Participation of L-Histidine in the Maintenance of Mitochondrial Integrity*

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ABSTRACT: L-Histidine has been identified as a unique factor in the mechanisms controlling the integrity of rat liver mitochondria. Histidine, while essentially ineffective alone, markedly increases the potency of respiratory substrate plus ADP in delaying the swelling of mitochondria, exposed to phosphate, calcium, thyroxine, or oleate-containing media. This effect is seen with all respiratory substrates tested, except succinate and is highly specific for the L isomer. In a media containing substrate, ADP, and oligomycin, histidine exhibits typical substrate concentration kinetics and has the same K_m in sucrose and KCl media. Respiratory rate, acceptor ratio, substrate transport, ATPase, and mitochondrial morphology are not significantly affected by histidine. It is concluded that energy, derived from the oxidation of substrate, with the auxiliary functioning of ADP, appears to be stabilized by L-histidine and is thus directed specifically toward the control of mitochondrial integrity.

The presence of the imidazole ring and its dissociable hydrogen confers upon L-histidine the ability, unique among amino acids, of effective buffering in the region of pH 6.1. Of the few suitable buffers having a pK in this region L-histidine is commonly used by several researchers. Because of its buffering properties, this compound was especially suitable for use in earlier studies on the mechanism of mitochondrial swelling (Connelly and Lardy, 1964). Since that time histidine has been used routinely even when the pH was near neutrality, where the buffer capacity was small, so as to keep the composition of the swelling media relatively consistent. Recent experiments have disclosed that in addition to its role as buffer L-histidine actually participates in the mechanism which maintains mitochondrial integrity in the presence of swelling agents. Attempts to ascertain the biochemical mechanisms which counteract the swelling process have disclosed primary roles for respiratory-linked substrate (D. R. Myron and J. L. Connelly, 1970, unpublished data) and ADP in a nonphosphorylating environment (Connelly and Hallstrom, 1967). This work shows that the combined effects of substrate and ADP are markedly enhanced by L-histidine, which could be a third primary factor in the maintenance of mitochondrial integrity.

Experimental Section

The preparation of Sprague–Dawley rat liver mitochondria and the conduct of mitochondrial swelling experiments was essentially as described by Connelly and Lardy (1964) and Connelly and Hallstrom (1966).

The rate of disappearance of α-ketoglutarate was followed spectrophotometrically according to a modification of the method described by Bergmeyer and Bernt (1963). Aliquots (1.0 ml) of the reaction mixture were added to 1 ml of cold 5.2% perchloric acid. After precipitation of the perchlorate by a quantity of potassium phosphate (tribasic) predetermined to bring the pH to 7.6, the supernatant solution was assayed for α-ketoglutarate. A Cary Model 15 recording spectrophotometer was used to follow the total change in absorbance at 340 mμ due to the oxidation of NADH. A linear relationship between absorbance and the quantity of α-ketoglutarate was obtained throughout the range from 0.01 to 0.20 μmole.

Composition of reaction media and the details of kinetic experiments are given in the figure and table legends. Water for all reagents was distilled and passed through mixed resin. All chemicals were of reagent grade and oligomycin A was a gift from Dr. H. A. Lardy, University of Wisconsin.

Results

Continuing efforts to characterize the chemical events which are associated with the swelling of rat liver mitochondria have led to concerted investigations in two areas. In logical sequence these are first, the mechanisms which determine how soon (time of onset, T_0) after exposure to swelling conditions mitochondria take up water and, second, the mechanisms which determine how much (extent) swelling will occur subsequent to T_0. Although some attempts have been made to characterize the latter phenomenon, current efforts have been centered on elucidating the more fundamental mechanisms controlling T_0.

