Controlled release of mercury into aqueous L-(-) histidine solutions: Biological implications of electrochemical studies

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Controlled release of mercury into aqueous L-(-) histidine solutions is examined in this study. The first effect of mercury on histidine is mercuration to yield two derivatives both of which are paramagnetic and contain monomeric Hg⁺ ion. In one of the compounds, mercuration is at the imidazole nitrogen while in the other it is at the alaninyl side chain. These two organomercurials undergo intramolecular transformations independently to yield urocanic and glutamic acids respectively. The net effect is the mercury-assisted deamination and oxidation of L-histidine. Urocanic acid also reacts with mercury to yield predominantly a Hg⁺ compound. The significance of the results to biological systems is considered. Monomeric mercurous mercury appears to be a potential intermediate in mercury toxification process. UV-vis, IR and EPR spectra of Hg⁺ compounds are discussed in the light of the results from cyclic voltammetry and dc and ac polarography.

Mercury is a pollutant of the environment and its toxicological effects are well-known. It has been noticed that there is often a time lag between the release of mercury into the environment and the onset of its effects. It has been estimated that ten to hundred years are required for inorganic mercury in sediments at the bottom of lakes and rivers to be converted into methyl mercury, an organic form dangerous to animal life. The generally less soluble inorganic mercurials are released in a slow and steady manner into the environment where they remain equilibrated with the biological molecules causing long term changes.

The thiophilicity of heavy metal ions makes S-containing protein systems very susceptible to their effects but biological systems contain a host of additional potential ligands, for example, the heterocyclic residues and the amino groups and the carboxylic acid groups. Therefore, the toxicity of organomercurials in living systems could be due as much to the Hg-non-S centres interactions as to the binding of Hg to S centres.

The imidazole ring is a fundamental component of nucleic acid (via the purines) and enzymes or proteins (via histidine) and is frequently involved in metal ion binding. Thus metalloenzymes often contain histidine bound metal ions and the structure and function of nucleic acids are mediated by certain metal ions that are believed to bind to the purine imidazole ring.

The present study provides an insight into the process that takes place when mercury comes in contact with biological molecules. We have examined the mercury binding sites of L-histidine (his) by dissolving mercury from a dropping mercury electrode (DME) into aqueous solutions of his. This technique of dissolution of mercury has been chosen since perfect potential control over the release of mercury can be achieved. Moreover, the ligand in the solution decides the type of mercury ion (mercurious or mercuric) released from mercury. Such a facility is strikingly superior to the generally adopted in vitro studies that make use only of compounds prepared in bulk from commercially available mercury salts (or oxides) and biological analogues. In other words, a more veritable simulation of mercury release into environment is achieved via controlled dissolution of mercury. This advantage is further
enhanced by the availability of a range of electroanalytical techniques to probe the mercury-electroactive species interaction. We have isolated and characterized Hg-his reaction products and identified the electrochemical events by dc and ac polarography and cyclic voltammetry.

The above technique of dissolution of metallic mercury is based on the fact that when a positive potential is applied to DME, mercury starts dissolving. The potential at which the dissolution begins depends on the medium. In non-complexing electrolyte solution such as KNO₃ or K₂SO₄, mercury dissolution begins at around +0.3 V (vs SCE) and a plot of current versus potential is exponential without limiting region(s). On the other hand species like halide ions or complexing ligands that react with mercury catalyse mercury dissolution; mercury dissolves at potentials less positive than +0.3V and polarographic waves with well-defined limiting regions appear. Thus, the technique of anodic dissolution of mercury serves the dual purpose of releasing mercury into the environment and also studying its interaction with molecules of interest by polarography or cyclic voltammetry as well as by a variety of other analytical tools.

