C, 70.6; H, 1.97; N, 27.4. Found: C, 71.0; H, 1.94; N, 27.4.

TCNQ readily undergoes a one electron reduction with a variety of reagents, including metal iodides, onium iodides, and metals to give stable salts, $M^{x+}(TCNQ^{\overline{\cdot}})_x$. In this respect it resembles tetracyanoethylene.[5] The reaction of LiI with TCNQ in acetonitrile provides a convenient synthesis of $Li^+TCNQ^{\overline{\cdot}}$, which undergoes metathetical reactions in water or ethanol to yield a wide variety of $TCNQ^{\overline{\cdot}}$ compounds containing metallic, organometallic, and onium ions. Specifically, reaction of $Li^+TCNQ^{\overline{\cdot}}$ with triethylammonium chloride in water has given $(C_2H_5)_3NH^+(TCNQ^{\overline{\cdot}})$ (III) as a blue powder, m.p. ~180° dec., resistivity (powder) = 10^9 ohm cm. *Anal.* Calcd. for $C_{18}H_{20}N_5$: C, 70.6; H, 6.54; N, 22.9. Found: C, 70.4; H, 6.27; N, 23.1.

Surprisingly, the addition of neutral TCNQ to an acetonitrile solution of III yields purple-black crystals identified as the anion-radical complex $(C_2H_5)_3NH^+(TCNQ^{\overline{\cdot}})(TCNQ)$ (IV), m.p. ~195°, resistivity (powder) = 20 ohm cm. *Anal.* Calcd. for $C_{30}H_{24}N_9$: C, 70.7; H, 4.55; N, 24.7. Found: C, 70.8; H, 4.89; N, 24.8. The anion-radical complex is stable, and TCNQ cannot be recovered from it by crystallization techniques. In addition, IV can be sublimed (200° at 0.1 mm.) with only slight decomposition.

The triethylammonium anion-radical complex IV has also been synthesized by methods 2, 3, and 4.

$$(C_2H_5)_3NH^+I^- + 2TCNQ \longrightarrow IV\ (50\%) + {}^1/_2I_2 \quad (2)$$

$$2(C_2H_5)_3N + H_2TCNQ + 3TCNQ \longrightarrow IV\ (87\%) \quad (3)$$

$$(C_2H_5)_3N + 2TCNQ \longrightarrow IV\ (77\%) \quad (4)$$

In the second preparation the electron is furnished by the iodide ion. In the third preparation the acid *p*-phenylenedimalononitrile (H_2TCNQ, m.p. 244–245°) is converted to its anion which transfers an electron to TCNQ. Reaction 4 is of special interest in that it occurs at room temperature in acetonitrile in the absence of added catalyst. The source of the ammonium proton has not been established but most probably it is derived from the amine.[6] The four reaction products have been shown to be identical by elemental analyses, absorption spectroscopy, polarography, and X-ray diffraction.

The above methods usually are applicable to synthesis of ion-radical complexes of the type IV, and a variety of products derived from aliphatic, aromatic, and heterocyclic amines have been prepared. However, in a few cases compounds lacking neutral TCNQ have been formed. For example, 5,8-dihydroxyquinoline with TCNQ gave the anion-radical salt, $C_9H_7NO_2H^+TCNQ^{\overline{\cdot}}$, m.p. 167–168° dec., resistivity (powder) = 14 ohm cm. However, quinoline with TCNQ gave $C_9H_7NH^+(TCNQ^{\overline{\cdot}})(TCNQ)$, m.p. ~ 220° dec., resistivity[3] (single crystal) = 0.01 ohm cm.

Reactions of one mole of phosphonium and arsonium iodides with two moles of TCNQ in acetonitrile have given the corresponding onium anion-radical complexes. Both $(C_6H_5)_3PCH_3^+[TCNQ^{\overline{\cdot}})(TCNQ)$ (V), (m.p. 231–233° dec., *Anal.* Calcd. for $C_{43}H_{26}N_8P$: C, 75.3; H, 3.8; N, 16.3; P, 4.5. Found: C, 75.4; H, 3.9; N, 16.3; P, 4.7) and $(C_6H_5)_3AsCH_3^+(TCNQ^{\overline{\cdot}})(TCNQ)$ (VI) (m.p. 224–227° dec., *Anal.* Calcd. for $C_{43}H_{26}N_8As$: C, 70.8; H, 3.6; N, 15.4; As, 10.3. Found: C, 70.9; H, 3.6; N, 15.4; As, 10.3) crystallized as large prisms. The triethylammonium derivative IV also was obtained in macrocrystalline form by slow crystallization from acetonitrile.

