

was added and the excess of iodine was determined with 0.1 *N* sodium thiosulphate. The sample had to be so small that less than half of the iodine was consumed. In this way the following values of the equivalent weight were obtained in different titrations: 118.3, 118.3, 118.9. Calculated for triethylsilane: 116.3.

When determining the amount of triethylsilane in the syntheses described above, a sample of about 0.2 ml was removed for titration. One test was made when the reaction components were mixed, and from this zero value the composition of the mixture could easily be determined at any time by titration.

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A Comment on the Ultraviolet Light Absorption Spectrum of Cytochrome c

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The light absorption spectrum of ferrocytochrome c at neutral pH shows an absorption band at $316 \text{ m}\mu$ in addition to the protein band at $280 \text{ m}\mu$, the Soret band, and the bands in visible light. The $316 \text{ m}\mu$ -band has been interpreted as indicating the presence of a sulphoxide group². This interpretation became unprobable when the six sulphur atoms in cytochrome c could be accounted for (two thioether, two methionine, and two cystine sulphur atoms)^{3,4,5}. We have examined the spectra in the near ultraviolet range of the

Table 1. Wavelengths in $\text{m}\mu$ of absorption maxima.

Species	Histidine		Ammonia	
	Ox.	Red.	Ox.	Red.
Proto	Shoulder	327	Shoulder	327
Meso	347	322	350	317
Deutero	337	320	340	315

histidine and ammonia ferri and ferro meso-, proto-, and deuteroporphyrins. The results are given in Table 1 and Fig. 1.

The solutions (20 ml) were $10 \mu\text{M}$ in the haemin in question, 0.1 *M* in histidine (free base without chloride in phosphate, resulting pH 7.5) or 5 *M* in ammonia. After deaeration with nitrogen reduction was performed with Pt-H_2 under spectroscopic control. The solution was filtered anaerobically and transferred via a capillary tube directly to the cuvette (Beckman). This was sealed with a 4 mm rubber square, provided with a capillary outlet. A system of taps permitted the closed system, in-

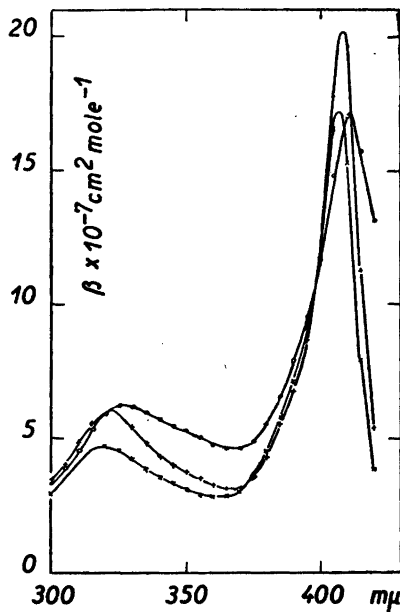


Fig. 1. Absorption spectra of ammonia proto- (O), meso- (+), and deuterohaemochromogens (x).

cluding the cuvette, to be washed free from air by means of nitrogen. The first 15 ml of the solution were used to rinse away remaining traces of oxygen. The solutions were stable in the cuvette for at least two hours. Fresh solutions were used for the determination of the base ferri porphyrin spectra, since reoxidation with air caused deterioration. We have found this arrangement — a number have been tried — to be most convenient for the handling of solutions of this kind.

The base ferro porphyrins (haemochromogens) gave one band within the range 315–330 $m\mu$. The substitution of the hydrogen atoms at the β -positions 2 and 4 in deuterohaemin by two vinyl or two ethyl groups to give protohaemin and mesohaemin caused shifts in wavelength of the band analogous to what is found in visible light. We conclude that the 316 $m\mu$ -band in ferrocytochrome c is to be attributed to its nature of being a haemochromogen. Notably the spectra of oxy- and carboxyhaemoglobin⁶ and of ferrocytochrome b₅⁷, which contain protohaemin, possess a band at 330–340 $m\mu$.

The ferricytochrome c spectrum shows an absorption band at 364 $m\mu$ ¹. It is present also in base ferri porphyrin and free ferri porphyrin spectra, and its position varies as above with the substitution at the positions 2 and 4. Most, possibly all, haemoproteids in the threevalent state show a peak at 350–370 $m\mu$ or a shoulder adjacent to the Soret band. The "sharpening" of the Soret band upon reduction is partly caused by its migration 10–15 $m\mu$ towards red and the vanishing of the band or shoulder at 350–370 $m\mu$.

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Note on the Potassium Bromide Disk Technique for Measurements of Infrared Spectra

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Attempts to find a general method for quantitative measurements of infrared absorption of solids have led to the development of the potassium bromide disk technique (Stimson¹, Schiedt^{2,3} and their associates, *cf.* also⁴). A few milligrams of the sample are mixed with some hundred milligrams of potassium bromide powder, the mixture pressed to a disk and the absorption of the disk measured. It is evident that the sample must be uniformly distributed throughout the disk in order to obtain quantitative results, and that the particles of the sample must be so small that further dispersion does not increase the absorption. The technique has been developed by Andersen and Woodall⁵ for samples as small as 0.01 mg by using a beam-condensing system of silver chloride lenses to reduce the area of the sample beam.

We have used the potassium bromide technique in an investigation of the infrared absorption of furan compounds. Our disks were prepared essentially after the directions of Stimson¹ and of Schiedt^{2,3}, but the mixture of sample and potassium bromide was not evacuated during the pressing (*cf.*⁵). The pressing tools described by Schiedt were simplified. The potassium bromide powder was prepared by precipitation of an aqueous solution of the salt with acetone. This way of preparing powder, which gives transparent disks, is simpler than grinding. The technique was not only used for solids but also for viscous liquids.

At first we found it difficult to distribute the sample uniformly and in sufficient dispersion throughout the disk. Grinding the sample in a mortar^{1,2} or in a small ball