Electron-transfer Reactions between Metallic Ions, Co-ordination Complexes, and Hæmoglobin.

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Single-electron transfer reactions between (i) a simple aquated ion and a complex ion, and (ii) pairs of complex ions, are extremely rapid with velocity constants $k > 10^5$ l. mole^{-1} sec.$^{-1}$ in perchloric or nitric acid solution at 18°; the ions used were $Fe^{2+}_{aq.}$, $Ce^{4+}_{aq.}$, $IrCl_6^{2-}$, $Fe(CN)_6^{4-}$, $Mo(CN)_8^{4-}$, and the dipyridyl and phenanthroline complexes of Fe^{2+}, Fe^{3+}, Ru^{2+}, and Ru^{3+}. In 1·0M-sulphuric acid the reduction of ceric sulphate by $Ru(dipy)_3^{2+}$ or $Fe(5\text{-nitrophenanthroline})_3^{2+}$ is slower ($k \sim 4 \times 10^3$ l. mole^{-1} sec.$^{-1}$), but with $Fe(phen)_3^{2+}$ k is again $> 10^5$ l. mole^{-1} sec.$^{-1}$. These results are discussed in terms of the charge type of the reacting ions, and in relation to Libby's suggestion that activation energy may be required for the reorganisation of solvation or co-ordination shells which must necessarily accompany the electron transfer.

The transfer of two electrons in similar reactions involving $Tl^{+}_{aq.}$ and $Tl^{3+}_{aq.}$ is very much slower. The reduction of $Tl^{3+}_{aq.}$ by $Fe(phen)_3^{2+}$ has a velocity constant of about 7×10^{-3} l. mole^{-1} sec.$^{-1}$ at 25°, and data for this reaction are compared with those for the reduction by $Fe^{2+}_{aq.}$.

In phosphate buffer solutions of pH 6·0 hæmoglobin and its oxy- and carbonmonoxy-complexes (all ferrous protoporphyrin derivatives) are oxidised very rapidly by $IrCl_6^{2-}$ to methæmoglobin (the ferric protoporphyrin derivative). It is clear that the covalent bonding of the iron in the oxy- and carbonmonoxy-complexes does not "protect" it from oxidation. Cationic oxidising agents like $Ru(dipy)_3^{3+}$ are ineffective, apparently because other groups on the globin molecule are oxidised preferentially.

(A) *Single-electron Transfer between Metal Ions and Co-ordination Complexes.*—The investigation of exchange reactions between metal ions and complexes by use of radioactive isotopes is providing data of great value in understanding the factors governing the rate of electron-transfer reactions in general. Typical examples with their velocity constants are given in Table 1. Reactions of this type are of particular interest since the reactants are ions of similar charges, and electrostatic repulsion would be expected to result in negative entropies of activation and appreciable activation energies, possibly as high as 15 kcal./mole (Gorin, *J. Amer. Chem. Soc.*, 1936, **58**, 1787). Both of these features are present in some exchange reactions which have been found to proceed at conveniently measurable rates, *e.g.*, Tl^{3+}–Tl^{+}, V^{4+}–V^{3+}, Ce^{4+}–Ce^{3+}, and $Co(en)_3^{3+}$–$Co(en)_3^{2+}$; the activation energies range from 10 to 16 kcal./mole, and entropies of activation from about −16 to −32 e.u. (Harbottle and Dodson, *ibid.*, 1951, **73**, 2442; Furman and Garner, *ibid.*, 1952, **74**, 2333; Gryder and Dodson, *ibid.*, 1949, **71**, 1894; see also Table 1, ref. *c*). However, several exchange reactions proceed far faster than expected on this basis, particularly $Fe(CN)_6^{3-}$–$Fe(CN)_6^{4-}$ and $Fe(\text{dimethylphenanthroline})_3^{3+}$–$Fe(\text{dimethylphenanthroline})_3^{2+}$. The slight doubt as to whether the separation or diffusion procedures,

Table 1. *Some electron-transfer reactions investigated by the radioactive-isotope method.*

Reactants	Velocity const. (l. mole^{-1} sec.$^{-1}$)	Ref.	Reactants	Velocity const. (l. mole^{-1} sec.$^{-1}$)	Ref.
Fe^{2+}, Fe^{3+}	~ 16	*a*	$Co(NH_3)_6^{2+}$, $Co(NH_3)_6^{3+}$	$< 6 \times 10^{-7}$	*c*
MnO_4^{2-}, MnO_4^{-}	> 15	*b*	* $Fe(Me_2phen)_3^{2+}$, $Fe(Me_2phen)_3^{3+}$	$> 2 \times 10^4$	*d*
$Co(en)_3^{2+}$, $Co(en)_3^{3+}$	$5{\cdot}2 \times 10^{-5}$	*c*	$Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$	$> 10^2$	*e*

* Me_2phen = 5 : 6-dimethylphenanthroline.