Materials and Methods

L-(-) histidine and L-glutamic acid (Loba Chem) were of biochemical grade purity. Urocanic acid (Aldrich) was used as such. The experimental details for dc and ac polarography are described elsewhere. Cyclic voltammograms were recorded on a PAR instrument with a potentiostat/galvanostat (model 173), a current-to-voltage converter (model 176), a universal programmer (model 175) and XY recorder (model RE 008). A hanging mercury drop electrode (HMDE) was fabricated by adopting the procedure of Bellamy. UV and visible spectra were recorded on a Carl-Zeiss spectrophotometer and also on a Hitachi 320 spectrophotometer using 1 cm quartz cells. IR spectra were recorded on a Perkin Elmer 983 spectrophotometer using KBr in the range 5000-600 cm⁻¹ and polyethylene in the range 600-200 cm⁻¹. A polytech FIR-30 spectrophotometer was used in recording FT-FIR spectra. The EPR spectra of the samples in the powder form were recorded on an E4 Varian X band spectrometer. Constant potential mercury dissolution was carried out on a large mercury pool electrode of 0.1m diameter. The solution was thoroughly deaerated before allowing mercury dissolution to commence while purified nitrogen was allowed to slowly bubble through during dissolution. The isolation of the product from the electrolyte solution was also done under nitrogen atmosphere to avoid contact with air. For all electrochemical measurements, the supporting electrolyte used was unbuffered 0.1 M KNO₃ (GR, Sarabhai Merck) as our aim was to obtain organo-mercury derivatives of histidine which are formed in unbuffered solutions. However, for comparison ac polarography was also carried out by varying the pH between 6.3 and 7.2. Triply distilled water was used throughout.

Results and Discussion

As is seen from the AC polarograms, the interaction between anodically dissolving mercury and L-(-) histidine in the solution involves three definite processes represented by S, A and B in Figs 1 and 2. As evident from the ac polarogram, process A is the most prominent one and occurs at a peak potential of 0.110 V. Process B occurring at 0.250 V is much less pronounced than A. S is the least prominent and occurs as a shoulder to process A at 0.020 V.

In the concentration range, 2-50 mM of his, all the peak potentials, viz, \( E_p \)A, \( E_p \)B and \( E_p \)S, remain constant. Below 2 mM, S and B disappear and only A is observed. Linear plots passing through the origin are obtained for variation in peak currents of S, A and B with change in [his]. The characteristic
feature of the dc polarogram is the sharp fall in the
limiting diffusion current \( I_d \) after 0.21 V. The cleft
in the limiting region gradually decreases with de-
creasing [his] and disappears below 5 mM. The
half-wave potential, \( E_{1/2} \), remains constant with [his]
at 0.145 V. \( I_d \) varies linearly with \( h^{1/2} \) (\( h \) = height of
the mercury column), indicating a diffusion con-
trolled process. Plot of \( I_d \) versus [his] is linear and passes
through origin.

The second harmonic ac polarogram shows two
peaks for each process with a peak separation of 70
mV with a minimum in current at the peak potential
of the fundamental harmonic ac polarogram. Thus
all the processes, S, A and B, are shown to be one-
electron processes. For the well-defined peak A,
plots of \( \log([I_p/I]^{1/2} + ([I_p-I]/I)^{1/2}) \) versus \( E_{dc} \) are linear
with slope of 118 mV, confirming the one-electron
nature of the process\(^{13}\). Peak B appears on the ac
polarogram only on a cathodic scan, i.e., from more
positive to less positive potentials. It disappears on
anodic sweep i.e., from less positive to more positive
potentials. This indicates that process B is due to the
reaction of a product obtained from his-mercury in-
teraction with mercury.

**Mercuration of histidine : Potential Range: - 0.1 to
0.3 V**

Disolution of mercury in 0.4 mM his in 0.1 M
KNO\(_3\) was studied at (i) ac shoulder potential of
0.020 V, (ii) ac peak potential of 0.115 V, (iii) half-
wave potential of 0.14 V, and (iv) ac peak potential
of 0.25 V. UV spectra of the solution were recorded
at regular time intervals during the course of mercury
dissolution. In each case, \( \lambda_{max} \) of 211 nm for his\(^{14}\) is
shifted to shorter wavelengths as mercury dissolves
while a new peak appears at 262 nm. This new peak
is attributed to mercury as it is being dissolved in his
and absorbs in the UV region. However, it should
be noted that this value of \( \lambda_{max} \) 262 nm does not agree
with that of Hg\(_{2}^{2+}/Hg^{2+}\) at a \( \lambda_{max} \) 235 nm\(^{15,16}\) but is
in closer agreement with \( \lambda_{max} \) of monomeric mercu-
rous mercury at 272 nm reported by Faraggi and
Amozig\(^{17}\) and by us\(^{10,18}\) in mercury-tryptophan
studies. Thus like typtophan, histidine elicits
monomeric Hg\(^+\) ion from mercury.

