the very great polarizability of the solvent molecules. The stabilizing influence which ordinarily forces the molecule into some planar configuration with respect to the single bonds is in a considerable measure the internal dispersion forces.[2] The solvent molecules can probably interact with the double bonds most energetically when the latter are out of coplanarity as a result of a partial twisting about the single bonds. Another effect observed by Zechmeister and co-workers[6] is isomerization at temperatures as low as the boiling point of hexane in the dark. It is believed that the activation energy for isomerization about a double bond is too high (the value for ethylene is 65 kcal./mole)[9] even with some lowering owing to exchange forces, so that what is observed are the end products of rotations about single bonds. Such rotated configurations would be possible from the standpoint of free energy because the s-*cis*, s-*trans* energy difference for a single rotation could well be of the order of kT at room temperature or slightly above. Thus the greater randomness of configurations with some rotation would become the determining factor. Even iodine-catalyzed isomerization[6] need not necessarily be rotations about the double bonds, as iodine atoms might serve very well to lower the potential barriers to s-*cis*, s-*trans* isomerization put up by the exchange forces.

Up until the present point the question of the exact configuration has been by-passed. It is almost certain that several naturally occurring pigments have elements of symmetry because they show the characteristic behavior: weak or virtually

(7) The situation is somewhat analogous to one encountered in atomic spectroscopy. The assumption of L, S coupling gives the most direct description of light atoms even though spin–orbit interaction is certainly present. Pursuing the analogy, to use the molecular orbital method in an attempt to describe carotenoid pigments would be like using functions appropriate for J, J coupling in an attempt to describe an atom of low Z.

(8) L. Zechmeister and A. Polgar, THIS JOURNAL, **67**, 108 (1945).

(9) J. E. Douglas, B. S. Rabinovitch and F. S. Looney, *J. Chem. Phys.*, **23**, 315 (1955).

non-existent absorption bands grow in intensity when the molecules are subject to isomerization which would destroy symmetry. The second transition is of this nature. Enhancement of the intensity of the second transition has been qualitatively understood for some time[6] and is called "development of the cis-peak." According to the foregoing, for compounds with low cis peak intensity, we are very likely dealing with a configuration trans with respect to double bonds and, in addition, either with the all s-cis or all s-trans. Which of the symmetrical configurations about the single bonds is the low energy form needs to be determined.

There is evidence both ways. In favor of s-trans is the fact that butadiene is believed to be s-trans.[10] Moreover the disposition of the methyl groups along the chain may raise the energy of the s-cis form through steric hindrance, though the effect would not be large
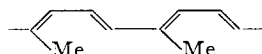
In favor of s-cis is the fact that there would be an internal dispersion force attraction greater for the cis arrangement on the basis of simple electrostatic considerations. What is involved is essentially a comparison of the following
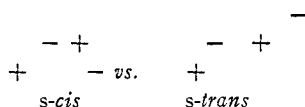
Likewise in favor of s-cis is the observation that the fourth transition energy for lycopene ($\lambda = 2580$ Å., see Table I, $n = 11$) and the energy for the main band for a compound with two double bonds, like butadiene ($\lambda = 2090$ Å.), are each given by

$$6.135 - 1.000H'$$

The absorption maximum for cyclohexadiene, in which the double bonds are s-cis, is 2560 Å.,[11] a value which is quite close to the value for the related fourth transition in lycopene.

In the next section, which is concerned with intensity, additional evidence will be developed having a bearing on the s-cis, s-trans question.

Intensity and How It May Change.—The intensity will be calculated from squares of the transition moment integrals assuming a pure resonance force model. The excited states are all of the form

$$\Sigma\, c_\nu \psi_\alpha^0 \psi_\beta^0 \ldots \psi_\nu^1 \ldots$$

where c_ν is the coefficient of the term with the one unit of excitation localized on the νth double bond. Coefficients c_ν for the $n = 11$ case are as follows

EXCITED STATE

Bond	1st	2nd	3rd
α	0.1057	0.2041	0.2887
β	.2041	.3536	.4082
γ	.2887	.4082	.2887
δ	.3536	.3536	.0000
ϵ	.3943	.2041	− .2887
ζ	.4083	.0000	− .4082
η	.3943	− .2041	− .2887

(10) See for example, R. G. Parr and R. S. Mulliken, J. Chem. Phys., **18**, 1338 (1950).
(11) V. Henri and L. W. Pickett, ibid., **7**, 440 (1939).