[a] Dodson, *J. Amer. Chem. Soc.*, 1950, **72**, 3315. [b] Adamson, *J. Phys. Coll. Chem.*, 1951, **55**, 293. [c] Lewis, Coryell, and Irvine, *J.*, 1949, S 386. [d] Eimer and Medalia, *J. Amer. Chem. Soc.*, 1952, **74**, 1592. [e] Cobble and Adamson, *ibid.*, 1950, **72**, 2276.

which have to be employed in the isotopic technique, themselves catalyse the exchange makes additional information on the speed of electron-transfer reactions between similarly charged ions of different species very desirable.

Taking advantage of the intense colour of many complex ions, easily detectable in 10^{-5}M-solutions, we examined the velocity of several such electron-transfer reactions, with the results recorded in Table 2.* All are fast reactions occurring " instantaneously " on mixing of the solutions, which enables a lower limit for their velocity constants to be put at 10^5 l. mole^{-1} sec.$^{-1}$. Fast reactions between the oppositely charged ions are to be expected, but it is surprising that those between the similarly charged ions are also very fast. Nine of those listed in Table 2 come into this category, seven between cationic species and two between anionic species. These results substantiate the behaviour found in the $Fe(CN)_6^{3-}$–$Fe(CN)_6^{4-}$ and Fe(dimethylphenanthroline)$_3^{3+}$–Fe(dimethylphenanthroline)$_3^{2+}$ systems by the isotopic method, and in the $Os(dipy)_3^{3+}$–$Os(dipy)_3^{2+}$ exchange reaction by utilising the optical rotatory power of the stereoisomers. In this case Dwyer and Gyarfas (*Nature*, 1950, **166**, 487) found the velocity constant at 10° to be about 10^3 l. mole^{-1} sec.$^{-1}$ by following the change in optical rotatory power when (+)- and (—)-stereoisomers of the Os^{3+} and Os^{2+} complexes respectively were mixed. Four of the fast reactions quoted in Table 2 are between pairs of similarly charged complex ions, so their speed cannot in part be accounted for through ion-pair complex formation of the type common in most exchange reactions between simple hydrated ions, an effect which reduces the charge product ZZ', *e.g.*, Cl^- catalysing the Fe^{3+}–Fe^{2+} and Eu^{3+}–Eu^{2+} exchanges *via* the ion-pair complexes $FeCl^{2+}$ and $EuCl^{2+}$ (Silverman and Dodson, *J. Phys. Chem.*, 1952, **56**, 846; Meier and Garner, *ibid.*, p. 853). The formation of conjugate acid species of the complex anions, *e.g.*, $HFe(CN)_6^{3-}$ from $Fe(CN)_6^{4-}$, would help to reduce the charge product in these systems, but an analogous reaction with the complex cations, *e.g.*, the production of $HFe(dipy)_3^{3+}$ from $Fe(dipy)_3^{2+}$ (Baxendale and George, *Trans. Faraday Soc.*, 1950, **46**, 736) would lead to even greater discrepancies.

TABLE 2. *Reactions between one-electron transfer oxidising ions and one-electron transfer reducing ions, all of which are fast with velocity constants* $>10^5$ *l. mole*$^{-1}$ *sec.*$^{-1}$ *at about 18°*: S^+ *represents simple cation,* C^+ *or* C^- *a complex cation or anion.**