It is now well established that ADP, in a nonphosphorylating environment, enhances the effectiveness of respiratory substrates as protective (delay T_0) agents opposing the swelling influence of inorganic phosphate, thyroxine, calcium, and oleate ions (Connelly and Hallstrom, 1967). During recent studies it was observed that replacement of L-histidine

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TABLE I: Influence of L-Histidine on the Time of Onset of Phosphate-Induced Swelling.[a]

Condition	Time of Onset (min)		
	Control	+L-His	L-His/ Control
No addition	4	7	1.8
Oligomycin	4	10	2.5
ADP	4	7	1.8
ADP + oligomycin	6	10[b]	1.7
Glutamate	5	8	1.6
Glutamate + oligomycin	5	24	4.8
Glutamate + ADP	11	56	5.1
Glutamate + ADP + oligo	26	177	6.8
ATP	20	42	2.1

[a] The reaction media contained sucrose (140 mM), K^+-phosphate (5 mM, pH 7.0),[c] and mitochondrial protein (0.3 mg/ml). Additions were oligomycin (1 μg/ml), ADP (0.5 mM), L-glutamate (0.4 mM), L-histidine (10 mM), and ATP (2 mM). In early experiments and some later work control mixtures contained 10 mM Hepes or Pipes as a replacement for 10 mM histidine in order to compensate for osmotic differences. (These buffer compounds are available commercially from Calbiochem, Los Angeles, Calif., and Sigma Chemical Co., St. Louis, Mo. Hepes is *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Pipes, piperazine-*N*,*N'*-bis(2-ethanesulfonic acid).) However, this precaution was found to be unnecessary since the P_i present buffered sufficiently and no osmotic effect was observed on T_0. That is, in the absence of histidine T_0's were the same in the presence or absence of Hepes. [b] The slight increase in T_0 over the "no addition" could be related to the presence of endogenous substrate. This contribution, however, was consistently found to be insignificant compared to the effect of added substrate (see glutamate + ATP + oligo + histidine condition). [c] pH 7.0 was occasionally used in experiments where high T_0's were expected. While the absolute T_0 is diminished somewhat (Connelly and Lardy, 1964), the relative T_0's set by other variables remained unchanged.

TABLE II: The Influence of L-Histidine on Swelling Induced by Phosphate, Ca^{2+}, and Thyroxine.[a]

Additions	Time of Onset (min)					
	Phosphate		Ca^{2+}		Thyroxine	
	Control	+His	Control	+His	Control	+His
Basic medium	5	9	11	10	4	7
Glutamate, ADP	6	22	22	127	6	16
Glutamate, ADP, oligomycin	8	41	30	100	12	43
Succinate, ADP	19	23	50	64	16	24
Succinate, ADP, oligomycin	56	56	42	48	21	25

[a] The reaction media contained 140 mM sucrose and 10 mM K^+-HEPES at pH 7.4. Additions include 5 mM K^+-phosphate, 0.040 mM $CaCl_2$, 0.025 mM thyroxine, 0.10 mM ADP, 0.33 mM substrate, 1 μg/ml of oligomycin, and 10 mM L-histidine; mitochondrial protein, 0.3 mg/ml.

with other buffers resulted in a marked loss of protection. Direct testing of histidine in a swelling situation clearly showed its ability to contribute a protective influence. A systematic study was carried out to attempt to determine whether histidine could act alone or whether it might provide a synergistic increase in the effects of substrate, or both. Table I indicates that histidine exerts its effect primarily on the combination of substrate and ADP. Furthermore, the greatest increase is seen in the system where phosphorylation is blocked by oligomycin. On the other hand, when ATP is the protective agent the per cent increase in T_0 provided by histidine is no more than seen in the absence of added protective systems. It follows then that maximum effectiveness of the substrate–ADP–oligomycin system is somehow dependent on certain of the characteristics of the histidine molecule. Since it is likely that the mechanism of swelling varies with different swelling agents, it was of interest to determine whether histidine would behave nonspecifically with regard to various swelling media. Data in Table II (glutamate) show that the influence of histidine is not unique to the phosphate system. Although small differences in the relative effects of histidine in the oligomycin-blocked system as opposed to the oligomycin-free system are seen, the major effect is a 3–6-fold increase in T_0 for all swelling agents tested. The lack of effect of histidine when succinate is substrate will be considered below (see text for Figure 1 and Table IV).

