

# GUANIDINE FERRIDEHEMOGLOBIN AND THE CARBIMID STRUCTURE OF "CYANAMIDE"

## THE RELATIONSHIP OF CHEMICAL STRUCTURE TO CHOLINERGIC ACTIVITY

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The capability of combination of ferrihemoglobin with the majority of known hydroacid anions suggested that this type of combination was due to the comparatively small ionic diameter of the latter with their consequent ability to penetrate the interstices of the porphyrin ring and come within sufficient proximity of the  $3^2$  electronic orbit of the iron atom to permit polar covalent combination.<sup>1,2,3</sup> This capacity would be facilitated by a distortion of the octahedral iron-porphyrin lattice whereby at least one of these interstices is widened. Such a lattice distortion for ferrihemoglobin has been postulated by Coryell and Stitt<sup>4</sup> who regard combinations of the ferrideheme type as exemplifying a heptacovalency of the iron. Since ferrideheme combinations of ferriheme and the ferrihemochromogens are rare while those of ferrihemoglobin are frequent, it is reasonable to assume a lattice distortion in the last case through its iminazolyl coordination<sup>5</sup> (Fig. 2). Heptacovalency would then account for combination with any of the ordinary anions. This, however, does not occur; ferrideheme formation potentiality is limited to a specific group; the hydroacid anions. With the discovery of O-benzoic sulfinide ferridehemoglobin<sup>2</sup>—the anion in this case being relatively large—it became apparent that size of the hydroacid anion was not the conditioning factor and search was made for other chemical common property characteristics of this class of anions. Stereochemical representation of the O-benzoic sulfinide radicle showed an acute polar tenaille. Since an acute tenaille would permit approximation of the polar group to the  $3^2$  orbit of the iron, the other ferridedheme formers were examined in this regard. Acute polar tenailles were demonstrable for all with the single exception of cyanamide as depicted by the ordinarily ascribed carbamonitrile configuration (Fig. 3).

However this configuration is at variance with the low dipole moment of cyanamide ( $\mu_{20^\circ\text{C}} = 3.8\text{ D}$ )<sup>6</sup> and the Raman spectrum suggests that some of the material in solution is in the carbimid form.<sup>7</sup> This form, rendered in Fig. 4 is isosteric with carbon dioxide and its ionized form would have two acute tenailles. Such a representation, if correct, would indicate that cyanamide is an anammonide of guanidine rather than (as the carbamonitrile) an anhydride of urea. There are some physiologic and pharmacologic bases for regarding the former relationship to exist. (1) Administration of arginine to cystinuric individuals results in the excretion of cyanamide.] (2) Cyanamide is available for the *in vitro* synthesis of creatinine.<sup>9</sup> (3) Cyanamide and guanidine are both cholinergic drugs; the fibrillary somatic twitching of cyanamide intoxication being abolished by atropine<sup>10</sup> as is the same feature of guanidine poisoning.<sup>11</sup> Because of the definitely close pharmacologic, and a possible chemical relationship of the two substances one might anticipate ferrideheme formation on the part of a solution of guanidine the aresonant form of which has an acute polar tenaille (the ionizable imino group). But since the bulk of such a solution would consist of the resonating guanidinium ion<sup>12</sup> a large molar excess should be necessary to demonstrate such combination.

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## EXPERIMENTAL

Ferrihemoglobin solutions were prepared by the treatment of laked, washed human erythrocytes with (A) ferricyanide solution, (B) azochloramide or (C) saturated aqueous iodine. All pigment solutions were then diluted to a ferrihemoglobin concentration of 0.4 millimolar. Samples of each solution were brought