Type	Oxidising ion	E_0 (v)	Reducing ion	E_0 (v)	ΔE_0 (v)	ZZ'
$S^+ + C^-$	$IrCl_6^{2-}$	1·02	Fe^{2+}	0·77	0·25	− 4
$S^+ + C^+$	$Fe(phen)_3^{3+}$	1·14	Fe^{2+}	0·77	0·37	+ 6
	$Ru(dipy)_3^{3+}$	1·30	Fe^{2+}	0·77	0·53	+ 6
	Ce^{4+}	1·61	$Fe(dipy)_3^{2+}$	1·06	0·55	+ 8
	Ce^{4+}	1·61	$Fe(phen)_3^{2+}$	1·14	0·47	+ 8
	Ce^{4+}	1·61	$Ru(dipy)_3^{2+}$	1·30	0·31	+ 8
$C^+ + C^+$	$Fe(phen)_3^{3+}$	1·14	$Fe(dipy)_3^{2+}$	1·06	0·08	+ 6
	$Ru(dipy)_3^{3+}$	1·30	$Fe(dipy)_3^{2+}$	1·06	0·24	+ 6
$C^+ + C^-$	$Fe(phen)_3^{3+}$	1·14	$Fe(CN)_6^{4-}$	0·36	0·78	−12
	$Ru(dipy)_3^{3+}$	1·30	$Fe(CN)_6^{4-}$	0·36	0·94	−12
$C^- + C^-$	$IrCl_6^{2-}$	1·02	$Fe(CN)_6^{4-}$	0·36	0·66	+ 8
	$IrCl_6^{2-}$	1·02	$Mo(CN)_8^{4-}$	0·74	0·28	+ 8

* Reactions involving Ce^{4+} in 1·0M-$HClO_4$, all others in 10^{-3}M-HNO_3.

The extent to which these reactions proceed more rapidly than expected from their charge type can be seen by referring to Table 3, which gives activation energies, E, and temperature-independent factors, A, for several typical reactions between ions, including some of the more normal exchange reactions. The low A factors are fairly satisfactorily accounted for by sizable negative entropies of activation, ΔS^* ranging from —10 to —30 e.u./mole, arising from the electrostatic interaction (Glasstone, Laidler, and Eyring, " The Theory of Rate Processes," McGraw-Hill, New York, 1941, chapter 8). With a lower limit of 10^5 l. mole^{-1} sec.$^{-1}$ for the velocity constant of the reactions listed in Table 2,

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* The rapid reaction between $Fe(phen)_3^{3+}$ and $Fe(dipy)_3^{2+}$ was previously reported by J. H. Baxendale and P. George at a discussion on " The Use of Radioactive Tracers in Chemistry " in London, April 1st, 1949, the main papers of which appear in *J.*, 1949, S 235; 420.

calculation shows that if A should have the value $1{\cdot}43 \times 10^{13}$, *i.e.*, $\Delta S^* = 0$, E is still less than 11·0 kcal./mole, whilst if A is put at $6{\cdot}5 \times 10^8$, *i.e.*, with a more reasonable value of -20 e.u./mole for ΔS^*, then E is less than 5·2 kcal./mole. It seems clear that in some way these electron-transfer reactions involving complex ions can occur more rapidly than similar reactions of aquated ions or reactions in which chemical bonds are broken and re-formed—for instance, the oxygen-atom transfer reaction at the top of Table 3.

TABLE 3. *Kinetic data for various reactions between similarly charged ions, including some from Table 2 for comparison where* S^{2+} *and* C^{3+}, *for instance, refer to a simple aquated ion and a complex ion, respectively.*

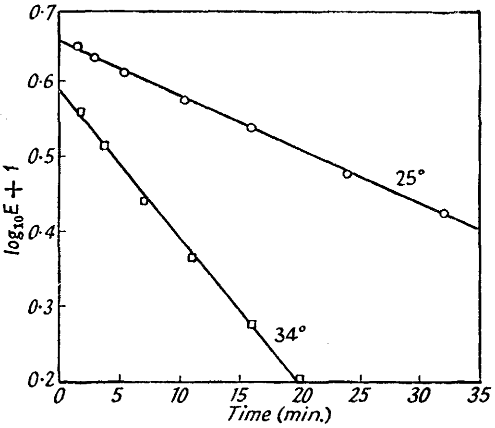
Reaction	ZZ'	Temp.	k, l. mole^{-1} sec.$^{-1}$	E, kcal./mole	A, l. mole^{-1} sec.$^{-1}$	Ref.
$AsO_3^{3-} + TeO_4^{2-}$	+ 6	89·6°	$3{\cdot}63 \times 10^{-3}$	14·4	$1{\cdot}7 \times 10^6$	*a*
$Co(NH_3)_5Br^{2+} + Hg^{2+}$	+ 4	25	3·3	12·3	$3{\cdot}5 \times 10^9$	*b*
$Co(en)_3^{3+} + Co(en)_3^{2+}$	+ 6	25	$5{\cdot}2 \times 10^{-5}$	~14·1	$1{\cdot}1 \times 10^6$	*c*
$VO^{2+} + VOH^{2+}$	+ 4	25	1	10·7	$1{\cdot}0 \times 10^8$	*d*
$Ce^{4+} + Ce^{3+}$	+12	25	16	13·4	$1{\cdot}1 \times 10^{11}$	*e*
$C^{3+} + S^{2+}$	+ 6	18	$>10^5$	$<11{\cdot}0$ if $A = 1{\cdot}43 \times 10^{13}$ $<5{\cdot}2$ if $A = 6{\cdot}5 \times 10^8$		
$S^{4+} + C^{2+}$	+ 8	18	$>10^5$			
$C^{3+} + C^{2+}$	+ 6	18	$>10^5$			
$C^{2-} + C^{4-}$	+ 8	18	$>10^5$			

