amounts of thiourea, diethyl sulfide, or I^-, the reaction of acid on $[Ru(NH_3)_5N_3](N_3)_2$ generated no dimer (B) (see Figure 1). This behavior may be readily rationalized in terms of the Lewis acid character of $[(NH_3)_5Ru-NH]^{3+}$ (C). Coordinated nitrene, like the similar carbene, is expected to be a soft acid.[7] Combination of $(NH_3)_5Ru-NH$ with itself to produce the dimer (B) is quenched by the more rapid reaction of the nitrene with the soft bases thiourea, $(C_2H_5)_2S$, or I^-. On the other hand, reagents such as Cl^-, PF_6^-, CH_3CN, or dimethyl sulfoxide had no observable effect on the course of the acid reaction.

The decrease in yield of the dimer (B) with increasing initial concentration of $[Ru(NH_3)_5N_3](N_3)_2$ (Table I) suggests that $[Ru(NH_3)_5N_3]^{2+}$ may also function as an effective trap for the nitrene (C) (eq 2). A combination of eq 1 and 2 explains the production of both A and B in the acid reaction of $[Ru(NH_3)_5N_3]^{2+}$.

$$[(NH_3)_5Ru{-}N{=}N{=}N]^{2+} + [HN{-}Ru(NH_3)_5]^{3+} \longrightarrow [(NH_3)_5Ru{-}N{=}N{-}\overset{H}{N}{\cdots}N{-}Ru(NH_3)_5]^{5+} \longrightarrow 2[Ru(NH_3)_5N_2]^{2+} + H^+ \quad (2)$$

A

The behavior of *cis*-$[Ru(en)_2(N_3)_2]PF_6$ and *cis*-$[Ru(trien)(N_3)_2]PF_6$ in acid is very similar to that of $[Ru(NH_3)_5N_3](N_3)_2$. The final spectra again exhibited two intense bands at 221 and 264 mμ. The band at 221 mμ agrees well with the known spectrum of *cis*-$[Ru(en)_2N_2H_2O]^{2+}$ (A′).[8] The bands at 264 mμ have been assigned to the dimers $[(A-A)_2H_2ORu-N_2-RuH_2O(A-A)_2]^{4+}$ (B′) (A–A = en, 0.5 trien). The latter compounds have been independently prepared *in situ*, and their spectra (ϵ_{264} = 48,000) agree closely with that reported for $[(NH_3)_5Ru-N_2-Ru(NH_3)_5]^{4+}$.[5] The presence of small amounts of thiourea or I^- in the acid reactions again eliminated the formation of the dimers (B′), supporting the presence of reactive nitrene intermediates.

Attempts to observe an esr signal for the postulated nitrene intermediates have been unsuccessful. Solutions of *cis*-$[Ru(en)_2(N_3)_2]PF_6$ (0.038 *M*) in H_2SO_4 (0.80 *M*) were frozen in liquid nitrogen after various reaction times, and their esr spectra recorded. All resonances observed in the region 1000–8000 G have been assigned to the unpaired electron of ruthenium(III). These bands decreased with increasing reaction time due to the formation of ruthenium(II). The failure to observe a nitrene resonance suggests it may be present in a singlet electronic state, though it may also be due to a low nitrene concentration.

A search has been made for similar acid-catalyzed behavior among other transition metal azide complexes. Azide complexes of Co(III), Rh(III), Pt(II), Pd(II), and Au(III) generated no gas when dissolved in 4 *M* H_2SO_4 at room temperature. However, similar behavior has been found in these laboratories for the Ir(III) complex *trans*-$[Ir(en)_2(N_3)_2]PF_6$.[9] Ultraviolet irradiation of metal azide complexes appears to be an alternative source of metalated nitrenes.[10]

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Compounds such as $ReCl_3(P(C_2H_5)_2C_6H_5)_2NC_6H_4X$ are known and their structures have been determined.[11] While these may formally be considered as arylnitrenes coordinated to metal ions, they are very stable and are best formulated as arylimino complexes.[12]

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A Model for the Interaction of Cytochrome P-450 with Carbon Monoxide[1]

Sir:

Although P-450 cytochromes are hemoproteins containing protoporphyrin IX, many of their spectral properties are exceptional for hemoproteins. In particular, the reduced P-450–CO complex shows a Soret peak at 450 mμ,[2] while the reduced P-450–ethyl isocyanide complex shows a pair of Soret peaks at 428 and 455 mμ.[3]

P-450 is converted to a modified form, P-420, by the action of organic solvents, detergents, or certain enzymes.[4] These modifications remove the anomalous spectral properties of the reduced cytochrome, and thus P-420 shows spectral properties of a typical *b*-type cytochrome (CO complex λ_{max} 421, 538, 565 mμ).

Since P-450 cytochromes of mammalian origin are tightly bound to cellular membranes, a separation from lipids and other components of membranes has not been achieved without accompanying conversion to P-420. For this reason, model compounds are particularly useful in understanding the anomalous spectral characteristics of P-450.

