PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Energetic Coupling of Acid Secretion in Gastric Mucosa

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The energetic coupling of acid secretion in gastric mucosa

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The relation between acid secretion and oxidative metabolism has been investigated using non-destructive optical techniques to monitor the redox level of respiratory chain components in the intact, isolated bull-frog gastric mucosa. High rates of acid secretion are found to be associated with cytochrome reduction while inhibition of secretion results in oxidation of the respiratory chain components. Changes in chloride secretion, independent of hydrogen ion secretion, result in opposite cytochrome redox changes indicating that the interaction with oxidative metabolism differs for the two secretory processes. Measurement of intracellular pH changes using indicator dyes shows a correlation between cytochrome reduction and increased pH. The results suggest that there exists a close coupling between the respiratory chain components and hydrogen ion secretion and that this coupling may involve changes in intracellular pH rather than changes in high energy phosphate compounds as previously suggested.

The secretion of hydrochloric acid by the gastric mucosa has interested investigators for over one hundred years. However, the abundant studies by noted investigators have to date failed to answer certain basic questions concerning this process. As yet, we do not know the mechanism by which acid is produced nor do we know the immediate source of the hydrogen ions which are secreted. While it is recognized that acid secretion is intimately linked to oxidative metabolism, we do not know the nature of this coupling. Again little is known of the site or mechanism of action of the various agents which affect acid secretion, e.g. histamine and thiocyanate. In short, we have a great deal of phenomenological information but very few definite answers to basic questions. I will not be so pretentious as to claim that the work I am presenting here offers answers to all or even one of the above questions. Indeed, it tends to add an additional, and perhaps more confusing, dimension to the problem. However, I do feel that this work represents a potential key to progress in that it offers a new approach which has already produced a number of significant observations tending to substantiate some previous ideas while raising serious doubts about others.

The present work has as its basis and central aim the question: how is acid secretion coupled to oxidative metabolism? It has been repeatedly shown that acid secretion is almost exclusively dependent upon respiration (Davenport 1947; Forte, Adams & Davies 1965) indicating a close linkage between these two processes. That this linkage is two-way is demonstrated by the ability of acid secretion to control in part respiration in gastric mucosa (Crane & Davies 1951; Davenport & Chavre 1953; Forte & Davies 1964; Villgeas & Durbin 1960). Such coupling requires at least one component to be common to both processes and for a simple mechanism implies that the component undergoes a cycle of production and utilization between the two processes. Since the active secretion requires a source of energy, it is generally felt that the common component serves as this source, although this is not necessarily the case. While several mechanisms which fit the general requirements have been offered, the most prevalent theory for metabolism–secretion coupling may be referred to as the 'ATPase' theory (Durbin & Kasbekar 1965; Forte *et al.* 1965; Kasbekar & Durbin 1965). This theory is familiar to most

workers since it has been postulated for many other active transport systems most notably; Na-K transport in erythrocytes (Hoffman 1962), squid axon (Mullins & Brindley 1969) and kidney slices (Whittam & Willis 1963). Briefly, this theory identifies the common component as adenosine triphosphate (ATP) which cycles between the transport mechanism or 'pump' and the energy conservation reactions of the mitochondrial respiratory chain. As depicted in figure 1, ATP serving as an energy source for the 'pump', is hydrolysed to adenosine diphosphate

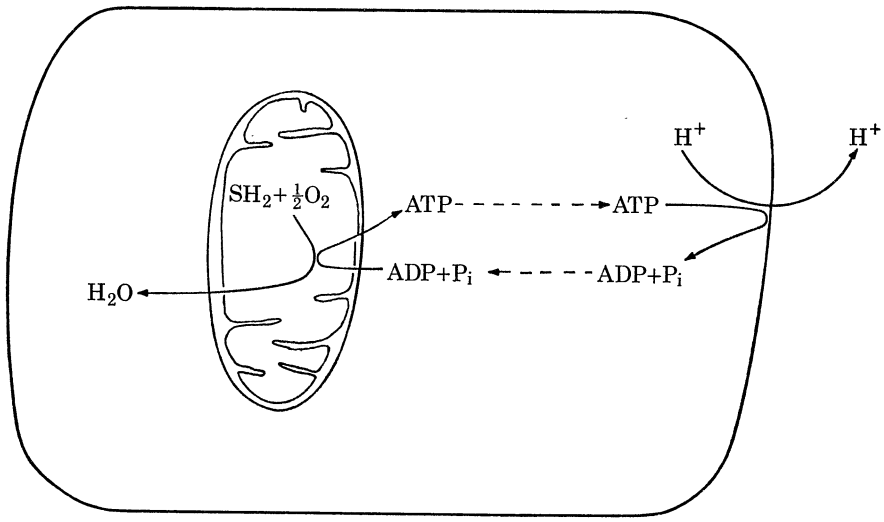

FIGURE 1. Hypothetical scheme for coupling between hydrogen in transport and mitochondrial respiration. The intramitochondrial reaction represents the overall activity of the respiratory chain.