The cyclic voltammogram (Fig.3) recorded in
this potential range indicates a reversible phenome-
non. The peak potentials of the anodic peak, \( E_{pa} \), and
cathodic peak \( E_{pc} \), are seen at 0.165 V and 0.100 V
respectively with a \( \Delta E_p \) of 65 mV indicating a
single electron transfer mechanism. The peak poten-

![Fig.2. Fundamental harmonic ac polarograms for the anodic dissolution of mercury in the presence of his in 0.1 M KNO\(_3\).](image-url)

![Fig.3. Cyclic voltammograms at different sweep rates for the anodic dissolution of mercury in the presence of 10 mM of his in 0.1 M KNO\(_3\). Inset is the plot of \( I_{pa} = I_{pc} \) versus v](image-url)
tials do not vary with scan rate. The ratio of the anodic to the cathodic peak current, \( i_a/i_c \) is \(-1\) with \( i_p \) varying linearly with \( v^{1/2} \), indicating a diffusion controlled process. Below a concentration of 15 mM of his, a cathodic shoulder appears at 0.02 V. Identical cyclic voltammograms are obtained irrespective of whether the potential is scanned from 0.1 V to 0.3 V or from 0.3 V to -0.1 V. Thus process A corresponding to peak A in the fundamental harmonic ac polarogram and to the peak in the cyclic voltammogram (Fig. 3) in the potential range -0.1 V to 0.3 V is conclusively proved to be a single-electron transfer reversible reaction.

In order to isolate the products formed by Hg-his interaction in this potential region, a series of constant potential mercury dissolution in 2.5 mM of his ion 0.1 M KNO\(_3\) was carried out separately at the ac peak potentials, \( E_pS \) 0.02 V and \( E_pA \) 0.110 V. The electrolysed solution was separated from mercury, concentrated by evaporation at 60°C, cooled to 10°C and then filtered. The solid residue was first repeatedly washed with water to remove nitrate and unreacted his, and then with acetone and finally dried in vacuum. A white solid, with an yellow tinge (S) and an yellow solid (A) were thus isolated from the constant potential mercury dissolution at \( E_pS \) and \( E_pA \) potentials respectively. The two compounds were characterised by diffuse reflectance, IR, FT-FIR and EPR spectra as compounds of monomeric mercurous mercury, Hg\(^+\) and his.

IR spectra of his and products S & A were recorded. The zwitterionic NH\(_3^+\) group or amine salts show absorption owing to N-H stretching at 3010 cm\(^{-1}\) (ref.19). This peak is unaffected in the IR spectrum of S while in A it becomes broader with a slight shift towards longer wavelength. This indicates a strong association of NH\(_3^+\) group with mercury\(^{20}\) in A. Imidazole exhibits three characteristic ring stretching frequencies at 1550, 1452 and 1451 cm\(^{-1}\) (ref. 19). Since his is an imidazole substituted amino acid, the frequencies at 1550, 1500 and 1458 cm\(^{-1}\) in the IR spectrum of his can be identified with those of the imidazole ring. The intensity of absorption of 1500 cm\(^{-1}\) is more for compound S than that for A. It is known that electron withdrawing substituents increase the intensity of ring stretching frequencies\(^{20}\), therefore, it can be inferred that in S, mercury is bonded at the imidazole moiety.

It should be mentioned that the ring stretching region of the imidazole ring is scanty in the IR spectrum of product A. The chelation of mercury by the \( \alpha \) -aminocarboxylic acid moiety can readily produce a five-membered ring. The similarity in geometry of the chelate ring with the imidazole ring, the presence of mercury and presence, if any, of traces of water might be some of the causes for masking of the imidazole stretching frequencies in A. Further, it is in this region that absorptions characteristics of NH\(_3\) and O-C=O also occur. Thus a clear-cut distinction between the functional groups becomes difficult.