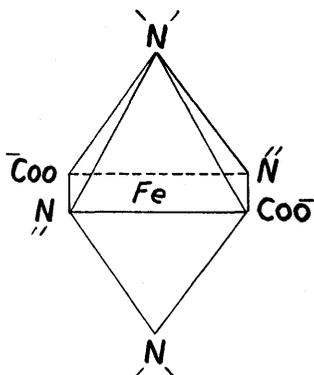


FIG. 1

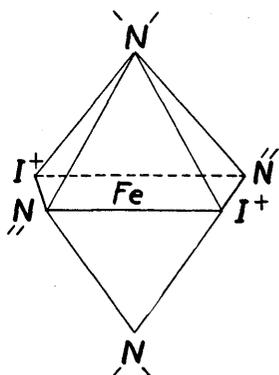


FIG. 2

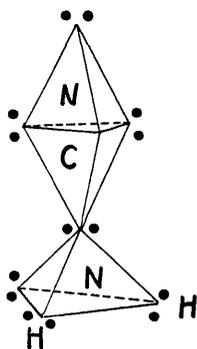


FIG. 3

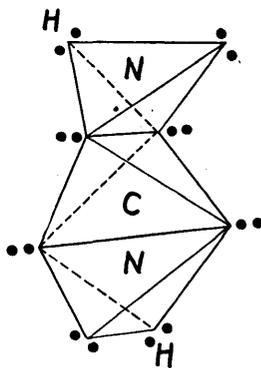


FIG. 4

FIG. 1. Stereochemical representation of axially symmetric octohedral hexagonal ferriheme pattern in which the carboxyls are coordinated in the  $4^{\circ}$  electronic orbit of the iron without distortion of the porphyrin lattice.

FIG. 2. Asymmetric porphyrin lattice of ferrihemoglobin. Distortion of one or more of the octohedral facets is brought about by coordination with the quarternary iminazolyl nitrogen of the globin histidine. By this agency one or more interstices in the  $4^{\circ}$  coordination shell is widened permitting polar covalent linkage with the iron in the  $3^2$  electronic orbit by anions or negative radicles with an acute polar tenaille.

FIG. 3. Carbamonitrile structure of cyanamide showing two obtuse polar tenailles on the same nitrogen atom. For purposes of representation in this and Fig. 4, the molecule is depicted in its undissociated state; the tenailles being in polar covalency with hydrogen.

FIG. 4. Carbimid structure of cyanamide; a symmetrical molecule with two acute polar tenailles each of which is on a separate nitrogen atom.

to varying guanidine concentrations ranging from 0.1 to 2.0 Molar by the addition of guanidine hydrochloride. After various intervals the reaction mixtures were subjected to spectroscopic examination.

Concentrations of guanidine less than 0.3 M are without effect on the color or spectroscopic features of ferrihemoglobin solution. Concentrations between 0.3 and 1 M produce a gradual color change from brownish to scarlet and the ferrihemoglobin absorption band in the red region of the spectrum is replaced by a diffuse single band with indefinite limits ranging from about 5400 to 5900  $\text{\AA}$ .

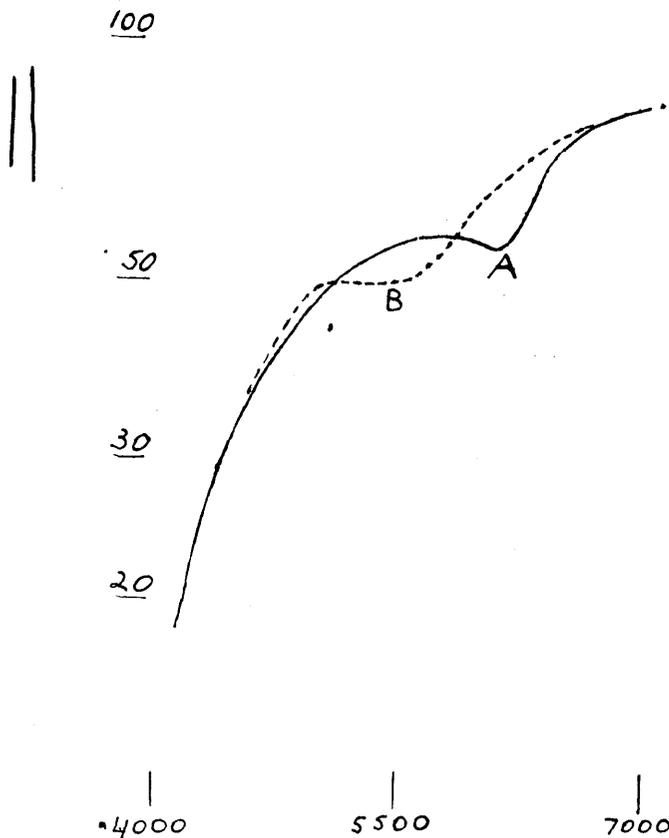


FIG. 5. The Effect of Sulfhydryl on the Spectrophotometric Absorption Curve of Lithium Ferriheme. The ordinates represent percentage transmissions and the abscissae, wavelength in decimicrons. Curve A shows the absorption of lithium ferriheme solution while curve B shows the absorption of an identical solution containing a molar equivalent of cysteine. The shift in spectral absorption which accompanies ferrideheme formation is qualitatively similar to that caused by the addition of guanidine or cyanamide to ferriheme and the direction and magnitude of this shift is best explained on the basis of polar covalent combination in the  $3d$  electronic orbit of the iron. Such inner orbital covalency may be an example of a theoretically possible interorbital type of resonating structure.