(ADP) and inorganic phosphate (P_i). The ADP generated is then rephosphorylated by the mitochondria during coupled respiration. This hypothesis is based on two important observations. First, it has been consistently demonstrated that respiration by mitochondria isolated from a variety of sources (Chance & Williams 1956) including gastric mucosa (Forte, Forte, Gee & Saltman 1967) may be partially controlled by the availability of ADP. It should be kept in mind, however, that respiratory control by ADP is crucially dependent upon the exact experimental conditions employed. Secondly, in certain tissues ATP has been shown to be an important, if not the only, source of energy for active transport (Hoffman 1962; Mullins & Brindley 1969), although this has not yet been clearly demonstrated for gastric mucosa.

In theory at least, these two major features of the 'ATPase' hypothesis are open to testing. However, it is technically very difficult if not impossible to directly test these features in the intact, functioning tissue. Indirect tests on subcellular components have been performed to some degree (Forte *et al.* 1967; Kasbekar & Durbin 1965), but these techniques require destroying the tissue and thus removing the distinction between the intracellular and extracellular fluid which is the basis of the transport problem. For this reason we chose to make use of certain recently developed non-destructive, optical techniques to monitor the respiratory chain in the intact, functioning tissue. These techniques involve the use of two absorption spectrophotometers developed by Dr Britton Chance and his collaborators, the splitbeam (Yang & Legallais 1954) and the dual-wavelength (Chance 1951). Both of these instruments are specially constructed for use with highly scattering samples, e.g. tissues and particle suspensions. The splitbeam is used to obtain steady-state difference spectra, i.e. a spectrum of the absorption difference between two tissue samples, while the dual-wavelength is used to monitor kinetic changes

of a single absorbing component. These instruments are capable of detecting small changes in the oxidation–reduction level of endogenous respiratory chain pigments and absorption changes in exogenous indicator dyes. As such the methods offer an enormous opportunity for measuring intracellular events in intact tissues, the full potential of which cannot as yet be estimated.

TABLE 1. PERCENTAGE REDUCTION OF RESPIRATORY CHAIN PIGMENTS IN ISOLATED MITOCHONDRIA

	NADH	Fp	cyt. *b*	cyt. *c*	cyt. *a*
state 3	54	24	16	8	5
state 4	100	41	36	13	0
net change (4 → 3)	ox.	ox.	ox.	ox.	red.

Use of the optical techniques allows one to perform a crucial, though non-conclusive, test of the 'ATPase' hypothesis. This test is based on the observations of Chance & Williams (1956) that when respiration of isolated mitochondria is altered by changes in the ADP level of the bathing medium, several members of the respiratory chain undergo characteristic changes in their oxidation–reduction state. The characteristic reduction levels of the respiratory chain members in two different respiratory states are given in table 1 which is adapted from Chance & Williams (1956). State 3 is the activity state where adequate levels of substrate, ADP and oxygen are available and respiration is rapid, limited by the intrinsic rate of the respiratory chain itself. State 4 is the resting state characterized by having adequate substrate and oxygen but no ADP. State 4 respiration is low and limited by lack of ADP. A transition from rest to activity (state 4 to state 3) brought about by addition of ADP results in increased respiration and characteristic oxidation–reduction changes, notably a net oxidation of most of the respiratory chain members. There are also characteristic 'cross-over' points where one member becomes oxidized while the subsequent member on the oxygen side becomes more reduced. This point may occur between cytochromes *c* and *a* or between cytochromes *b* and *c* depending on the exact experimental conditions (Chance & Williams 1955). While these oxidation-reduction transitions have been well documented for isolated mitochondria, the question might be raised whether similar changes occur in tissues or whether they are an artefact of isolation. This question has been answered most notably for skeletal muscle by F. F. Jobsis and collaborators (Jobsis 1963; Jobsis & Duffield 1967) who showed that the respiratory chain responds to changes in ADP resulting from contractile activity in a manner which is almost identical to the responses observed in isolated mitochondria. Therefore, we felt justified in using the response of the respiratory chain components as an important test of the 'ATPase' hypothesis for coupling between oxidative metabolism and acid secretion. Accordingly, an increase in acid secretion ought to increase the ADP level resulting in a transition away from state 4 toward state 3 and an accompanying oxidation of at least certain respiratory chain members. Conversely, a decrease in secretion ought to result in reduction of the same components. The actual percentage change cannot be predicted due to lack of information on initial ADP levels and the extent to which secretion contributes ADP as compared with other reactions.