The compounds S and A absorb in the lower frequency region at 560 cm\(^{-1}\) and 455 cm\(^{-1}\) respectively which can be attributed to Hg-N bonding\(^{20}\). FT-FIR spectra of products S and A do not show any absorption, that can be associated with the Hg-Hg bonding.

EPR spectra of S and A are shown in Fig.4. They are identical and exhibit sharp EPR signals. The hyperfine lines flanking the DPPH singal are indicative of paramagnetism of the compounds. The spectra are similar to the EPR spectrum of Hg\(^{+}\)-trp compound\(^{10}\), the multiplicities and intensities on either side of the standard singal do not coincide indicating anisotropic nature of the products. The EPR signals confirm the existence Hg\(^{+}\) (6s\(^1\) ion) in both the compounds S and A\(^{20}\).

Based on the above electrochemical and spectroscopic data, the following mechanism (Scheme 1) can be proposed for processes S and A.

A comparison of the electron densities at the nitrogens of pyrrole\(^{22}\), pyridine\(^{23}\) and imidazole\(^{24}\) and other properties has led to the understanding that in imidazole the nitrogen of C=N-C behaves like pyri-
dine nitrogen and that of C=N-H behaves like pyrrole nitrogen. Imidazole is generally regarded as being aromatic. The π-electrons of pyrrole nitrogen of imidazole are part of the aromatic sextet and the one pair of electrons on the pyridine nitrogen of imidazole is the only unshared pair. Hence an important conclusion is that the neutral imidazole molecule presents a single energetically favourable coordination site for a proton, the unshared pair of electrons on the pyridine nitrogen and the same is true for a metal ion.

It has been already shown that the shoulder S appears in the fundamental harmonic ac polarogram and cyclic voltammogram in the concentration range 50 to 2 mM of his. The change in pH in this concentration range as determined by external pH measurements of unbuffered solutions is from 7.53 at 25 mM his to 7.30 at 2 mM. The zwitterionic pH of his is 7.56 (ref.25) indicating that a major portion of the his molecule will be in the dipolar form. Under such conditions, mercuration of his may take place at the pyridine nitrogen at $E_{pS}$ potential as given below.

In unbuffered solutions, as in the present study, process A accompanies the above reaction in the higher concentration range of his and also continues to take place at low concentration region where the pH is much lower than the isoelectric pH. This indicates that process A is preferred over process S. This is once again proved when basicities of the three nitrogen of his are considered, i.e., $\text{NH}_2 >> \text{C-N} = \text{N-H}$. Hence the preferential attack of Hg will be on the primary amino nitrogen. The possibility of protonation of imidazole nitrogen at slightly acidic pHs cannot be ignored. In such a case, his becomes more cationic through the formation of imidazolium cation.

In the ac polarograms recorded for 5 mM of his, in the pH range 6.3-7.2, only process A was observed while the shoulder S was completely absent. This observation supports the above possibility.

**Decomposition of the Hg$^+$- his derivatives**

The two paramagnetic compounds described above were stable under inert atmosphere or in fresh deaerated chloroform solutions. Upon exposure to atmosphere or upon ageing in solution, they decomposed, expelling fine droplets of mercury and eventually turned diamagnetic, with a UV absorption maximum at 235 nm, characteristic of Hg$_2$S$^2+/Hg_2^+$ species. A similar observation has been made with Hg- tryptophan derivative in our previous study.

The polarogram of the decomposed samples gave a 2e reduction wave characteristic of Hg$^+$ (II) species.