The lack of activity of the variety of analogs, metabolites and substituted histidines shown in Table III attests to the remarkable specificity for the histidine structure. Moreover, the D isomer of histidine proved to behave as a swelling agent, a fact which indirectly supports the possibility that the molecular structure of the L isomer is uniquely important in the chemical machinery which opposes swelling. In addition to L-histidine, imidazole pyruvate, the keto acid of histidine markedly increases the T_0. These chemicals are rapidly interconvertible (Spolter and Baldridge, 1963) in mitochondria and attempts to establish the amino or keto form as the primary reagent were unsuccessful. Finally, the partial activity seen with the histidine methyl ester may have been due to the presence of some free histidine as a product of hydrolysis. The increase in T_0 resulting from the presence of L-histidine or imidazole pyruvate was consistently evident, often five to ten times over the control.

The delayed discovery of the effects of histidine prompted a reevaluation of the interdependence between this amino acid and succinate, which had been used as substrate in most of the early work. When compared to glutamate in the ADP–oligomycin system succinate, in the absence of histidine, was much more protective (Figure 1). However, whereas the presence of histidine greatly stimulated the effectiveness of glutamate at all substrate concentrations, the T_0's obtained using succinate were only slightly increased. It is further noteworthy that the T_0 of the succinate system, even with histidine, is quite low compared to that of the histidine-stimulated glutamate system.

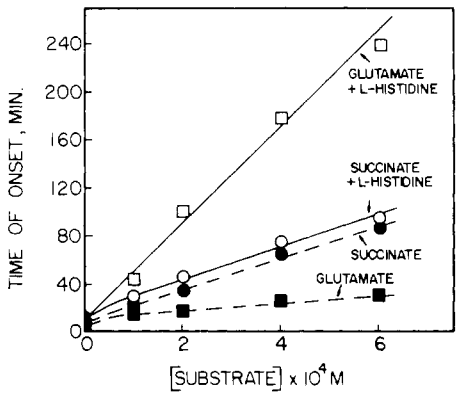

FIGURE 1: The effect of L-histidine on substrate-supported increase in the time of onset. The reaction media contained sucrose, K-phosphate, ADP, and oligomycin as noted in Table I. Histidine, where added, was 10 mM and substrate concentration was varied as shown.

The question of substrate specificity, and the relative effectiveness of NAD-linked and other respiratory substrates was examined. In preliminary experiments, concentrations of the various substrates were kept equal. In general these studies confirmed previous findings concerning succinate and indicated that the action of all other substrates tested was influenced by L-histidine (Table IV). Most interesting and perhaps somewhat misleading was the observation that α-ketoglutarate and to a lesser degree citrate, isocitrate and glutamate were highly effective while β-hydroxybutyrate, pyruvate, and malate were about half as potent. A more extensive testing of these compounds showed that the capabilities of these substrates, again with the single exception of succinate, were nearly the same when compared at infinite concentration ($T_{0,max}$). The results of this study are shown in Table IV. As a consequence of this information, it appears that the utilization of substrate and not its availability or permeability is the critical feature of the role of substrate. Furthermore, since histidine seems to affect each substrate (except succinate) media the same, it is suggested that histidine is acting in conjunction with a product of substrate utilization.

Figure 2 demonstrates that in the presence of sufficient respiratory substrate and ADP, time of onset relates to histidine in the hyperbolic form of a typical substrate concentration curve. The effect of histidine on T_0 in either potassium chloride or sucrose media is markedly dependent on the presence of substrate. If the T_0 value is considered to be an index of velocity, the data appear to follow a typical enzyme-substrate relationship when plotted according to the method of Lineweaver and Burk (1934). The typical inverse plot (inset) indicates that the maximum influence of histidine is identical in either potassium chloride or sucrose media. However, at the concentration of ADP and glutamate employed, the concentration required for half-maximal stimulation was calculated to be 2.5 mM in the salt medium, and 6.7 mM in sucrose.[1] It is noteworthy that if glutamate is replaced by

TABLE IV: The Influence of L-Histidine on Substrate-Induced Delay of Onset of Swelling.[a]

Substrate	Time of Onset (min)		
	Control	+L-Histidine	Histidine/Control
None	5	9	2
DL-β-Hydroxybutyrate	24 (77)[b]	47 (400)	2 (5)
Pyruvate	43 (74)	73 (300)	2 (4)
Citrate	37 (100)	167 (400)	4 (4)
DL-Isocitrate	37 (100)	125 (300)	3 (3)
α-Ketoglutarate	27 (67)	144 (200)	5 (3)
L-Glutamate	24 (67)	125 (200)	5 (3)
Succinate	45 (100)	52 (120)	1 (1)
L-Malate	17 (40)	33 (120)	2 (3)