In the initial experiments acid secretion was altered by stimulating with histamine and inhibiting with thiocyanate (Hersey & Jobsis 1969). Although the mechanism of action of these compounds is unknown they were chosen because of their well established actions on secretion.

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Figure 2 shows the steady-state redox changes of the respiratory chain associated with histamine stimulation (curve *a*) and thiocyanate inhibition (curve *b*). The various members of the respiratory chain are identified by their characteristic absorption peaks. Two very remarkable features of this result are immediately obvious. First, histamine produces a more reduced steady state while thiocyanate results in a reoxidation. Secondly, all of the respiratory chain components including NADH and cytochrome a_3 undergo changes in the same direction. These results are

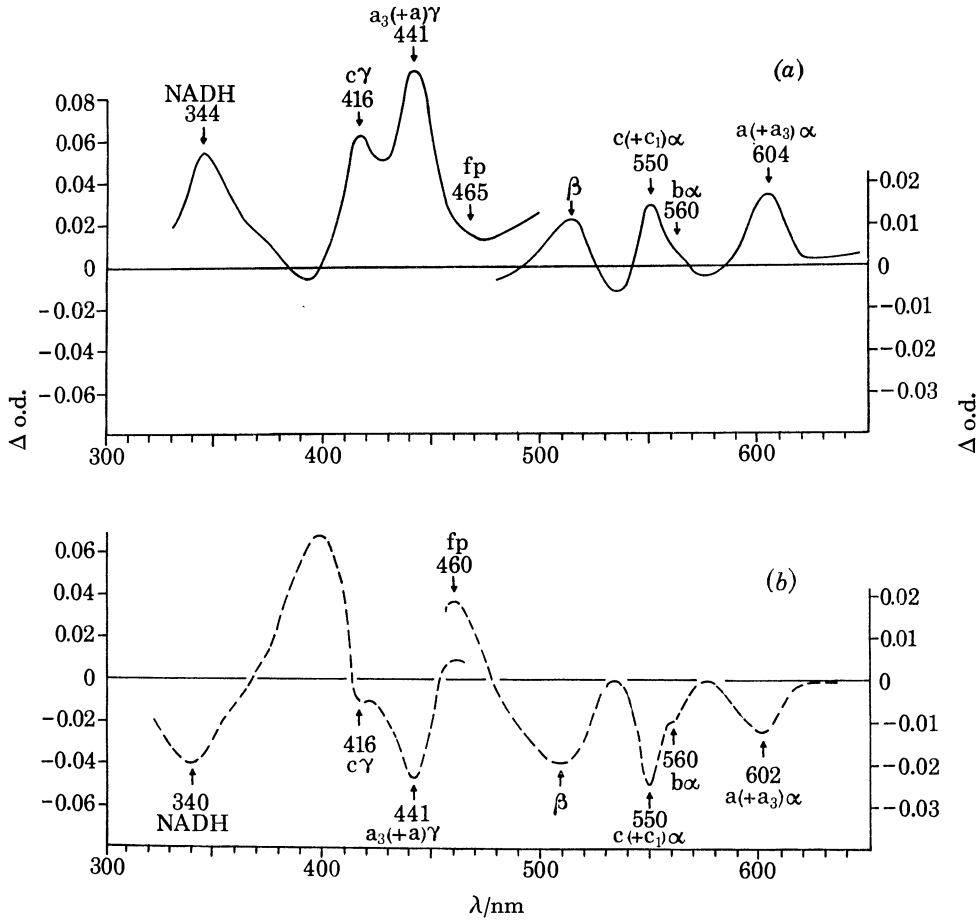

FIGURE 2. Steady-state absorption changes in intact gastric mucosa induced by histamine and thiocyanate. (*a*) optical density changes at 40 min after addition of histamine (10^{-4} mol/l) as compared with spontaneously secreting condition (baseline). (*b*) optical density changes at 20 min after addition of thiocyanate (10^{-2} mol/l) as compared with the histamine stimulated condition (baseline). Respiratory chain components identified by absorption peaks. Note optical density scale change at break in the curves. Upward change corresponds to reduction of cytochromes and NADH and oxidation of flavoprotein (Hersey & Jobsis 1969).