**Deamination of histidine to form urocanic acid : Potential range -0.1 to 0.4V**

The cyclic voltammogram of 10 mM his in 0.1 M KNO$_3$ with 0.1 V as the starting potential and 0.4V as the final potential shows two anodic peaks: $a_1$ around 0.11V and $a_2$ around 0.32V in the first anodic sweep (Fig.5). On reversing the scan at 0.4V, five cathodic peaks (C$_1$ to C$_5$) are seen. In the second anodic scan, new anodic peak, $a_3$, at 0.22 V is noticed. The couples are $a_1/c_1$, $a_3/c_2$, and $a_3/c_3$. In the subsequent sweeps peak heights of $a_1$, $c_1$ and $c_5$ decrease while those of others increase until a steady state is reached. The peaks $a_1$, $c_1$ and $c_5$ are also present in the cyclic voltammogram recorded in the potential range -0.1 to 0.3V. It has been already established that no new peaks appear and the existing peaks remain unaffected in this potential region either on changing the initial scan direction or on repeating the sweeps several times. Therefore, it can be concluded
that the new peaks \( a_2, a_3, c_2, c_3 \) and \( c_4 \) are due to the products formed from the oxidation of his at potential > 0.3 V and < 0.4 V. This is further confirmed by the same cyclic voltammogram of his recorded in the cathodic direction starting from 0.4 V (Fig. 5). All the anodic peaks appear in the initial scan itself and the peak heights of \( a_1, c_1 \) and \( c_4 \) are much reduced indicating that the other peaks \( (a_2/c_2, a_3/c_3 \) and \( c_4 \) develop at the expense of the former peaks.

UV spectra (Fig. 6) of the products of dissolution of mercury into 0.4 mM his in 0.1 M KNO\(_3\) at constant potential 0.375 V at \( t = 30 \) min show that absorption due to mercury at 262 nm grows. Biological as well as many of the chemical reactions of his produce urocanic acid (uro) and glutamic acid (glu) as major oxidation products. The \( \lambda_{\text{max}} \) of uro is seen at 278 nm while that of glu at 206 nm. Therefore, it is concluded that the peak at 280 nm is due to uro.

Cyclic voltammograms recorded (between -0.1 V and 0.3 V) during the course of mercury dissolution are also shown in Fig. 6. The couple \( a_1/c_1 \) characteristic of his decreases in height while a new couple, \( a_2/c_3 \) develops at 0.22 V/0.14 V. This new couple can be identified with the couple \( a_2/c_3 \) in the representative cyclic voltammogram of uro (Fig. 5, curve 6).

**Reaction of urocanic acid with mercury**

Cyclic voltammogram of uro (Fig. 5) shows two anodic peaks, \( a_1 \) at 0.210 V and \( a_2 \) at 0.325 V and three cathodic peaks, \( c_1, c_2 \) and \( c_3 \) at 0.140, 0.250 and 0.185 V respectively. The couple at 0.200 V/0.140 V is more prominent when compared to the couple \( a_2/c_2 \) at 0.315 V/0.250 V. The one at 0.185 V is poorly developed and does not have a corresponding anodic peak indicating the irreversible nature of the process at this potential. This cathodic peak disappears when [uro] falls below 0.1 mM. The peak potentials of uro coincide with those of peaks \( a_2, a_3, c_2, c_3 \) and \( c_4 \) of his recorded between -0.1 V and 0.4 V. Cyclic voltammograms of uro are identical with respect \( E_p \) and \( I_p \) values irrespective of whether the potential is scanned from -0.1 V to 0.4 V or from 0.4 V to - 0.1 V.

The ac polarogram of uro (Fig. 7) shows two small peaks C and B at 0.250 V and 0.180 V respectively and a prominent one (A) at 0.145 V corresponding to the peaks \( c_2, c_3 \) and \( c_1 \) of the cyclic voltammogram.
Fig. 7. Fundamental harmonic ac polarograms for the anodic dissolution of mercury in the presence of uro (—) second harmonic ac polarogram of uro (•).

Fig. 8. UV spectra recorded during the macroelectrolysis of 0.005 mM of uro in 0.1M KNO₃ at (a) 0.25V, (b) 0.14V. [Reference cell contains 0.1M KNO₃]

of uro respectively. These peaks also coincide with the peaks c₂, c₃ and c₄ of his scanned in the potential region of -0.1 to 0.4 V. Thus the formation of uro from his at potentials between 0.3 V and 0.4 V is confirmed.