In solution the above compounds are dissociated to the cation, neutral TCNQ, and $TCNQ^{\overline{\cdot}}$. Thus, the ultraviolet absorption spectrum of $(C_2H_5)_3NH^+(TCNQ^{\overline{\cdot}})(TCNQ)$ (ϵ_{395} = 85,800 and ϵ_{842} = 43,400) is essentially a composite of that of $(C_2H_5)_3NH^+TCNQ^{\overline{\cdot}}$ (ϵ_{394} = 21,500 and ϵ_{842} = 43,500) and TCNQ (ϵ_{395} = 63,600). Furthermore, the molecular weight determination of IV in acetonitrile showed dissociation into three fragments (mol. wt. found, 147), and the compound is a strong electrolyte in acetonitrile. In the solid state, however, it is believed that there is complete electron delocalization between $TCNQ^{\overline{\cdot}}$ and TCNQ.

The relationship between a π-complex and an anion-radical complex derived from TCNQ has been illustrated dramatically in the case of diaminodurene. This amine with TCNQ in tetrahydrofuran gives the purple π-complex, whereas its hydroiodide with TCNQ yields the blue-black anion radical complex.

DIAMINODURENE-TCNQ DERIVATIVES

Product	Composition	Resistivity (powder) ohm cm.	Magnetic character
π-Complex	$C_{10}H_{16}N_2(TCNQ)$	10^9	Diamagnetic
Anion radical complex	$C_{10}H_{16}N_2H^+(TCNQ^{\overline{\cdot}})(TCNQ)$	8	Paramagnetic

Other quinodimethanes bearing electron-withdrawing substituents, *e.g.*, 7,7,8,8-tetrakis-(methoxycarbonyl)-quinodimethane, m.p. ~ 147°, and 7,7,8,8 - tetrakis - (ethylsulfonyl) - quinodimethane m.p. 195.5–198° dec., have been synthesized, and our studies in this area are continuing. A complete description of this work will be published shortly.

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THE EFFECT OF CHAIN LENGTH UPON HYPOCHROMISM IN NUCLEIC ACIDS AND POLYNUCLEOTIDES

Sir:

The hypochromic effect is well known in nucleic acids and polynucleotides. It is a lowering of the

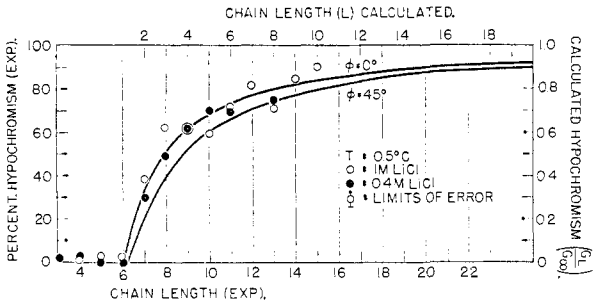

Fig. 1.—Theoretical and experimental hypochromism is plotted as a function of chain length. The calculated curve plots hypochromism in terms of the ratio G_L/G_∞ where G is the geometry-dependent absorption inhibition due to dipole interaction for a two stranded helix with L base pairs (G_L) or infinitely long (G_∞). The calculated results are plotted in the smooth curves for two different orientations (ϕ) of the induced dipole moment relative to the helix axis. The experimental points record per cent. hypochromism at 2625 Å. for the 1:1 helical complex of long polyriboadenylic and short lengths (L) of polydeoxyribothymidylic acid. The solutions are all at *p*H 6.9.

optical density in the ultraviolet absorption spectrum which is associated with stacking of purine and pyrimidine residues in the nucleic acid. In deoxyribosenucleic acid (DNA) this hypochromism partially disappears when the molecule is denatured by heat or strong acid, and fully disappears when the molecule is hydrolyzed to its consistent nucleotides. Measurement of hypochromism also has been used frequently in polynucleotide studies to indicate the formation of helical complexes,[1] many of which are similar in structure to DNA. It is therefore of importance to understand fully the relation between helices and hypochromism in nucleic acids.