in direct contrast to changes predicted by the 'ATPase' hypothesis both as to the direction of the redox changes and the lack of any crossover points. Thus it would appear that the entire respiratory chain is responding as a unit to some factor other than or in addition to a change in the ADP concentration. The exact nature of this response and the factor or factors responsible for it remain for the moment unknown. In the case of histamine, the reduction of NADH together with the known increase in oxygen consumption (Alonso & Harris 1965; Croft & Ingelfinger 1969; Villgeas & Durbin 1960) require an increased input of reducing equivalents from substrate, i.e. a substrate mobilization occurs. Moreover, the reduction of cytochrome a_3

indicates that the respiratory chain is limited at the oxygen terminal end. The action of thiocyanate might then be interpreted as removing the terminal limitation. If thiocyanate also reverses the substrate mobilization, a decrease in oxygen consumption should be observed. Such a decrease apparently does not occur until well after the effects of thiocyanate on acid secretion and cytochrome reduction have taken place (Bannister 1964; Forte & Davies 1964; Moody 1968) indicating that this compound does not act directly on substrate metabolism.

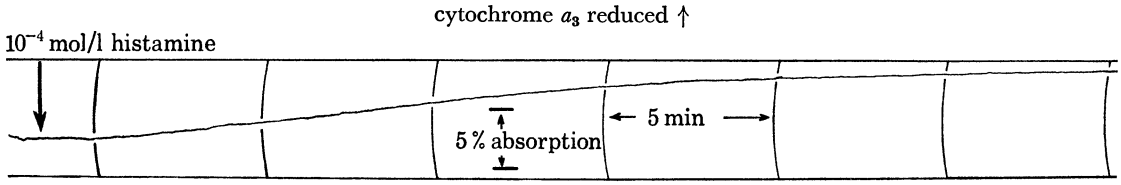

FIGURE 3. Response of cytochrome a_3 to histamine addition. Histamine added to secosal bathing medium at arrow. Sample and reference wavelengths are 445 and 465 nm respectively. Intact gastric mucosa, 18 °C.

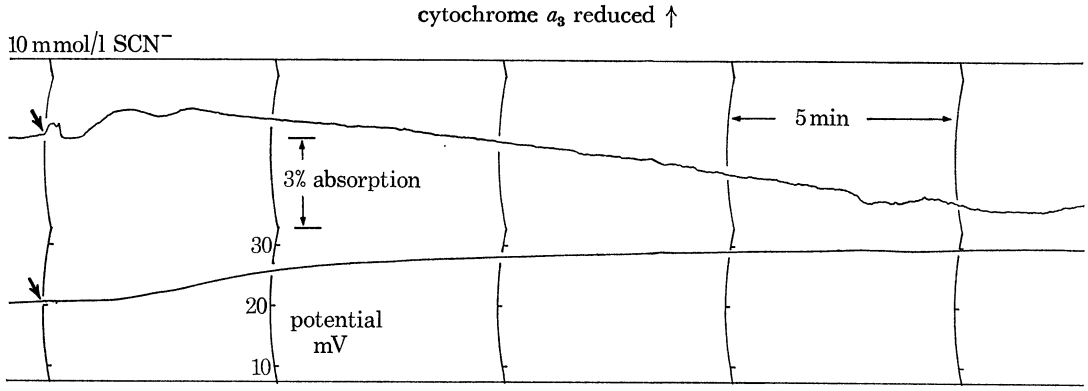

FIGURE 4. Response of cytochrome a_3 to thiocyanate addition. Tissue was previously stimulated to secrete with histamine, 18 °C. Upper trace, cytochrome a_3; lower trace, spontaneous potential difference. Increasing potential is serosal surface positive relative to mucosal surface. Thiocyanate added at arrows, note addition artefact in cytochrome trace. Wavelengths as in figure 3.