It is quite obvious now that peak B of the ac polarogram of his (Fig.1) is identical in all respects to the peak at 0.250 V of uro. Its appearance and disappearance depending on the starting potential, i.e., whether 0.6 V or -0.1 V respectively, can now be explained. The potential at which uro is produced from his is higher than the potential (0.25V) of its electrochemical reaction. When the potential is scanned anodically, i.e., starting from -0.1 V, the product of oxidation of his in the range 0.3-0.4 V is not available for reaction at 0.25 V. Hence no corresponding peak is seen on the anodic scan of the ac polarogram. On the other hand, with a starting potential of 0.6 V, the product is already formed for it to act as the reactant for the process at 0.25 V and hence the peak can be seen. The shift of $E_{pA}$ with dilution of his below a concentration of 2 mM may be explained on the basis of overlap of various reactions of the products.

The peak currents decrease and the peak potentials remain constant with decreasing [uro]. The peak at 0.185 V disappears below an [uro] of 0.01 mM. The peak-width at $1/2 I_p$ of peaks, A, B and C is 90 mV at [uro] < 0.04 mM. A plot of log $[(I_p/I_p)^{1/2} + (I_p/I_p)^{1/2}]$ versus $E_{dc}$ is linear with a slope of 111 mV for a one-electron process. This analysis could be done only for peak A. The second harmonic ac polarograms indicate that processes A and C of uro are one-electron process (Fig.7 curve 7).

Constant potential mercury dissolution of 0.05 mM uro in 0.1M KNO₃ was carried out at $E_{pA}$ potential of 0.140 V of uro. In the UV spectra, it is seen that $\lambda_{max}$ of uro at 278 nm decreases steadily while a new peak at 270 nm corresponding to Hg⁺ develops (Fig.8a). Optical density is very high for this absorption. The same dissolution carried out at a constant potential of $E_{pC}$ (0.250 V) shows UV spectra (Fig.8b) which are similar to that recorded at 0.140 V in the first 1 hr dissolution. As time lapses a new peak develops at 300 nm. However, the magnitude of absorbance at 300 nm is smaller indicating that the species responsible for absorption at 300 nm is produced in small amounts.

On the basis of the above observations, the following mechanisms may be proposed for processes A and C of uro.

Process B of uro at 0.185 V is too poorly defined for any precise measurement and can be attributed to some product of chemical reaction of mercurated uro.
These evidences adequately prove the formation of uro due to reaction of his with mercury at DME. The mechanism for the formation of uro from his is shown in Scheme 2.

**Mercury-ammonia reactions**

The deamination of mercurated his to form uro is accompanied by the liberation of ammonia. Ammonia reacts readily with mercury to form a highly insoluble mercury-ammonia complex having a stability constant of $pK_a = 17$ (ref.27). The formation of such an insoluble film of the complex blocks the electrode surface thus inhibiting the anodic reaction. Hence there is a sharp fall in the limiting current of the dc polarogram. The adsorption phenomenon is also revealed in the electrocapillary curves (Fig.9).

**Oxidation of histidine to glutamic acid : Potential range : -0.1 to 0.6V**

In addition to the peaks present in the potential range -0.1 to 0.4V, two new peaks are observed in the cyclic voltammogram recorded between -0.1V and 0.6V (Fig.10). The anodic peaks x and y occur at 0.41 V and 0.46 V with the corresponding cathodic peak x′ and y′ at 0.35 V and 0.42 V respectively. The couples x/x′ and y/y′ can be attributed to the products from his at potentials between 0.4V and 0.6V.

UV spectra recorded at regular intervals during the course of mercury dissolution at 0.55 V show that absorption at $\lambda_{max}$ of 211 nm due to his decreases.

Fig.9. Electrocapillary curves for L-histidine in 0.1 M KNO$_3$: [1, 0.1M KNO$_3$; 2, 12 mM his in 0.1 M KNO$_3$; 3, 50 mM his in 0.1 M KNO$_3$]

Fig.10. Cyclic voltammograms for the anodic dissolution of mercury in the presence of 10 mM of his (1 to 6) and of 10 mM of glu (7) in 0.1 M KNO$_3$ recorded between -0.1V and 0.6V at a sweep rate of 50 mV s$^{-1}$ [1 to 6 represent repeated cycles.]
while that at 204 nm increases with the duration of mercury dissolution. The latter wavelength corresponds to $\lambda_{\text{max}}$ of 206 nm of glutamic acid, glu.