[a] Stroup and Meloche, *J. Amer. Chem. Soc.*, 1931, **53**, 3331. [b] Bronsted and Livingston, *ibid.*, 1927, **49**, 435. [c] Lewis, Coryell, and Irvine, *J.*, 1949, S 386. [d] Furman and Garner, *J. Amer. Chem. Soc.*, 1952, **74**, 2333. [e] Gryder and Dodson, *ibid.*, 1949, **71**, 1894.

Although these reactions in 10^{-3}M-nitric acid and 1·0N-perchloric acid were too fast for kinetic measurements, some reactions of the ceric ion in sulphuric acid solution were slow enough: in 1·0M-acid the velocity constant for reduction by $Ru(dipy)_3^{2+}$ was approximately $4{\cdot}0 \times 10^3$ l. mole^{-1} sec.$^{-1}$ at 18°. With the 5-nitrophenanthroline complex of ferrous iron, the velocity constant was the same within experimental error, but with the unsubstituted phenanthroline complex $Fe(phen)_3^{2+}$ the reduction was again too fast for kinetic measurements. Moore and Anderson (*J. Amer. Chem. Soc.*, 1945, **67**, 167) showed that, at concentrations of less than 0·01M in ceric and sulphate ion in 2N-perchloric acid, the first ion-pair complex $CeSO_4^{2+}$ predominates with a formation equilibrium constant of 2×10^3 mole^{-1} at 24°, but that at higher concentrations other complex ions are formed with more sulphate bound. Hardwick and Robertson (*Canad. J. Res.*, 1951, **29**, 828) obtained values for the equilibrium constants of 3500, 200, and 20 for the successive formation of the species $CeSO_4^{2+}$, $Ce(SO_4)_2$, and $Ce(SO_4)_3^{2-}$ at 25° in solution of ionic strength 2·0. Thus in the present reduction experiments certainly less than 1 part in 2000, and probably as little as 1 part in 10^7, of the ceric ion is present as the simple aquated ion. From Hardwick and Robertson's values it follows that about 5% of the ceric ion is present as $Ce(SO_4)_2$ and 95% as $Ce(SO_4)_3^{2-}$. The slow reduction under these conditions is somewhat surprising in view of the widespread catalysis of electron transfer between metal ions by complex-forming anions, especially since the temperature-independent factor for reaction of $Ru(dipy)_3^{2+}$ with Ce^{4+} might be expected to be several powers of ten lower than that for its reaction with $Ce(SO_4)_3^{2-}$, where the reacting ions are of opposite charge.

These results are particularly interesting in connection with Libby's recent theoretical discussion of the factors governing the speed of electron transfer (*J. Phys. Chem.*, 1952, **56**, 863). They may be summarised thus: (i) Electron exchange between simple aquated ions like $Fe^{3+}_{aq.}$ and $Fe^{2+}_{aq.}$ is relatively slow, since it results in the production of a ferrous ion in the solvation environment of a ferric ion and *vice versa*, entailing activation energy to bring about the necessary rearrangement of the water molecules in the solvation shells. (ii) The magnitude of this activation energy is reduced if the ions form firm complexes so that both valency states are symmetrical and of the same dimensions to within the amplitude of the zero-point motion in the ground state. If this conditions holds, then no rearrangement of the co-ordination shell accompanies the transfer. (iii) In an exothermic oxidation–reduction between different species, the energy being liberated can lower the energy of activation otherwise required for the rearrangement of solvation and co-ordination shells.