The kinetics of the cytochrome response to histamine (figure 3) and thiocyanate (figures 4, 5) are of interest in relation to the time course of their action on acid secretion. Histamine has a very slow time course requiring 20 to 30 min to reach a steady state of cytochrome reduction. This is consistent with its action on acid secretion (Rehm 1962) and oxygen consumption (Alonso & Harris 1965) for *in vitro* preparations. Unfortunately, the long time course tends to mask inflexion points making it difficult to perform a detailed analysis of the time relations between acid secretion and cytochrome reduction. Within the rather broad limits of resolution the two processes appear to change concominantly. In the case of thiocyanate, the time course of action is much more rapid both for acid secretion and cytochrome changes. Analysis of the time relation is assisted by measuring the change in spontaneous potential difference which accompanies thiocyanate inhibition of acid secretion (Durbin & Heinz 1958; Rehm 1962). While the exact cause of the potential change is uncertain it is assumed to represent the earliest detectable change in acid secretion and provides a fairly sharp inflexion point. The cytochrome response to thiocyanate, shown in figure 4, reveals a feature not seen in the steady-state measurement, namely an initial reduction followed by a slower change to a more oxidized

steady-state level. This initial phase is shown in more detail in figure 5. The oscillation observed in the cytochrome a_3 response is typical of about half of the preparations tested while a single reduction peak is observed in the others. Of particular interest is the fact that the initial reduction clearly occurs before any change in the potential. The latter shows an inflexion point which consistently coincides with the peak of the reduction phase of the cytochromes. In the case of those preparations which exhibit an oscillation of cytochrome a_3 the potential change occurs at the peak of the first cycle. These unexpected kinetics suggest that thiocyanate exerts a dual

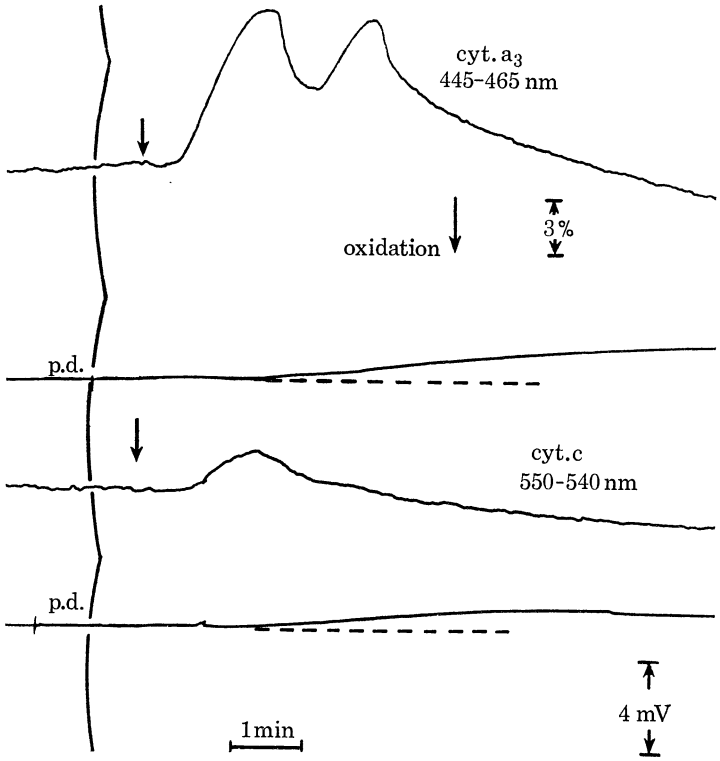

FIGURE 5. Initial response of two cytochromes to thiocyanate addition. Tissue was previously stimulated to secrete with histamine, 18 °C. From top to bottom, trace 1, cytochrome a_3; trace 2, potential difference corresponding to trace 1; trace 3, cytochrome c; trace 4, potential difference corresponding to trace 3. Absorption scale corresponds to both cytochromes, potential scale corresponds to both potentials. Time scale corresponds to all traces. Thiocyanate 2×10^{-2} mol/l to maximize response, added to serosal bathing medium at arrows. Dashed lines are extrapolation of initial potential to emphasize inflexion point.

effect. The initial effect, giving rise to a reduction of cytochromes, is primarily on the respiratory chain without affecting secretion while a second action affects both secretion and the cytochromes. Alternatively these two actions may be linked in some as yet unknown manner. If there are two independent actions, the second is of greater interest because of its relation to secretion. The exact onset of the oxidizing effect cannot be determined but it must occur at or before the peak of reduction. Thus the secretion-linked effect of thiocyanate on the cytochromes must occur before or simultaneous with the affect on secretion. The resolution of this time relation is within a few seconds which is good enough to demand a very close linkage between secretion and cytochromes but insufficient to warrant postulating a direct physical link.