[a] The swelling medium contained sucrose (140 mM), K^+-phosphate (5 mM, pH 7.0), oligomycin (1 μg/ml), ADP (0.5 mM), substrate (0.3 mM of optically active isomer), L-histidine when added (10 mM), and mitochondrial protein (0.3 mg/ml). [b] Values in parentheses are $T_{0,max}$: which represent the theoretical time of onset when substrate is extrapolated to infinite concentration. $T_{0,max}$ was determined by plotting the inverse relationship between time of onset and substrate concentration from 2×10^{-5} to 10^{-3} M.

TABLE III: The influence of L-Histidine and Related Compounds on Glutamate-Delayed T_0.[a]

Addition	Time of Onset (min)		
	Expt 1	Expt 2	Expt 3
None	25	17	13
L-Histidine	270	180	70
D-Histidine	1	2	..
L-Histidinol	44	...	..
Imidazole acetate	36	...	..
L-Histidine methyl ester	90	...	..
Urocanate	12	10	..
Imidazole	...	10	..
Imidazole pyruvate	...	220	..
Histamine	...	16	..
Carnosine	...	23	..
Anserine	...	26	..
L-Ergothionine	..	22	..
L-3-Methylhistidine	...	25	..
L-1-Methylhistidine	...	24	..
L-2-Thiolhistidine	...	25	..
Glycine	...	...	21
L-Alanine	...	...	46
L-Aspartate	...	...	28
L-Arginine	...	...	17
L-Tryptophan	...	...	13
L-Serine	...	...	32
L-Proline	...	...	33

[a] The reaction media contained sucrose, K^+-phosphate, ADP, oligomycin, substrate, and mitochondria as described in Table I. L-Histidine or related compounds were present at 10 mM. Leaders indicate no determination.

[1] The K_m of histidine varies with [S] + [ADP]. They are given for [S] = 0.4 mM, [ADP] = 0.5 mM.

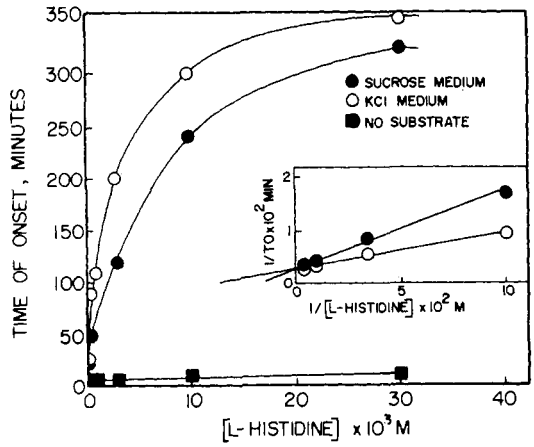

FIGURE 2: The influence of L-histidine on time of onset. The reaction media was the same as noted in Table III. Where noted KCl at 150 mM replaced sucrose.

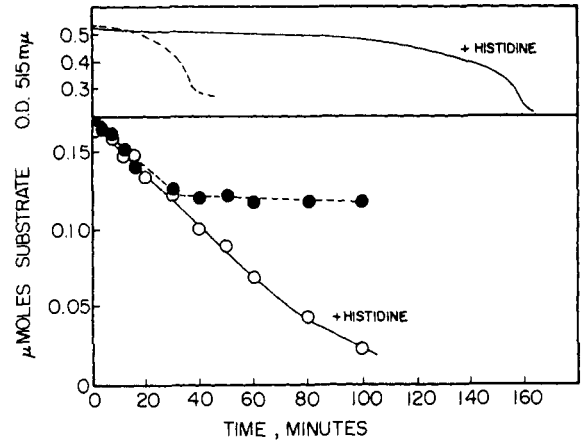

FIGURE 3: A comparison of the influence of L-histidine on swelling and substrate utilization. The reaction media was the same as in Table III except that 0.3 mM and ketoglutarate replaces glutamate. L-Histidine, where added, was 10 mM. Utilization was followed as described in the Experimental Section.

succinate, little increase in T_0 is observed over the entire range of histidine concentrations.