The cyclic voltammograms (Fig. 11) recorded during mercury dissolution in 1.0 mM his at 0.55V and cyclic voltammograms of his in the potential range -0.1 V to 0.6 V (Fig. 10) are identical except that the shoulders $c_4$ and $c_5$ (which do not appear when [his] $<2$ mM) are absent in the former. The peaks $a_1$ and $c_1$, characteristic of his steadily decrease in height while the couples $x/x_1$ and $y/y_1$ develop. Since the formation of glu is suspected, the cyclic voltammogram of 10 mM glu in 0.1 M KNO$_3$ was recorded. Cyclic voltammogram of glu shows two reversible couples $x/x_1$ and $y/y_1$, at 0.400 V/0.355 V and 0.420 V/0.380V respectively (Fig. 10 curve 7). The same couples at the respective redox potentials are seen in the cyclic voltammogram of his (recorded between -0.1 V and 0.6V) and of electrolysed (at 0.55V) solution of his. This observation and the UV data confirm the formation of glu from his. The absence of absorption at 280 nm in the UV spectra indicates that uro is not formed at the potentials at which glu is formed.

A separate investigation on glu$^{11}$ has shown that glu produces anodic polarograms only when its concentration is above 0.5 mM. Therefore, it can be concluded that the amount of glu produced by his is less than 0.5 mM and hence the formation of glu is not clearly indicated in the ac polarogram of his. The probable mechanistic pathway of formation of glu from his is shown in Scheme 3.

The nature of the amino acid appears to determine the type of mercury ion released from elemental mercury. Histidine resembles tryptophan in that it generates Hg$^+$ ion from elemental mercury. This behaviour is contrary to that exhibited by other non-sulphur amino acids$^8$. Aspartic acid, glutamic acid, lysine and arginine produce only Hg$^{2+}$ or Hg$_2^{2+}$ ions from mercury, whereas glycine, alanine, valine, leucine, isoleucine, threonine, proline, hydroxyproline, asparagine, glutamine, phenyl alanine and tyrosine do not bring about any change on reaction with elemental mercury. Aspartic acid and glutamic acid each produce one mercurous (Hg$_2^{2+}$) and one mercuric (Hg$^{2+}$) compound during dissolution of mercury. The reactions of lysine and arginine are essentially the formation of hydroxy complexes of mercuric mercury. Therefore, it seems reasonable to presume that monomeric Hg$^+$ ion is an important

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Fig. 11. Cyclic voltammograms recorded every 1/2 hour during macroelectrolysis of his in 0.1M KNO$_3$ at 0.55V. Reference cell contains 0.1M KNO$_3$. [1 to 5 represent...
reactive intermediate in the biological toxification of histidine residues by metallic mercury. The situation with $\text{Hg}_2^{2+}$ or $\text{Hg}^{2+}$ contamination is different; almost all amino acid would precipitate these ions. In other words, amino acids exhibit selectivity in their reaction towards metallic mercury.

The technique of anodic dissolution of mercury has been successfully employed in the present study to effect a controlled release of mercury in aqueous L-(−) histidine solutions. The first effect mercury on L-(−) histidine is mercuration. Atleast two different mercuration modes have been identified; one at the imidazole ring and the other at the alaninyl side chain. Both the organomercury derivatives are paramagnetic and contain $\text{Hg}^+$ ion. The two Hg-his derivatives undergo intramolecular transformations separately to yield urocanic acid and glutamic acid respectively. In other words, mercury-assisted deamination and oxidation of his take place to produce respectively urocanic acid and glutamic acid. This is in agreement with the known applications of mercuration modes have been identified; one at the imidazole ring and the other at the alaninyl side chain.

Urocanic acid is not an intermediate in the conversion of L- histidine to glutamic acid. Urocanic acid also reacts with mercury to form atleast two Hg(I) compounds, the formation of one of the compounds being more pronounced than that of the other.

The conversion of L-(−) histidine by mercury into urocanic acid or glutamic acid suggests that the foremost effect of mercury poisoning would be the impedement of the normal catalytic functions of enzymes because histidine is an important active centre of enzymes such as ribonuclease. Similar disturbances could be envisaged wherever histidine residues are present.

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