| θ | .3536 | − .3536 | .0000 |
ι	.2887	− .4082	.2887
κ	.2041	− .3535	.4082
λ	.1057	− .2041	.2887

The transition moment integrals involve the electric moment vector operator

$$\vec{q}_{op} = \sum_\nu \sum_i e_{i\nu}\, \vec{q}_{i\nu}$$

where summation over i includes the electrons in the νth region, and summation over ν is a summation over the various double bonds. Expressions for the integrals reduce to sums of the coefficients c_ν weighted by trigonometric factors. For example, for the s-trans case, for the 1st transition, the integral is

$$\int (\psi_\alpha^0 \psi_\beta^0 \psi_\gamma^0 \ldots)^* \vec{q}_{op}\, (0.1057\, \psi_\alpha^1 \psi_\beta^0 \psi_\gamma^0 \ldots + \ldots) d\tau$$

The directions will be selected according to the diagram
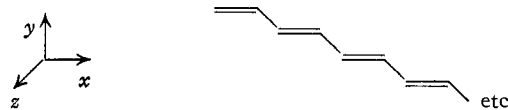
The only part of q_{op} giving a contribution is then the x component. The first term in the transition moment expression is

$$0.1057 \int \psi_\alpha^{0*} \sum_i e_{i\alpha} \vec{q}_{i\alpha} \psi_\alpha^1 d\tau_\alpha$$

(terms involving $e_\beta q_\beta$, etc., drop out, because of the local symmetry). The α-integral is the dipole moment integral for an ethylenic double bond, taken along the carbon–carbon axis. It will be called $\bar{x}$. The total transition moment integral here is simply $\bar{x}\sum_\nu c_\nu = 3.1011\,\bar{x}$, so the relative intensity is the square, $9.617\,\bar{x}^2$. A similar calculation gives zero for the second transition intensity and $0.971\,\bar{x}^2$ for the third. A study of spectra in ref. 6 shows that the second transition is often very low in intensity, as noted above, and the third roughly one tenth the first, as predicted by the resonance force model.

The calculation for a configuration of all s-cis gives almost exactly the same result on a relative basis, though the intensities are all lower by ca. $1/4$ on an absolute basis. We next consider development of the cis-peak, with the notion that the results may help to differentiate between the s-cis and s-trans configuration as the low energy one.

We begin by assuming that rotation about a single bond gives a new configuration which is energetically unstable by the quantity ΔE. Such a rotation could, for example, take the molecule from

(s-cis case) or from
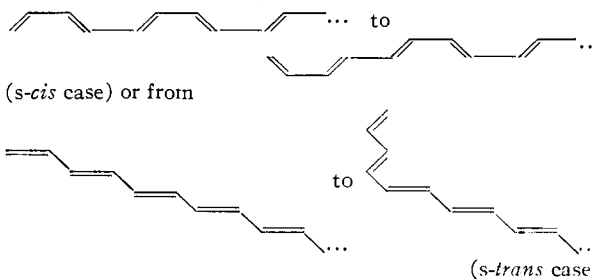
(s-trans case)

There are ten single bonds for the $n = 11$ case and to a first approximation one may consider rotation to be equally likely about any one of them. Whichever single bond is involved, the energy of the molecule goes up by ΔE. The configurations reached after a single rotation will be lumped together and called "the unsymmetrical isomer." The ratio of molecules in the unsymmetrical and symmetrical isomeric forms is given by

$$n_u/n_s = 10e^{-\Delta E/RT}$$

The intensities for configurations reached after a rotation may be calculated approximately by assuming the coefficients c_v remain constant. The intensities of the bands for the unsymmetrical isomer are the simple averages of intensities calculated for all configurations reached in a single rotation.

For the case of all s-cis, the intensities for the isomers are (units of $\bar{x}^2/4$)

Isomer	1st	Excited State 2nd	3rd
Symmetrical	9.625	0	1.057
Unsymmetrical	9.920	0.365	1.305

It is seen that assuming $\Delta E/RT$ is appreciable there will be developed a cis peak less than one third the 3rd band intensity, and concomitantly the 1st and especially the 3rd intensities will increase slightly.