To ascertain whether histidine is acting directly on some aspect of respiratory substrate metabolism, several experimental approaches were utilized. These include a direct measurement on substrate utilization, respiration, substrate uptake by mitochondria, and kinetic interrelationships.

The utilization of α-ketoglutarate in the ADP–oligomycin system was examined in the presence and absence of L-histidine. Figure 3 shows that both the optical density curves (T_0) and the substrate utilization relationships are altered by histidine. However, prior to T_0, the rates of α-ketoglutarate utilization are identical. The sharp break in the rate of α-ketoglutarate disappearance occurring subsequent to swelling in the absence of histidine, is attributed to such factors as the change in the ionic environment of the catabolic enzymes, and the dilution of cofactors occurring with the rupturing of the mitochondria. Note that in the presence of histidine, T_0 does not occur until well after the complete disappearance of the substrate. The unrelatedness of the rate of substrate utilization to histidine is further supported by the lack of influence by histidine on respiratory function and the coupling process as indicated by oxygen electrode studies carried out in this laboratory.

Studies were conducted on the effect of histidine on the penetration of mitochondria by various respiratory substrates using the techniques of Chappell and Haarhoff (1967). In contrast to malonate, known to stimulate the rate of uptake of citric acid cycle substrates, L-histidine had little influence on the rate of substrate uptake. In conjunction with these experiments, the uptake of [^{14}C]histidine by mitochondria was examined with regard to agents that influence T_0. Although histidine is rapidly bound by mitochondria, neither the swelling agent nor conditions that delay swelling influence the quantity of histidine bound. No effort was made to determine the distribution of the bound histidine.

The relative ineffectiveness of either respiratory substrate, ADP or histidine (independently), on T_0 indicates a specific interaction among these three influences which function together to delay the swelling process. In an effort to distinguish the relationship among histidine, substrate, ADP, and their collective effects on T_0, advantage was taken of the applicability of the system to kinetic study. The use of kinetics to relate the influence of certain agents to the time factor in the swelling of mitochondria has been described (Gosch *et al.*, 1962; Arcos *et al.*, 1969). If T_0 is considered to represent the velocity as a function of both glutamate and ADP, the entire system can be treated according to the two-substrate system described by Florini and Vestling (1957). Under this condition, the influence of histidine can be determined on the kinetic parameters for each substrate at saturating levels of the other.

The secondary plot (Figure 4) describes the influence of histidine on T_0 in relation to the combined effects of glutamate and ADP. At infinite substrate concentration, histidine acts to increase the T_0 over the entire range of ADP concentrations. Likewise, at an infinite level of ADP, a dependency on histidine is observed at all concentrations of substrate. It appears that histidine influences, rather equally, the functions of both glutamate and ADP. The $K_{m,app}$ for glutamate and for ADP were increased at the higher level of histidine, which is in contrast to what was expected on the basis of the specific stimulatory effect of histidine on systems containing substrate and ADP. More significantly, it is noted that at infinite concentrations of glutamate and ADP, histidine acts independently to increase the T_0 of the system. The latter effect accounts for the calculated increase in maximum T_0 from 130 to 670 min in response to a rise in the histidine level from 5 to 17 mM. Thus, from the kinetic interpretation, the major influence of histidine on swelling appears to be a direct effect on the combined functions of ADP and substrate.

Discussion

Although the histidine structure has been implicated in a number of important instances by virtue of its location at or near the active site of an enzyme (Hoffee *et al.*, 1967; Crestfield *et al.*, 1963; Martinez-Carrion *et al.*, 1967; Bradbury, 1969; Dobry-Duclaux and May, 1968; Schollmann, 1965; Cottam and Srere, 1969; Wagner *et al.*, 1967; Mitchell *et al.*, 1964), there are few reported instances, outside of the reactions related to histidine metabolism, in which free histidine specifically affects enzymatic activities.