Libby suggests that (i) and (ii) account for the slowness of the $Fe^{3+}{}_{aq.}$–$Fe^{2+}{}_{aq.}$ exchange compared with $Fe(CN)_6{}^{3-}$–$Fe(CN)_6{}^{4-}$. The speed of the reactions between complex ions listed in Table 2 would also follow from (ii) and (iii). However, the relative slowness of the $Os(dipy)_3{}^{3+}$–$Os(dipy)_3{}^{2+}$ exchange compared with the speed of the reaction between $Fe(phen)_3{}^{3+}$ and $Fe(dipy)_3{}^{2+}$, $Ru(dipy)_3{}^{3+}$ and $Fe(dipy)_3{}^{2+}$, etc., is surprising, for all these species apparently involve firm bonding in Libby's sense, like the $Fe(CN)_6{}^{4-}$ and $Fe(CN)_6{}^{3-}$ ions. This could be explained in terms of (iii) if activation energy were still an important consideration in these reactions, for whereas the exchange is thermoneutral, the reactions between the different complex ions are very likely to be exothermic, as a consideration of the entropy changes in relation to the overall free-energy decrease shows (see Baxendale and George, *loc. cit.*). In two cases, however, the exothermicity is not likely to be very large. In the $Fe(phen)_3{}^{3+}$–$Fe(dipy)_3{}^{2+}$ reaction it will be about 2 kcal./mole in view of the small difference in oxidation–reduction potential and the great similarity between the structures of the complex ions. Calculations, details of which are given later, suggest that the Ce^{4+}–$Ru(dipy)_3{}^{2+}$ reaction is also exothermic but only to the extent of 1·3—3·3 kcal./mole. This reaction is one where Libby's considerations would predict a relatively slow rate, since a cerous ion is formed in the unfavourable solvation environment of the ceric ion : yet it is far faster than the Ce^{4+}–Ce^{2+} exchange. These two cases suggest that a small favourable free-energy or heat change can result in a great increase in the rate of the electron exchange. The possibility of small changes being significant in this way is borne out by a comparison of the speed of reduction of ceric sulphate by $Ru(dipy)_3{}^{2+}$, $Fe(5\text{-nitrophenanthroline})_3{}^{2+}$, and $Fe(phen)_3{}^{2+}$. Although the mechanism is obscure, the change in rate constant from about 4×10^3 to $>10^5$ l. mole^{-1} sec.$^{-1}$ can be correlated with the increasingly favourable free-energy change which has values of —3·2, —4·4, and —6·9 kcal./mole respectively; and in all probability, since the three complex ions have very similar structures, the exothermic heats of the reactions increase likewise.



*First-order plots for the reaction between thallic perchlorate and tris-*o-*phenanthrolineferrous perchlorate at 25° and 34° in 2·7*M-$HClO_4$ *solution. In each case the thallic perchlorate was in excess, 3·6 × 10^{-2}*M *being present compared with an initial concentration of the ferrous complex of about 5 × 10^{-5}*M; E *is the optical density of the solution at 510 mμ.*

Whatever the detailed physical mechanism of these reactions may be, it is abundantly clear that high charges of the same sign on the two reacting species do not necessarily entail slow electron transfer. There is, however, always the possibility that special features are present, such as far larger effective collision diameters, so that no strict comparison is possible with the behaviour observed in bond-breaking reactions.

(B) *Two-electron Transfer Reactions between a Metal Ion and Co-ordination Complexes.*— The other slow reactions of complex ions encountered in this investigation were those involving the Tl^{3+}–Tl^{+} couple, which requires the transfer of two electrons. The oxidation of Tl^{+} by $Ru(dipy)_3{}^{3+}$ in 0·5M-nitric acid is extremely slow, with a half-time of several hours at room temperature. The reduction of Tl^{3+} by $Fe(phen)_3{}^{2+}$ in perchloric acid solution is faster and the progress of the reaction can be followed easily (see Figure). The bimolecular velocity constant in 2·7M-perchloric acid at 25° is approx. $7{\cdot}3 \times 10^{-3}$ l. mole^{-1} sec.$^{-1}$. The reaction has an apparent activation energy of about 20 kcal., and is faster in less acid solution, with a hydrogen-ion dependence of approximately inverse first power, indicating that a partly hydrolysed thallic ion is an important reacting species. This behaviour is in many respects similar to that found for the reduction of Tl^{3+} by simple aquated Fe^{2+} ions (Johnson, *J. Amer. Chem. Soc.*, 1952, **74**, 959), where the observed bimolecular velocity constant can be expressed as the sum of two terms appropriate to two paths the reaction can take. If *h* stands for the hydrogen-ion concentration, then

$$k_{\text{obs.}} = \frac{K_1 k_1}{K_1 + h} + \frac{k_2 K_2}{h} \frac{K_1}{K_1 + h}$$