For the case of all s-trans the intensities are (units of $\bar{x}^2$)

Isomer	1st	Excited State 2nd	3rd
Symmetrical	9.617	0	0.971
Unsymmetrical	5.364	3.208	.609

In this case the cis peak grows much faster as the amount of unsymmetrical isomer present at equilibrium increases, and for high T or small ΔE may

surpass in intensity that of the third transition. As the cis peak grows the intensities of the 1st and 3rd bands decrease.

Examination of experiments[6] shows that there is an equilibrium value for cis peak development, reached from above (Figs. 19, 32)[6] and below (Figs. 7–9).[6] The observed behavior can be qualitatively reproduced by the theory, provided the configuration of the symmetrical isomer is chosen to be all s-trans. For example, the main band and third band are observed to decrease in intensity as the cis peak grows. The equilibrium mixture at room temperature corresponds roughly to the case where $n_s = {}^2/_3$ and $n_u = {}^1/_3$. This gives the following intensities, which compare favorably with the experimental values

Isomer	1st	Excited State 2nd	3rd
Symmetrical	9.617	0	0.971
Equilibrium mixture	8.201	1.068	.850

We may therefore now take $n_u/n_s \approx {}^1/_2$ giving for the heat of isomerization at 300°K. the estimated value $\Delta E = 3.2$ kcal./mole.

The intensity calculations are for a pure resonance force model, and are based on the approximation that the coefficients c_v remain constant after isomerization. Nevertheless the agreement with experiment is probably not accidental, serving as evidence that the ground state configuration is all s-trans, and supporting the explanation of the cis peak effect given here.

The writer wishes to thank D. L. Peterson for suggesting that the all s-trans ground state configuration was not considered carefully enough in the early stages of the preparation of this manuscript.

Seattle, Washington

Equilibria in the Fibrinogen–Fibrin Conversion. III. Heats of Polymerization and Clotting of Fibrin Monomer[1]

By Julian M. Sturtevant, Michael Laskowski, Jr.,[2] Thomas H. Donnelly and Harold A. Scheraga

The heat evolution during the polymerization and clotting of monomeric fibrin f, with no thrombin added, was studied calorimetrically. The heat evolution on polymerization at pH 6.08 in 1 M NaBr followed first-order kinetics with $k = 0.090 \pm 0.010$ min.$^{-1}$ and $\Delta H = -21 \pm 1$ kcal./mole of monomer. The heat evolution on clotting at pH 6.88 in 1 M NaBr was first order with $k = 0.13 \pm 0.01$ min.$^{-1}$ and $\Delta H = -54 \pm 2$ kcal./mole of monomer; when this process was essentially complete there appeared to be an onset of a slower endothermic reaction, the existence of which, however, may have been due to experimental difficulties. The ΔH values for the initial first-order process should be corrected since a small exothermic reaction occurs in pure fibrinogen F. The corrected values for step 2 of the fibrinogen–fibrin conversion at pH 6.08 and 6.88 are -19 and -44.5 kcal./mole, respectively, and are compatible with a mechanism in which the polymerization of step 2 is regarded to take place by formation of intermolecular hydrogen bonds between approximately 19 histidyl acceptors and 19 tyrosyl (or amino) donors.

Introduction

The solubility of fibrin increases with increasing

(1) This work has been supported at Yale University by grants from the National Institutes of Health (U. S. Public Health Service) and the National Science Foundation, and at Cornell University by grants from the National Institutes of Health (U. S. Public Health Service), the Office of Naval Research, and Eli Lilly and Co. Reproduction in whole or in part is permitted for any purpose of the United States Government.

(2) U. S. Public Health Service Fellow of the National Heart Institute, 1952–1954.

temperature[3,4] suggesting that the association of fibrin monomers, f (previously designated "activated fibrinogen"[5]) into intermediate polymers f_n,

(3) M. Laskowski, Jr., D. H. Rakowitz and H. A. Scheraga, This Journal, 74, 280 (1952).

(4) K. Laki, personal communication.

(5) (a) T. H. Donnelly, M. Laskowski, Jr., N. Notley and H. A. Scheraga, Arch. Biochem. Biophys., 56, 369 (1955). This paper should also be consulted for a more detailed discussion of the mechanism of the fibrinogen–fibrin conversion and for a more extensive bibliography. (b) The term "fibrin monomer" has been suggested by Laki.[6]

(6) K. Laki, Arch. Biochem. Biophys., 32, 317 (1951).