The observations reported here are thus singularly striking since the actions of histidine most certainly involve enzymes, probably closely related to the energy-trapping system of

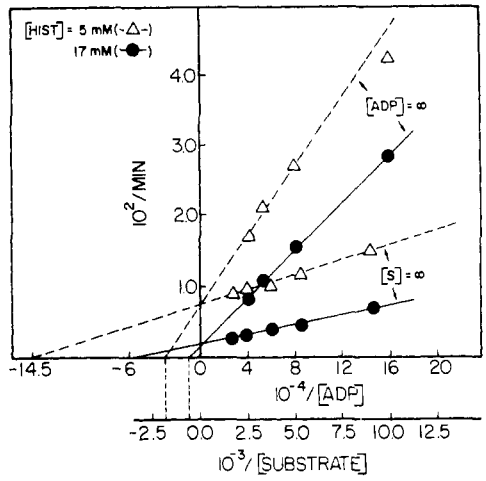

FIGURE 4: The kinetic relationships among T_0, L-histidine, glutamate, and ADP. The basic medium contained 140 mM sucrose, 5 mM K^+-phosphate (pH 7.4), and 1 μg/ml of oligomycin. This secondary plot describes the influence of histidine on T_0 at concentrations of glutamate and ADP ranging to infinity. The respective influences of glutamate and ADP on T_0 (velocity), at saturating levels of each, are obtained at two concentrations of histidine. The experimental approach is as follows. In the presence of oligomycin, and at a single level of histidine, a glutamate titration was carried out at several concentrations of ADP. The latter range of concentrations was from above to below the $K_{m,app}$ for ADP. From a double-reciprocal plot of the data, values were obtained for the maximum T_0 at infinite glutamate concentration at each of several concentrations of ADP. Using an identical design, but with the exchange of substrates, the maximum T_0 at infinite ADP was obtained for several levels of glutamate. The entire protocol was repeated at a second concentration of histidine with similar treatment of the data. Concentrations of histidine were chosen so that one level of histidine was below its $K_{m,app}$ while the other was above.

mitochondria and moreover, these particular effects are highly specific for L-histidine. Nevertheless, the possible physiological significance must await the complete elucidation of the mechanism of histidine involvement. To this end a variety of possibilities have been investigated. Since histidine has a protective influence on mitochondria in a swelling environment, three general mechanisms must be considered. Histidine may be antagonistic toward the effects of the swelling agent or condition; it may exert an independent protective influence; or it may act in conjunction with other protective agents to enhance their influence. The first of these seems an unlikely possibility in view of the fact that histidine influences the T_0 similarly in the presence of several swelling agents whose effective concentrations and probable mechanisms of action are quite different. Moreover, the pattern of substrate specificity of histidine (see Table II, glutamate *vs.* succinate) is similar under conditions of swelling induced by these different agents. These results indicate that the direct action of histidine is more closely related to the function of agents that delay swelling rather than to agents that induce swelling. Direct examination of the relation of histidine concentration to T_0, in the absence of added substrate and ADP, readily shows that the second of the above possibilities is inappropriate. The slight increase in T_0 noted with histidine alone (Table I, first line) is likely related to the presence of endogenous substrate and adenine nucleotides.[2] It is pertinent, however, that under theoretical conditions where substrate and ADP are at infinite concentrations, histidine exerts a marked effect on T_0 (see Figure 4).

In contrast to the two possible mechanisms noted above, there is considerable evidence to support the idea that histidine is acting to enhance the influence of other protective agents. In these studies this process manifests itself maximally as a 5–10-fold increase in T_0 of the system protected by respiratory substrate, ADP, and oligomycin.[3]

Efforts to discern the mode of action of histidine were, to a large degree, dependent on parallel studies on the role of respiratory substrate, the results of which are reported elsewhere (D. R. Myron and J. L. Connelly, 1970, unpublished data). Those studies revealed that: (a) T_0 was proportional to the amount of substrate utilized; (b) with the exception of succinate and malate, the effectiveness of all substrates tested was approximately equal; and (c) maximal effectiveness for all substrates required the presence of ADP and oligomycin. These findings support the idea that an energy-rich status, generated by the oxidation of substrate and stabilized by ADP, functions in the control of mitochondrial integrity.

Subsequent studies indicated that histidine does not affect general substrate uptake, utilization, or respiratory functions. As evidenced by the absence of influence by both D and L isomers on respiratory control, histidine does not alter the ability of respiratory substrate to generate energy-rich intermediates. The stabilization or conservation of an energy-rich form for structural control seems a more likely role.