Johnson identified the first term with the reaction between Fe^{2+} and $TlOH^{2+}$, where k_1 is the velocity constant and K_1 the equilibrium constant for the ionisation $Tl^{3+} + H_2O \rightleftharpoons TlOH^{2+} + H^+$, and the second term with the reaction between Fe^{2+} and TlO^+, where k_2 is the velocity constant and K_2 the equilibrium constant for the second ionisation $TlOH^{2+} \rightleftharpoons TlO^+ + H^+$. K_1, k_1, and k_2K_2 have the values $1{\cdot}43 \times 10^5$ exp. $(-5890/\boldsymbol{R}T)$ l. mole^{-1}, $4{\cdot}33 \times 10^{11}$ exp. $(-18{,}400/\boldsymbol{R}T)$ l. mole^{-1} sec.$^{-1}$, and $1{\cdot}89 \times 10^{14}$ exp. $(-22{,}060/\boldsymbol{R}T)$ sec.$^{-1}$, respectively. Calculation shows that under the above conditions used for the reduction by $Fe(phen)_3^{2+}$, simple ferrous ions would react preferentially with $TlOH^{2+}$ rather than with TlO^+, for the velocity constants have the values $9{\cdot}8 \times 10^{-3}$ and $3{\cdot}2 \times 10^{-3}$ l. mole^{-1} sec.$^{-1}$, respectively. There is very little difference therefore between the net velocity constant for reduction by simple ferrous ions and the $Fe(phen)_3^{2+}$ complex, *i.e.*, $13{\cdot}0 \times 10^{-3}$ and $7{\cdot}3 \times 10^{-3}$ l. mole^{-1} sec.$^{-1}$. However, since in 2·7M-perchloric acid the factor $K_1/(K_1 + h)$ is close to unity, any marked hydrogen-ion dependence must arise through the second ionisation, and these exploratory experiments thus suggest that TlO^+ is the chief species reacting with the complex-ion reducing agent. The overall activation energy of 20 kcal. is close to that of 22·0 kcal. for the simple ferrous ion reacting with TlO^+. This is another example where the activation energy for the reduction of an ion is greater than that of the electron-exchange reaction which, for the Tl^{3+}–Tl^+ system, has a value of 14·7 kcal. (Harbottle and Dodson, *loc. cit.*). The different features which the reduction by the complex ion presents, particularly considerations of hydration energy and its transfer along the lines of Libby's theoretical discussion summarised above, make it a very interesting system for further investigation.

(C) *Electron-transfer Reactions of Hæmoglobin.*—We have recently shown (*Biochem. J.*, 1953, **53**, xxv; *Science*, 1953, **117**, 220) that the ferric forms of the hæmoproteins myoglobin and peroxidase can be oxidised by the single-electron transfer reagents $IrCl_6^{2-}$ and $Mo(CN)_8^{3-}$ to compounds having the redox properties of quadrivalent iron compounds. These appear to be identical in physical and chemical properties with the intermediate compounds formed by hydrogen peroxide and alkyl hydroperoxides, which were long considered to be the peroxide complexes, Fe_p^{3+}–O·OH and Fe_p^{3+}–O·OR, where Fe_p^{3+} denotes the ferriprotoporphyrin iron atom. Cationic oxidising agents of comparable or greater oxidising power like $Fe(phen)_3^{3+}$ and $Ru(dipy)_3^{3+}$ failed to give these compounds, so an examination of the oxidation of other hæmoprotein derivatives, where the more familiar valency change $Fe_p^{2+} \longrightarrow Fe_p^{3+}$ occurs, was undertaken.

TABLE 4. *Reaction of hæmoglobin and myoglobin derivatives with oxidising agents in* 0·04M-*phosphate buffer solution* (pH 6·0) *at room temperature.* (Fe_p^{2+} and Fe_p^{3+} represent the ferrous and ferric protoporphyrin iron atoms.)

Reactants	Hb, *i.e.*, Fe_p^{2+}	HbO_2, *i.e.*, $Fe_p^{2+}O_2$	HbCO, *i.e.*, $Fe_p^{2+}CO$	MetMb, *i.e.*, Fe_p^{3+}
$Fe(CN)_6^{3-}$	Fast	Fast	Slow, dissociation determines rate	No reaction
$IrCl_6^{2-}$	Fast	Fast	Fast	Fast (pH > 8)
$Ru(dipy)_3^{3+}$	Alternative reactions of the ruthenium complex ion occur with all four derivatives			

