of 2-keto-3-deoxyarabonate to α-ketoglutarate by *P. saccharophila* (*10*).

Our preliminary observations indicate that KGSA in the presence of NH_3 is converted nonenzymatically to pyrrole-2-carboxylate and other compounds, including a probable Δ^1-pyrroline which is reducible to proline by sodium borohydride. KGSA appears also to be a substrate for reduction by DPNH with crystalline rabbit muscle lactic dehydrogenase, although the reduction product has not been characterized. We are investigating other possible involvement of this reactive dicarbonyl in both bacterial and mammalian metabolism.

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4. We thank Dr. Clauson-Kaas for a generous gift of 2,5-dimethoxy tetrahydro-2-furoic acid methyl ester (*5*), from which KGSA is obtained after mild acid hydrolysis and separation by chromatography through Dowex-1-Cl.
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Spectra of Deoxygenated Hemoglobin in the Soret Region

Abstract. *Methemoglobin is easily formed during the deoxygenation of hemoglobin. It can be removed with methemoglobin reductase. The Soret spectrum of pure deoxyhemoglobin is reported. Comparison of these data with the published spectra shows that some of these are incorrect since they must represent mixtures of deoxy- and methemoglobin.*

The preparation of deoxygenated ferrohemoglobin (deoxyhemoglobin) from oxyhemoglobin presents certain difficulties. In general, three methods have been used for this purpose. The most commonly employed chemical reagent for the reduction of oxygen is sodium dithionite. Although this method removes oxygen rapidly and completely, there is little doubt that it produces chemical changes in the hemoglobin molecule, probably as a result of the action of hydrogen peroxide which is a product of the reduction of oxygen by sodium dithionite. Dalziel and O'Brien (*1*) have shown that this results in secondary changes in the extinction at 430 mμ within a very short time (less than 1 minute). They also found that the yield of deoxyhemoglobin decreases with decreasing hemoglobin concentration, decreasing concentrations of dithionite and increasing oxygen concentration. This method is therefore particularly unsuitable for preparing known concentrations of deoxyhemoglobin from exyhemoglobin for measurements in the Soret region.

The alternative method for converting oxy- to deoxyhemoglobin is the physical removal of oxygen, either by passage of an oxygen-free inert gas over the solution or by repeated evacuation in a high vacuum. Displacement with an inert gas involves relatively long exposures of the hemoglobin to low oxygen pressures. This is undesirable since, as Brooks has shown (*2*), the rate of oxidation of hemoglobin to methemoglobin is at a maximum at low oxygen pressures. This apparent paradox is due to the fact that deoxyhemoglobin is oxidized very much more rapidly than oxyhemoglobin. Such protection against oxidation by oxygen in oxygenated ferrous complexes is well-known and has been discussed by Williams (*3*). The spectra of deoxyhemoglobin prepared by slow deoxygenation are thus likely to contain contributions from methemoglobin.

Both the spectra published by Sidwell *et al.* (*4*) and those in Lemberg and Legge's textbook (*5*) show a shoulder between 410 and 415 mμ. The deoxyhemoglobin in both cases was prepared by slow deoxygenation. It thus appeared likely that this shoulder was due to methemoglobin.

In our experience a shoulder between 410 and 415 mμ was always observed in the spectra of deoxyhemoglobin prepared by slow deoxygenation. The magnitude of this effect (ε412/ε430) was quite variable and not reproducible. On the assumption that methemoglobin was responsible for the absorption in this region, the influence of methemoglobin reductase was investigated. This enzyme catalyzes the reduction of ferri- to ferrohemoglobin by DPNH (reduced diphosphopyridine nucleotide) in the presence of trace amounts of methylene blue (*6*). Three samples of identical hemoglobin concentration (0.0085 percent) were deoxygenated by passage of oxygen-free nitrogen over the solution for 2 hours, and the spectra were recorded. In each case the spectrum was again recorded after reoxygenation of the solution. The results are shown in Fig. 1. It can be seen that sample A, which initially contained only oxyhemoglobin, shows, after deoxygenation, a shoulder 412 mμ and an extinction coefficient ε of 1.20 at 430 mμ. The absorption maximum after reoxygenation was at 412 mμ. Sample B differed from sample A only by the addition of methemoglobin reductase, DPNH, and methylene blue before deoxygenation. Under these conditions the shoulder is absent and the extinction coefficient at 430 mμ has increased to 1.42. Reoxygenation results in a spectrum which is indistinguishable from that of the original oxyhemoglobin sample with a maximum at 415 mμ. The third sample (C) which was identical with sample B except for the substitution of methemoglobin for oxyhemoglobin, gives spectra which are identical with those shown for sample B. The points shown on Fig. 1*A* are calculated for a mixture of 20 percent methemoglobin and 80 percent deoxyhemoglobin. This was done by using the extinction coefficients of the deoxyhemoglobin spectrum shown in Fig. 1*B* and of a spectrum of methemoglobin prepared as described here. The observed spectrum can thus be accounted for by an impurity of 20 percent methemoglobin that is formed during the deoxygenation and

Table 1. Absorption of hemoglobins.