The requirement of ADP (+ oligomycin) for maximal influence, also suggests the direct influence of histidine on an energy-rich status. Influences of histidine on certain reactions related to oxidative phosphorylation have been reported. Swanson (1956) observed an inhibitory influence of histidine on Mg^{2+}-ATPase in the region pH 6–7. In addition, histidine appeared to help sustain the P_i–ATP exchange, in the presence of Mg^{2+}, in contrast to succinate-Tris buffer. Reports by Cereijo-Santalo (1967, 1968) of an inhibitory influence of histidine on the DNP-stimulated ATPase of mitochondria indicate that histidine may act to conserve energy-rich intermediates. In our hands only slight inhibition of DNP-stimulated ATPase by histidine was observed, and furthermore, no differences in influence by different respiratory substrates (succinate *vs.* glutamate) were found. Consequently, it is unlikely that the small influence on ATPase is related to the effect of histidine on T_0.

The kinetic approach to the role of histidine indicates that the target of interaction is a product of the combined function of substrate and ADP. At infinite concentrations of ADP and substrate, a large independent influence of histidine on T_0 is obtained. This result is consistent with the concept of histidine

[2] Preliminary experiments indicate that histidine is without effect on systems containing the respiratory inhibitor rotenone which would block endogenous respiratory activity.

[3] While other conditions and agents, such as respiratory inhibitors and EDTA, have been shown to be capable of delaying T_0, none have exhibited the interdependence with ADP and respiratory substrate which is manditory for L-histidine. It would appear then that more than one mechanism is available for delaying swelling and that, for example, metal chelation, a probable mechanism with EDTA-delayed swelling, is not applicable in the histidine–substrate–ADP–oligomycin system since none of these is effective alone even at high concentrations. The case of respiratory inhibitor-blocked swelling represents an unresolved engima. On the one hand, T_0 is delayed by blocking respiration (presumably endogenous) and, on the other hand, in the histidine–ADP–oligomycin system utilization of added substrate is required for increased T_0.

stabilizing an energy-rich condition (X-ADP) generated by respiration as shown by Figure 5. It is of interest to note that a plot of the $1/T_{0,max}$ intercepts of Figure 4 *vs.* the inverse of histidine concentration passes through the origin ($T_0 = \infty$).

The unique variation seen in the lack of interrelation between histidine and succinate remains unexplained. Recent experimentation by Klingenberg (1970) regarding the sidedness of location of various dehydrogenases in mitochondria suggests the possibility that the locale of X-ADP generated by succinic acid dehydrogenase, located in the inside of the inner membrane, might make X-ADP less accessible to histidine.

Efforts to characterize the relationship between L-histidine and the preservation of mitochondrial integrity included an examination, using electron microscopy, of the morphology of the swelling process. In the glutamate–ADP–oligomycin system, which is most influenced by histidine, the sequence of ultrastructural changes accompanying swelling, in both the presence and absence of histidine is preceded by conformational changes from "condensed to twisted" and from "twisted through orthodox to swollen." No differences in major conformations were observed between the control and histidine-treated runs. The ultimate difference between the two conditions was the duration of the twisted conformation which essentially paralled the corresponding T_0. It may be pertinent to the role of histidine that the twisted conformation reflects an energy-rich status of the mitochondrion (Green *et al.*, 1968). Nevertheless, it appears that the mechanism of action of L-histidine does not manifest itself in gross morphological alterations.

A recent report by Estrada-O *et al.* (1970) concerning the latency of certain mitochondrial enzymes, suggests roles for ADP and oxidizable substrate in the "generation of an energy supply, necessary to preserve mitochondrial membrane integrity, thus favoring the maintenance of latent enzymes within the mitochondria." Since these experiments utilized histidine buffer, it is tempting to consider the participation of this molecule in the protective process.

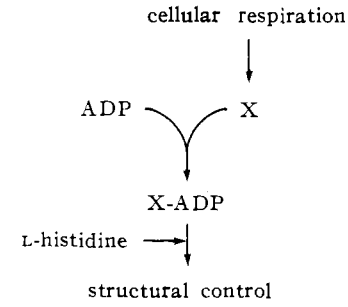

FIGURE 5: A schematic interrelationship of respiration, ADP, histidine, and mitochondrial integrity.

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