Recent theoretical work has quantitatively interpreted the hypochromic effect in terms of the interactions between the dipoles which are induced in the chromophores by the light.[2] The induced dipole in a given purine or pyrimidine is influenced by all of the neighboring induced dipoles on the polynucleotide chains; however, the neighbors which are closer have a greater effect. Thus we can calculate the hypochromism which is predicted in a DNA-like helical structure for various chain lengths. If we assume that the oscillator strength (proportional to extinction coefficient) for the 2,600 Å. band is equal to that of the 2,000 Å. band, and also assume that the bases in the polymer are the same, we can calculate $F_{\text{polymer}}/f_{\text{monomer}} = 1\text{-}G_L$ where F = oscillator strength of polymer, f = oscillator strength of monomer, and G_L = the geometry-dependent absorption inhibition due to dipole interaction (Eq. 1, ref. 2) for a polynucleotide with L base pairs. Using the dimensions of DNA we have plotted in Fig. 1 $G_L/G_\infty = (\epsilon - \epsilon_L)/(\epsilon - \epsilon_\infty)$, where ϵ = an average absorption coefficient for the monomer, ϵ_L = the absorption coefficient for a polymer with L base pairs, and ϵ_∞ = the absorption coefficient for a very long polymer. The continuous curves in Fig. 1 represent this ratio as a function of chain length for two different directions of the transition dipole moments, $\phi = 0°$ (along the radius of the helix) and $\phi = 45°$.

The availability of a series of short polynucleotides[3] has provided an opportunity to test the predictions of the theory. It has been shown recently that a long strand of polyriboadenylic acid (polyribo A) with approximately 2000 nucleotides will combine with short strands of polydeoxyribothymidylic acid (polydeoxy T) to form two-stranded helical complexes which are similar to DNA.[4] Fractions of polydeoxy T containing from 1 to 16 residues were isolated on a diethylaminoethyl-(DEAE)-cellulose column. These were each added to an equimolar solution of polyribo A at *p*H 6.9 in a medium with a high salt concentration. Two types of behavior were noted: either no reaction occurs, or a 1:1 helical complex is formed involving all the thymine and adenine residues. Experiments were carried out at several temperatures; at 0.5° the drop in optical density, plotted as percentage hypochromism, is given in Fig. 1 as a function of chain length L. Zero % is the optical density of the unreacted mixture of polyribo A and polydeoxy T. For 100% hypochromism a figure is used which represents the 21% lowering of optical density which is observed for the 1:1 complex of polyribo A plus polyribouridylic acid when both chains are very long.[1a] This is similar to the amount of hypochromism observed when long polyribo A combines with long polyribothymidylic acid and therefore we assume it will be similar in the present system.

The excellent agreement between the experimental observations and the theoretical curve allows us to draw certain conclusions. In the 1:1 complex, one polyribo A strand is combined with a large number of polydeoxy T oligomers lying end to end. However, apparently, the ends of the short thymine polymers are not bound tightly enough to give rise to the dipole–dipole interactions which are responsible for the hypochromism. These loose ends also provide enough flexibility to make the individual helix segments optically independent. Thus, although the smallest thymine polymer which reacts has seven residues, it only produces a hypochromism such as one would expect to find theoretically for two sets of base pairs. Similarly, each of the longer thymine polymers has approximately 2–3 loose residues at each end. Experiments carried out at 24° show a similar dependence of hypochromism on chain length, except that there are four loose thymine residues at each end. The number of loosely bound nucleotides at each end appears to be independent of the length of the polydeoxy T, but is a function of temperature.

It is possible that these "frayed" ends on the two-stranded DNA molecule may play an important role in the initial attachment of the DNA polymerase enzyme.

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These data are different from those found for the three-stranded helical complex between an adenine tetraribonucleotide and two long strands of polyribouridylic acid.[5] Apparently in this system, when the complex forms, it becomes a complete, rigid helix with no flexibility at the ends of the short adenine polymers.

The results presented here corroborate the theoretical analysis of the hypochromic effect. They also point out that one must be cautious in assuming that the amount of hypochromism is a valid index of helix content. For example, a partially denatured DNA sample with 50% in the form of a helix would have 50% hypochromism if the helix part of each molecule was in very long continuous segments. However, if there were on the average six optically interacting base pairs in a helical region and then an equal number of optically independent residues in a coil, the observed hypochromism would be near 35%.

We wish to thank Professor H. G. Khorana for his generous gift of deoxyribothymidylic acid oligonucleotides.[6]

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GAS CHROMATOGRAPHIC BEHAVIOR OF VITAMINS D_2 AND D_3

Sir:

The detection and estimation in natural materials of individual members of the Vitamin D family is a difficult problem, particularly if more than one D vitamin is present. Since the high resolving power and high sensitivity of gas chromatographic methods may now be applied to steroid problems, the behavior of vitamins D_2 and D_3 was investigated to determine whether these substances could be distinguished from each other and from provitamins D_2 and D_3 by gas chromatographic procedures.