Our study		Sidwell *et al.*	
λ_{max} (mμ)	10^{-5} ϵ_{max}	λ_{max} (mμ)	10^{-5} ϵ_{max}
	Methemoglobin		
405	1.53		
	Oxyhemoglobin		
415	1.32	415	1.29
	Deoxyhemoglobin		
430	1.40	430	1.18

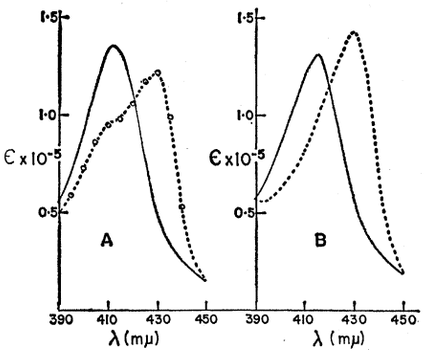

Fig. 1. Spectra after deoxygenation (dotted lines) and spectra after reoxygenation (solid lines). The points are calculated for a mixture of 20 percent methemoglobin and 80 percent deoxyhemoglobin. The initial composition of solution *A* was 0.0085 percent oxyhemoglobin in 0.04*M* phosphate buffer, *p*H 7.4. The initial composition of solution *B* was 0.0085 percent oxyhemoglobin, $1.9 \times 10^{-4}M$ DPNH, $5 \times 10^{-7}M$ methylene blue, 0.017 mg of methemoglobin reductase per milliliter, in 0.04*M* phosphate buffer, *p*H 7.4. A mixture of the last four components of solution *B* showed no significant absorption in the spectral region shown.

which is quantitatively reduced in the presence of methemoglobin reductase. Rapid deoxygenation in a high vacuum minimizes the formation of methemoglobin, although, even by this method, some methemoglobin is frequently formed.

In summary, therefore, the presence of methemoglobin in a sample of deoxyhemoglobin can be recognized from the spectrum in the Soret region by three features. These are (i) an increase in absorption between 410 and 415 mμ, causing a shoulder, (ii) a decrease in absorption at 430 mμ, and (iii) a shift to wavelengths shorter than 415 mμ in the absorption maximum of the oxyhemoglobin formed on reoxygenation. These spectral features are explained by the fact that the absorption maximum of methemoglobin at 405 mμ is separated by 25 mμ from that of deoxyhemoglobin but only by 10 mμ from that of oxyhemoglobin.

The absorption maxima and extinction coefficients of oxyhemoglobin, deoxyhemoglobin, and methemoglobin found in the course of this work are listed in Table 1. It is clear from these values that the maximum extinction coefficient of deoxyhemoglobin is higher than that of oxyhemoglobin. This relationship is reversed in some of the published spectra (*4*, *5*) which is undoubtedly due to contamination with methemoglobin.

Oxyhemoglobin was prepared from normal human blood as described previously (*7*). Methemoglobin was prepared by oxidizing a 4 percent solution of oxyhemoglobin in 0.04*M* phosphate buffer, *p*H 6.8, with 1.2 equivalents of potassium ferricyanide for 1 hour at room temperature. The protein was then freed of ferri- and ferrocyanide by dialysis against three changes of phosphate buffer, *p*H 6.8, 0.2 ionic strength. This was followed by exhaustive dialysis against distilled water. Dialysis against phosphate buffer was effective in removing ferrocyanide which, in contrast to ferricyanide, is strongly bound by hemoglobin. The absence of ferrocyanide in the final solution was proved by a negative Prussian blue test in the filtrate after precipitation of the protein with trichloroacetic acid. The Prussian blue test was strongly positive if the dialysis against phosphate buffer was omitted.

Deoxyhemoglobin was prepared by passage of "prepurified nitrogen," further purified by the sulfate vanadous method (*8*) over the oxyhemoglobin solution in 0.04*M* phosphate buffer, *p*H 7.4, at room temperature for 2 hours. Transfer to cylindrical cuvettes of 1-cm light path was then carried out under nitrogen by an arrangement similar to that described (*9*). All spectra were recorded on a Cary model 14 spectrophotometer. Extinction coefficients are expressed per atom of iron and are based on spectrophotometric determination of hemoglobin concentration as methemoglobin cyanide by using the extinction coefficient of 1.15×10^4 at 540 mμ. The concentration of reagents used (see Fig. 1) reduced methemoglobin completely in 10 minutes (*10*).

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Allotropy in Some Rare-Earth Metals at High Pressures

Abstract. *Allotropes of lanthanum, cerium, praseodymium, and neodymium have been observed at elevated pressures with an x-ray diffraction camera which incorporates a diamond-anvil, high-pressure cell. In each case a high-pressure modification was observed which has a face-centered cubic structure. At room temperature the unit cell dimensions (a_0) for the high-pressure face-centered cubic structures and approximate pressures at which they were determined are as follows: La, 5.17 Å (23 kb); Ce, 4.82 Å (15 kb); Pr, 4.88 Å (40 kb); and Nd, 4.80 Å (50 kb). The unit cell dimensions for the high-pressure forms of La, Pr, and Nd apparently have never been reported.*

In recent years a notable increase in activity in high-pressure research has taken place which is due in part to the successful development of new experimental techniques for conducting studies at high and ultrahigh pressures (*1*). With the advent of increased accessibility of high-pressure data, a renewed interest in electronic transitions such as the one observed in Ce metal (*2*) at elevated pressures has developed both from an experimental as well as theoretical viewpoint (*3*). Cerium metal transforms from a face-centered cubic structure to a "collapsed" face-centered cubic structure at 7.5 kb (1 kb $\approx 10^3$ atm) (*2*). It is generally accepted that this transition involves the promotion of a 4*f* electron to the 5*d* band. Although a number of other metals such as Cs and Rb are suspected of having electronic transitions at elevated pressures (*3*), to date, it appears that only the electronic transition in Ce has been verified by x-ray diffraction studies (*2*).

The determination of the crystallographic nature of the phase transformations observed in La and Pr at elevated