Imai and Sato[5] have recently shown that ethyl isocyanide can form a complex with protoheme in which the 455-mμ Soret peak of the reduced P-450–ethyl isocyanide is reproduced. They have shown that this unusual protoheme complex requires an association of two or more protohemes. Consequently, they suggest that the anomalous spectral properties of reduced P-450 complexes are due to a direct interaction of two hemes. However, no evidence has been provided about possible interaction of two hemes in the corresponding CO complex of either reduced protoheme or P-450.

Several years ago, Holden and Lemberg[6] reported that the complex of reduced protoheme, pyridine, and CO showed a Soret peak at 440 mμ. When this work was repeated, we found that the visible absorption

(1) This work was supported in part by Research Grant AM-10767 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service, and in part by funds made available from the State University of New York.
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spectrum showed two Soret peaks absorbing at 437 to 439[7] and 417 mμ, corresponding to two distinct complexes. The spectrum was critically dependent upon the method of mixing the solutions. In our experiments, protoheme (1% in 2.50 *M* pyridine) was diluted 12.5 times with a solution of 3.75 *M* pyridine, and then the resulting solution was further diluted (10 times) with an aqueous solution of sodium dithionite (0.02 *M*) that was saturated with CO. In the absence of CO, a different pair of Soret peaks (433 and 420 mμ) was observed, and the α,β absorbancies were quite different from those of the CO complexes. Similar pairs of Soret peaks were observed when pyridine was replaced by equivalent quantities of either *n*-butylamine or imidazole (Table I).

Table I. Absorption Maxima (mμ) of Reduced Protoheme Complexes

Base	Monomer state		Associated state	
	Base–base	Base–CO	Base–base	Base–CO
Butylamine	422	420	442	450
(0.3 *M*)	528	537	532	548
	557	568	563	580
Imidazole	408	417	434	442
(0.5 *M*)	538	545		
	565	577		
Pyridine	420	418	433	437–439
(0.37 *M*)	527	540	530	543
	558	568	563	570

Increase in the concentration of heme decisively changed the proportion of the CO complexes (Table II) in favor of the complex providing the long-wavelength Soret peak. Thus, association of hemes was strongly indicated as the cause of this absorption. The tendency to association was only slightly affected by the ligand (pyridine > *n*-butylamine > imidazole ≅ CO), suggesting that most of the association energy derived from a direct heme–heme interaction.

Table II. Effect of Heme Concentration on Reduced Protoheme–CO Complexes

Concentration of heme, $M \times 10^5$	OD associated form/OD monomer form[a]		
	Pyridine	*n*-Butylamine	Imidazole
0.32	0.33		
0.64	0.63		
1.22	1.10	0.58	
2.02	1.27	0.97	0.69

[a] Ratios of the respective Soret peaks (Table I).

The relative proportion of the two forms of the hemochrome–CO complexes was affected by the same substances that convert P-450 into P-420. Thus, in general the complex providing a long-wavelength Soret peak was converted to the form that absorbed at around 420 mμ in the presence of sodium deoxycholate (1 mg/ml) and organic reagents such as alcohols, while the reverse change was effected by an increase in salt concentration. In addition, the effect of alcohols became more pronounced as the alkyl chain increased in length. The pyridine–heme–CO complex was almost completely converted to the associated form by 0.1 *M* KCl, while, however, increase concentrations of salt resulted in a pronounced and specific hyperchromicity of the 439-mμ absorbancy.

The tendency of sodium deoxycholate and alcohols to dissociate the reduced protoheme–CO complexes while increased ionic strength favors association both suggest that the association energy in these complexes derives from a hydrophobic interaction of a pair of hemes which are oriented to maximize lipophilic overlap.

The relationship between the two types of hemochrome–CO complex is very similar to that between the CO complexes of P-450 and P-420. Thus, this model is in agreement with evidence that the anomalous spectral properties of reduced P-450 may arise from a direct contact of the hemes of the same or separate subunits which can be specifically oriented by the cellular membrane. In addition, either an imidazole group of histidine or, particularly, an ε-amino group of lysine is indicated as a protein ligand for the hemes of P-450 and P-420 in the reduced state. This is consistent with our recent evidence[8] that the epr spectrum of oxidized P-450 can be attributed to a single heme coordinated by a sulfhydryl group and either histidine or lysine, and that the sulfhydryl interaction is lost on reduction.

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Oriented Smectic Liquid Crystal Solutions

Sir:

The orientation of molecules dissolved in nematic liquid crystal solvents has been exploited in studies of the anisotropy in nuclear and electron spin, electronic, and vibrational interactions;[1] it has also been shown that molecules can be oriented in mixtures of cholesteric liquid crystals which have been "compensated" to a nematic structure.[2]

An ordered smectic solution would offer the possibility of arbitrary control of the direction of orientation, and would therefore be of great use in nmr experiments.[1a] Despite this advantage, no results obtained with smectic solutions have been reported.[3] It has commonly been thought that the nmr lines obtained from smectic solutions would be too broad to be useful.[4] Nevertheless, since pure smectic materials have been oriented,[5] one may conclude that the preparation of ordered solutions should be possible. The purpose of this communication is to report the confirmation of this conclusion, and some of the interesting properties of oriented smectic solutions.

A 20 mole % solution of 1,1,1-trifluorotrichloro-

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