Vitamin D_2 gave two distinct peaks, with no evidence of decomposition on the column. For comparison purposes, the relative (to cholestane) retention times were determined on a silicone SE-30 phase (non-polar) and a neopentyl glycol succinate phase (polar) for ergosterol, lumisterol, pyrocalciferol and isopyrocalciferol. Vitamin D_2 is known to undergo a thermal cyclization reaction yielding pyrocalciferol and isopyrocalciferol, and the observed relative retention times indicated that the vitamin was transformed in the "flash heating" zone of the column into a mixture of these two compounds. This was confirmed by a comparison of the infrared and ultraviolet spectra of the initial material and the corresponding spectra for material collected after gas chromatography. The infrared spectrum changes showed loss of the terminal methylene group, and the ultraviolet spectrum changes were

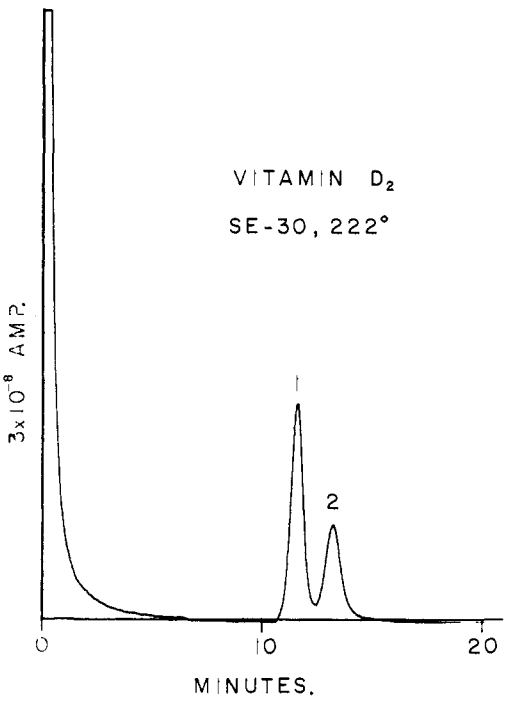

Fig. 1.—Gas chromatographic behavior of vitamin D_2, showing pyrocalciferol (1) and isopyrocalciferol (2): column, 6 ft. × 4 mm.; 0.75% SE-30 on 100–140 mesh Gas-Chrom P, 222°; 19 psi.; argon ionization detector.

those resulting from transformation of vitamin D_2 into the tetracyclic systems of the "pyro" isomers. The collected material was re-run on both phases; the observed retention times were those of pyrocalciferol and isopyrocalciferol, and no further changes were seen.

TABLE I

RELATIVE RETENTION TIMES ON NON-POLAR AND POLAR PHASES

Compound	Structure 9-H	Structure 10-CH_3	Phases SE-30[a]	Phases NGS[b]
Ergosterol	α	β	2.35	10.45
Lumisterol	β	α	1.68	6.19
Pyrocalciferol	α	α	1.83	6.10
Isopyrocalciferol	β	β	2.08	9.22
Vitamin D_2			1.84, 2.08	6.13, 9.17
7-Dehydrocholesterol	α	β	2.05	9.37
Vitamin D_3			1.66, 1.88	5.52, 8.19
Cholestane			1.00[c]	1.00[d]

[a] Column conditions: 0.75% SE-30 silicone on 100–140 mesh Gas-Chrom P; 222°; 19 p.s.i.; 6 ft. × 4 mm. col. [b] Column conditions: 0.75% neopentyl glycol succinate on 100–140 mesh Gas-Chrom P; 210°; 22 p.s.i.; 6 ft. × 3 mm. col. [c] Time, 6.2 minutes. [d] Time, 4.4 minutes.

The influence of the "flash heating" temperature on the transformation was investigated. The ratio of areas of the two peaks (pyrocalciferol: isopyrocalciferol) was compared (SE-30 phase) for three temperatures, with these results: the ratio was 1.86 at 230°, 1.78 at 267° and 1.73 at 300°. The data indicate that the ratio of products is only slightly dependent on the temperature of the cyclization reaction under these conditions.

When these methods were used for vitamin D_3 a comparable result was found. Two compounds