Since the bullfrog gastric mucosa actively transports chloride as well as hydrogen (Heinz & Durbin 1957; Hogben 1955), it seemed desirable to determine how this process might affect the

redox level of the cytochromes independent of hydrogen-ion secretion. The rate of chloride transport was altered by substituting glucuronate (Durbin 1964) for chloride in the bathing media in the presence of thiocyanate. The influence of increasing chloride concentrations on the steady-state oxidation of cytochrome *c* is shown in figure 6. Increasing chloride concentrations result in oxidation of the cytochrome and the shape of the curve reveals saturation phenomena, although the exact shape of the curve must be interpreted with great caution. While the result is clear, the cause is uncertain since the oxidation might be due to the presence of the chloride ion *per se* rather than chloride transport. In this regard it is noted that the relation between cytochrome oxidation and chloride closely resembles the relation between chloride

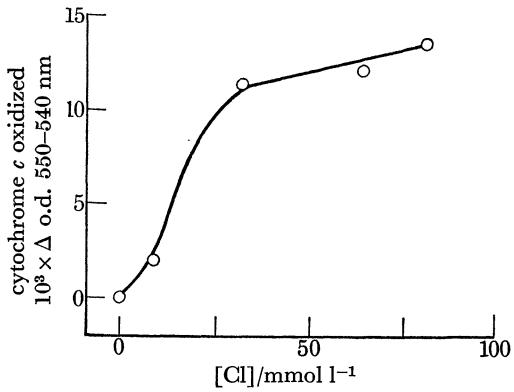

FIGURE 6. Effect of external chloride concentration on cytochrome *c*. Chloride concentration was varied by substitution for glucuronate in both mucosal and serosal media. All solutions contained 10^{-2} mol/l thiocyanate. Each point is the mean of duplicate determinations. Values normalized to zero chloride level of oxidation, 18 °C.

concentration and short-circuit current (Durbin & Kasbekar 1965) which under most conditions is a measure of active chloride transport (Hogben 1955). Assuming the effect is one of chloride transport, the results might be interpreted as supporting the idea of an 'ATPase'-type coupling mechanism for chloride transport, suggesting that the metabolic link for this process is different than that for the hydrogen ion secretion. The situation is not so simple, however, since all of the cytochromes undergo oxidation in response to increasing chloride concentration, i.e. there is no crossover point. An alternative explanation is that both transport processes influence the cytochrome redox levels by the same mechanism but do so in opposite directions and to different extents. The actual redox level observed would then be determined by whichever process dominated under a given set of circumstances.

There are at least two possible explanations for the discrepancy between the observed results and those predicted by an 'ATPase' mechanism. First, the mechanism for coupling between metabolism and secretion in gastric mucosa may not involve the ATP/ADP couple. Secondly, the results may be due to specific effects of the agents used to alter the transport process. In order to further test these possibilities, two additional agents were employed, applied external potential and acetazolamide.

It has been demonstrated that the application of current from an external source so as to change the potential across the gastric mucosa will inhibit or stimulate acid secretion depending upon the direction of current flow (Rehm 1962). Moreover, the change in secretion is accompanied by a proportional change in oxygen consumption (Forte & Davies 1964). This method

of altering secretion has the advantage of being rapid and reversible and therefore seemed well suited for correlation with cytochrome changes. The single most important difficulty is that no method is presently available to measure acid secretion with sufficiently rapid time resolution, the pH-stat method being far too slow. The affect of an applied potential on cytochromes *c* and *b* is shown in figure 7. The applied potentials are sufficiently large to either completely inhibit (mucosa positive) or markedly stimulate (mucosa negative) acid secretion. Upon applying a potential so as to inhibit secretion there is characteristically observed a lag time of 5 to 7 s followed by a rapid and large reduction of the cytochromes. When the current is turned off the reduction is seen to continue, again for about 5 to 7 s, before returning to about the original level of oxidation. On the other hand when the potential is applied so as to stimulate secretion, no cytochrome response is observed at all.