The results are given in Table 4, which includes for comparison the action of $Fe(CN)_6^{3-}$ well established by many biochemical studies. $IrCl_6^{2-}$ was found to oxidise all the ferrous derivatives, hæmoglobin, oxyhæmoglobin, and carbonmonoxyhæmoglobin, extremely rapidly to methæmoglobin, the corresponding ferric compound, with velocity constants greater than 10^5 mole^{-1} sec.$^{-1}$. The further oxidation of methæmoglobin to its higher oxidation state, Fe_p^{IV}, was avoided by keeping the pH <7, for there is evidence that the effective redox potential of the Fe_p^{IV}–Fe_p^{3+} couple in such systems is hydrogen-ion dependent such that $IrCl_6^{2-}$ is not a sufficiently strong oxidising agent to effect this oxidation step in more acid solutions. For the same reason $Fe(CN)_6^{3-}$ at all pH values is too weak an oxidising agent to give the Fe_p^{IV} compound. In contrast, $Ru(dipy)_3^{3+}$ was without effect on all these ferrous derivatives, which suggests that other reducing groups in the protein react preferentially. Tyrosine, tryptophan, and cysteine were all found to reduce $Ru(dipy)_3^{3+}$ extremely rapidly. On the other hand, $IrCl_6^{2-}$ reacted relatively slowly with these amino-acids. The slow oxidation of tyrosine and tryptophan in proteins even by mild oxidising agents like $Fe(CN)_6^{3-}$ is well known from the work of Mirsky and Anson (*J. Gen. Physiol.*, 1936, **19**, 451). The different action of the strong cationic and anionic oxidising agents may therefore be attributed to competition reactions of this sort.

A comparison of the rates at which $IrCl_6^{2-}$ and $Fe(CN)_6^{3-}$ react with carbonmonoxy-hæmoglobin suggests that the reaction follows a different path in the two cases. It is generally accepted that $Fe(CN)_6^{3-}$ only reacts with free Hb according to the mechanism

$$\text{HbCO } (i.e., \text{ Fe}_p{}^{+2}\text{CO}) \overset{\text{Slow}}{\rightleftharpoons} \text{Hb } (i.e., \text{ Fe}_p{}^{2+}) + \text{CO}$$

$$\text{Hb } (i.e., \text{ Fe}_p{}^{2+}) + \text{Fe(CN)}_6{}^{3-} \xrightarrow{\text{Fast}} \text{MetHb } (i.e., \text{ Fe}_p{}^{3+}) + \text{Fe(CN)}_6{}^{4-}$$

in which the rate-determining step is the slow dissociation of HbCO. Good evidence for this comes from Millikan's study of the corresponding reaction with muscle hæmoglobin, *i.e.*, myoglobin, which may be denoted by Mb (*Proc. Roy. Soc.*, 1936, *B*, **120**, 366). The rate of oxidation of MbCO by ferricyanide was found to increase by only 60% for a ten-fold increase in ferricyanide concentration. On the basis that the rate at the lower concentration corresponded to the dissociation rate of MbCO, the equilibrium constant for the reaction $\text{Mb} + \text{CO} \rightleftharpoons \text{MbCO}$, calculated by using the value for the forward velocity constant, was numerically consistent with a direct determination of the equilibrium constant. Whilst the slight increase in rate as the ferricyanide concentration is increased may be evidence for some direct reaction between MbCO and $Fe(CN)_6^{3-}$, the far greater speed of the $IrCl_6^{2-}$ oxidation of HbCO compared with its dissociation rate is good evidence that a direct oxidation of the carbonmonoxy-compound as such is possible. This is significant because it shows that the covalent bonding of the iron in the carbonmonoxy-complex does not protect it against oxidation. The ready oxidation of ferrocytochrome-*c*, where the iron is also covalently bonded, affords another example with a hæmoprotein (Theorell, *J. Amer. Chem. Soc.*, 1941, **63**, 182), and many more can be found amongst co-ordination complexes of ferrous ion, *e.g.*, $Fe(dipy)_3^{2+}$ and $Fe(phen)_3^{2+}$. This conclusion is of importance with regard to the mechanism of the oxidation of hæmoglobin and myoglobin to their respective ferric states by molecular oxygen; for these reactions, which show extremely complicated kinetic behaviour, can in part be accounted for by a reaction scheme in which oxyhæmoglobin and oxymyoglobin, both of which also contain the iron covalently bound, are oxidised directly by some free-radical intermediate (George and Stratmann, *Biochem. J.*, 1952, **51**, 418).

