

0.5 micron apart are clearly resolved. The specimen was deposited on a collodion film placed 0.2 mm. in front of the plate. The X-ray tube had a 10-micron aluminium window, and the space between this window and the plate was evacuated. The reflector was 4 cm. long and the specimen placed 16 cm. from the focus. These dimensions were chosen to make use of existing gear. The focus-specimen distance can be reduced to at least half without affecting the resolution. This would reduce the exposure time to about 10 min. Another factor of 2 could be gained by using a tungsten anti-cathode instead of the copper target of our tube. The glancing angle by which the X-rays are reflected, and hence $\lambda(\text{min.})$, changes slightly across the object plane. The effect is easily calculated and amounts to about 2 A./mm. across the specimen for a focus to specimen distance of 16 cm.

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¹ Ehrenberg and Spear, *Proc. Phys. Soc.*, B, **64**, 67 (1951).

² Spear, *Proc. Phys. Soc.*, B, **64**, 233 (1951).

³ Engström, "Progress in Biophysics" (Butterworth, London, 1950).

⁴ Lamarque, *C.R. Acad. Sci., Paris*, **202**, 684 (1936).

⁵ Dauvillier, *C.R. Acad. Sci., Paris*, **190**, 1287 (1930).

⁶ Kirkpatrick and Baez, *J. Opt. Soc. Amer.*, **38**, 766 (1948).

⁷ Kirkpatrick, *Nature*, **166**, 251 (1950).

⁸ Ehrenberg, *J. Opt. Soc. Amer.*, **39**, 746 (1949).

Phosphorus Trifluoride-Haemoglobin

THERE are numerous examples of complex compounds formed by trivalent phosphorus compounds which have an electron pair available for the formation of dative bonds¹, and recently this knowledge has been extended by the preparation of phosphorus trifluoride complexes of divalent platinum². There is a close analogy between the lower halides of phosphorus and carbon monoxide in such compounds, and this analogy has been strengthened by the recent preparation of the tetrakis-trihalogenophosphine nickels, $\text{Ni}(\text{PCl}_2)_4^3$, $\text{Ni}(\text{PBr}_2)_4^4$, and $\text{Ni}(\text{PF}_2)_4^4$; the latter is a volatile liquid similar to nickel carbonyl, $\text{Ni}(\text{CO})_4$. It was therefore thought that phosphorus trifluoride might form a complex with ferrohæmoglobin similar to the well-known covalent complexes with carbon monoxide, nitric oxide, isocyanides, etc.⁵. The existence of such a phosphorus trifluoride-hæmoglobin compound has been established, providing further evidence for the covalent bonding ability of phosphorus trifluoride.

A solution of partially oxygenated human hæmoglobin was prepared (cf. ref. 5, p. 211) and portions of this solution were diluted with disodium hydrogen phosphate citric acid buffer solutions to give a hæmoglobin concentration about 1/200 that in whole blood. Hæmoglobin was then formed by reduction with sodium dithionate ($\text{Na}_2\text{S}_2\text{O}_4$), and portions of the solution were agitated with phosphorus trifluoride at various partial pressures in pure nitrogen. A pink colour, visually different from the colour of either oxyhæmoglobin or carbonmonoxyhæmoglobin, rapidly developed and the broad band of the hæmoglobin absorption spectrum centred around 560 $\text{m}\mu$ changed to give two sharp bands centred about 540 and 575 $\text{m}\mu$; a hand spectroscope with a scale was used for detection. No change in colour or absorption bands was evident on addition of excess dithionate.

The formation of the phosphorus trifluoride-hæmoglobin complex is not critically dependent on the pH of the solution in the range 5.0-8.1. The complex is unstable, persisting for only a few minutes. In alkaline solutions, the rate of formation is slower and the rate of decomposition faster than in acid solutions, presumably due to the increase in the rate of hydrolysis of phosphorus trifluoride with increasing pH.

It has proved impossible to measure the absorption spectrum of the compound with any accuracy due to the rapid hydrolysis; but a rapid survey of the spectrum in the region from 520 to 590 $\text{m}\mu$ was made on a Beckman spectrophotometer. Comparison with the spectra of oxyhæmoglobin (peak maxima at 576-578 $\text{m}\mu$ and 540-542 $\text{m}\mu$) and carbonmonoxyhæmoglobin (peak maxima at 568-572 $\text{m}\mu$ and 538-540 $\text{m}\mu$) measured in the same conditions shows the close similarity of the phosphorus trifluoride hæmoglobin (peak maxima 574-577 $\text{m}\mu$ and 540-543 $\text{m}\mu$) with the former. The intensity of the peaks appears to be similar and the α -peak is the sharper.

Again due to hydrolysis, estimation of the half-saturation pressure is impossible; however, the compound is readily formed with phosphorus trifluoride partial pressures of the order of 1 cm. mercury.

No evidence has been obtained for the formation of a hæmoglobin complex of nitrogen trifluoride, and the reported⁶ cyanosis due to nitrogen trifluoride was probably due to impurities. The inability of nitrogen trifluoride to form a compound with hæmoglobin is probably due to the absence of vacant $2d$ orbitals in the nitrogen atom, so that there is no possibility of the π -bonding which occurs in the phosphorus trifluoride complexes, where there is a vacant $3d$ orbital in the phosphorus atom. The nitrogen trifluoride used in this work was kindly given by Prof. E. G. Rochow of Harvard University.

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¹ Sidgwick, N. V., "The Chemical Elements and their Compounds" (Oxford, 1950).

² Chatt, J., *Nature*, **165**, 637 (1950).

³ Irvine, jun., J. W., and Wilkinson, G., *Science* (in course of publication).

⁴ Wilkinson, G., M.I.T. Laboratory for Nuclear Science and Engineering Progress Reports (1951).

⁵ Cf. Lemberg, R., and Legge, J. W., "Hematin Compounds and Bile Pigments" (Interscience Publishers, New York, 1949).

⁶ Ruff, O., *Z. anorg. Chem.*, **197**, 273 (1931).

Free Amino Groups of Collagen

USING the technique originated by Sanger¹ for the determination of end-groups in proteins by reaction with dinitrofluorobenzene, no free α -amino- or imino-groups have been detected in ox hide collagen², even when the equivalent of 2-3 gm. dinitrophenyl-collagen was placed on the silica gel columns.

After heating in water at 70° C., and treating with alkali, or urea, a small number of α -amino groups were found.