(This is the region in which all of the ferridehemoglobins exhibit their single absorption band). Guanidine concentrations above 1 M cause a rapid but evanescent appearance of this band after which denaturation occurs. Apparently guanidine reacts with ferrihemoglobin in a twofold manner; by ferrihemoglobin formation and

denaturation, the former being a zone reaction because of the intrusion of the latter. By stratification of a 0.4 M ferrihemoglobin solution upon a saturated guanidine hydrochloride solution in a test tube, a ring color reaction gradually develops within the ferrihemoglobin stratum and at the time of its greatest clarity and breadth (about 10 hours after stratification) is amenable to spectroscopic examination, upon which it is found to be practically indistinguishable from cyanamide ferridehemoglobin.

The experiments were controlled by testing the effect of guanidine on the pH of ferrihemoglobin solutions. This was deemed advisable even though the colored reaction product bore no spectroscopic resemblance to ferrihemoglobin hydroxide because the similarity of the guanidine and cyanamide-ferrihemoglobin reaction products might give rise to the suspicion that guanidine was converted to cyanamide during the course of the reaction. While the possibility of such a conversion is highly remote in this instance (on purely chemical grounds) it is ruled out by the absence of pH change in the reaction mixture as any such change must be accompanied by the liberation of ammonia. Cyanamide in 5 Molar concentration does not denature ferrihemoglobin while denaturation is evident in the presence of one-tenth this molar concentration of guanidine.

#### DISCUSSION

It appears probable that the colored reaction products of cyanamide and ferrihemoglobin on the one hand and of guanidine and ferrihemoglobin on the other, while not identical have a similarity conditioned by that of their respective prosthetic groups. An actual similarity of these groups would be possible only on the assignment of the carbimid structure to cyanamide.

The manner in which ferrideheme formation potentiality governs the cholinergic activity of a compound is not clear though, with the exception of acetylcholine (which might, *in vivo*, assume a transitional resonant state) this feature is so common to cholinergic drugs that the property is difficult to regard as an artefact. It has been suggested that cholinesterase owes its enzymatic activity to a transitional element prosthetic nucleus.<sup>13</sup> This might explain the role of the acute polar tenaille in the anti-esterase cholinergics but it does not explain histamine, guanidine or pilocarpine activity unless it is assumed that the cell receptors, themselves, are also heme (or other transitional element prosthetic nucleus) conditioned. In this connection it is curious that those three amino-acids exclusively, which possess acute tenailles; arginine, histidine and cysteine, can block histamine action presumably by usurping its destined position in or on the receptor substance.<sup>14</sup> The formation of a ferrideheme by cysteine and the soluble iron-porphyrin derivative, lithium ferriheme<sup>15</sup> is shown by the comparative spectrophotometric curves in Fig. 5. In this case the determinant role of the acute polar tenaille (sulfhydryl) and the subsidiary role of general molecular species is further exemplified.

Whether or not the hypothesis, that polar covalent linkage in the juxtaperipheral orbit of a transitional element prosthetic nucleus, conditions cholinergic activity, should prove to be correct it will nevertheless have been a useful one. As a single example of this utility it may readily explain on a structural basis the verified histaminic effect of colloidal silica<sup>16</sup> which Habeeb<sup>17</sup> regards as the important pathodynamic feature of human silicosis. Any particle of colloidal silica, whether from quartz, cristobalite or tridymite is bound to have half of its presenting facets in the form of an acute tenaille composed of the electropositive silicyl ion. The latter is practically isomorphous with carbon monoxide (of definite cholinergic activity) and should have similar potentialities for ferrylheme formation.<sup>18</sup>

#### CONCLUSIONS

Guanidine reacts with ferrihemoglobin to form a pigment spectroscopically similar to cyanamide ferridehemoglobin. From this fact as well as from the

parallel cholinergic activities of cyanamide and guanidine the former is believed to have a carbimid structure.

The stereochemistry of cyanamide and certain other cholinergic substances is analyzed and it is postulated that cholinergic activity may be due to possession of an acute tenaille which may form a polar covalent linkage with the transitional element in the prosthetic nucleus of the cell receptor.

Colloidal silicon dioxide has a structure permitting combination with heme and it may be in this potentiality that the cholinergic effects of silica reside.

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