According to the above equation the direct oxidation of HbCO should lead to the liberation of gaseous carbon monoxide. Evolution of the appropriate amount of gas was confirmed by an experiment using a Warburg manometer. The use of $IrCl_6^{2-}$ in this way offers a possible method for the gasometric estimation of HbCO similar to that of HbO_2, where oxidation by $Fe(CN)_6^{3-}$ can be used successfully because the dissociation step is extremely rapid.

EXPERIMENTAL

Dipyridyl- and Phenanthroline-ferrous Complexes.—These were prepared by accurately weighing ferrous sulphate to give the desired concentration, dissolving it in dilute acid, and adding the requisite amounts of 2 : 2′-dipyridyl and 1 : 10-phenanthroline respectively. The ferric derivatives were prepared from these by oxidation with lead dioxide and a little concentrated nitric acid and filtering through a sintered-glass funnel to remove the excess of dioxide.

Dipyridyl Ruthenium Complex Chloride.—The bivalent salt was prepared according to Burstall (*J.*, 1936, 173), and a stock solution converted into the tervalent compound by the same procedure as above.

Potassium Molybdocyanide.—This was prepared by Willard and Thielke's method (*J. Amer. Chem. Soc.*, 1935, **57**, 2609). After three recrystallisations of the crude product, titration with potassium permanganate showed the sample to be about 99% pure. Stock solutions were accordingly made up by weight.

Thallic Perchlorate Solution.—This was made by diluting a solution of thallic hydroxide in 60% perchloric acid to a suitable concentration. The hydroxide was prepared according to the method of Sherrill and Haas (*ibid.*, 1936, **58**, 953). The thallic perchlorate was standardised with potassium bromate (Noyes *et al.*, *ibid.*, 1935, **57**, 1232).

Hæmoglobin Derivatives.—These were obtained from pure oxyhæmoglobin prepared from horse blood and purified according to Keilin and Hartree's method (*Proc. Roy. Soc.*, 1935, *B*, **117**, 1).

All other reagents used were of the highest analytical grades.

All the reactions mentioned in this paper involved a highly coloured reagent, so their rates could be followed by observing colour changes. In all cases, except in the reaction of thallic perchlorate with ferrous phenanthroline, a lower limit is given for the velocity constant. In these cases the reaction was instantaneous with the lowest convenient concentration of reactants, *viz.*, about 10^{-5}M. In this reaction the colour change of the ferrous complex at 510 mμ was followed by using a Unicam quartz spectrophotometer. The concentration of the thallic salt in these experiments was $3{\cdot}6 \times 10^{-2}$M, and that of the ferrous complex $5{\cdot}0 \times 10^{-5}$M. Under these conditions reasonably good first-order coefficients were obtained. Examples are given of runs at 25° and 34° (see Figure).

Except reactions involving ceric ion, which were carried out in approximately M-perchloric acid, all reactions were carried out in approximately 0·001M-nitric acid.

Calculation of Heat of Reaction between $Ce^{4+}_{aq.}$ *and* $Ru(dipy)_3^{2+}$.—The entropy change in the Ce^{4+}–Ce^{3+} cell reaction is $+22{\cdot}4$ e.u., based on values for the partial molal entropies of $Ce^{4+}_{aq.}$ and $Ce^{3+}_{aq.}$ of $-75{\cdot}6$ and $-37{\cdot}6$ e.u. calculated from Powell and Latimer's empirical equation (*J. Chem. Phys.*, 1951, **19**, 1139) and 31·2 e.u. for the entropy of gaseous hydrogen. The oxidation reduction potential in about 1·0M-acid is 1·61 v for nitric acid and 1·70 v for perchloric acid. From these data ΔH for the cell reaction is found to be $-30{\cdot}5$ and $-32{\cdot}5$ kcal./mole, respectively.

The entropy change in the $Ru(dipy)_3^{3+}$–$Ru(dipy)_3^{2+}$ cell reactions may be assumed to be identical with that for the corresponding osmium complexes, *i.e.*, 2·6 e.u. as calculated from the data of Barnes, Dwyer, and Gyarfas (*Trans. Faraday Soc.*, 1952, **48**, 269). Its oxidation–reduction potential is 1·30 v (Dwyer, *J. Proc. Roy. Soc. N.S.W.*, 1949, **83**, 134), and hence ΔH for the cell reaction is $-29{\cdot}2$ kcal./mole.

From these two ΔH values it follows that the electron-transfer reaction

$$Ce^{4+}_{aq.} + Ru(dipy)_3^{2+} \longrightarrow Ce^{3+}_{aq.} + Ru(dipy)_3^{3+}$$

is exothermic to the extent of about 1·3—3·3 kcal./mole.

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