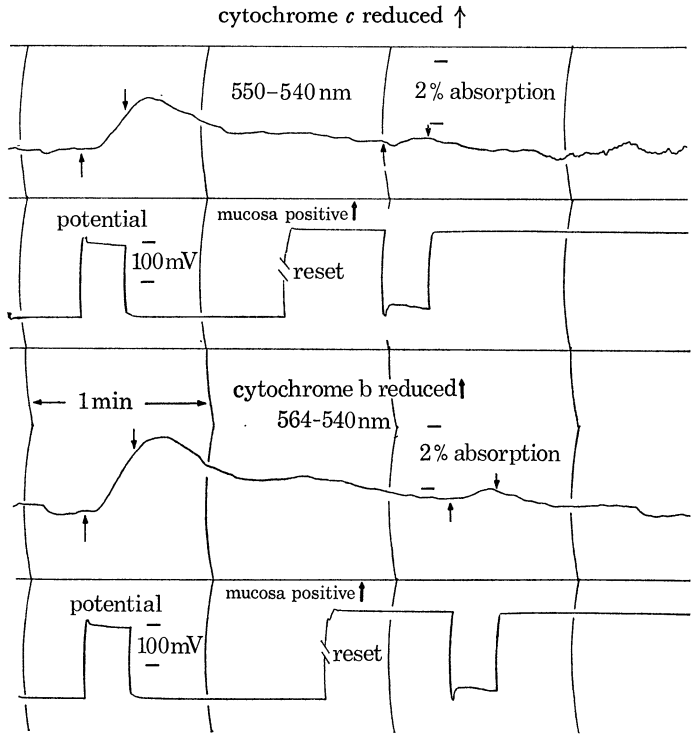

FIGURE 7. Response of two cytochromes to applied potential. Arrows indicate points at which potential is applied and removed. Note baseline reset for potential between applications of opposite polarity. Tissue was secreting under the influence of histamine, 18 °C.

The application of a potential is thought to directly affect the hydrogen ion transport mechanism by making the electrochemical gradient more or less favourable for secretion. If this is the case, a reduction of cytochromes *c* and *b* would be expected with secretory inhibition according to the 'ATPase' hypothesis. The lag of the cytochrome response might then be due to a delay necessitated by a diffusion time for ADP, although the consistency of the lag, its symmetry with respect to the on and off points of the potential, and its rather long time would be difficult to explain by diffusion delays alone. An alternative possibility is that there is a lag between potential change and acid secretory response. This possibility is equally difficult to conceive of and cannot be tested until we devise a more rapid method for measuring secretion. Whatever the cause of the delay, the 'ATPase' hypothesis cannot explain fully the cytochrome response to applied potentials since cytochrome a_3 also responds (Jobsis, Hersey & High 1971).

Moreover, the 'ATPase' hypothesis does not predict rectification with regard to the direction of current flow. This feature of the response is perhaps the most remarkable. Since neither the acid secretory response nor oxygen consumption rectify to anything like the same extent, it would indicate that the cytochrome changes are not responding directly to either of those processes. This is consistent with the observation that thiocyanate produces little change in respiration while the cytochromes undergo a marked oxidation, again indicating that the cytochrome redox level is not dependent upon the respiratory rate.

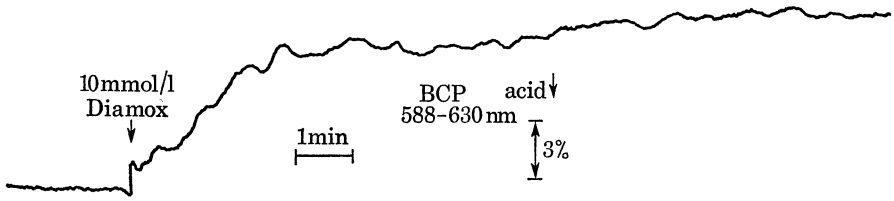

FIGURE 8. Response of bromcresol purple to Diamox. Diamox added to serosal medium at arrow. Addition artefact masks onset of response. Upward deflexion indicates alkaline change. Tissue was stimulated with histamine 60 min before Diamox, 18 °C (Hersey & High 1971).

A possible means of correlating, though in no way a full explanation, of our rather diverse results was suggested by a consideration of the inhibitory action of acetazolamide (Diamox) on acid secretion. This compound has long been known to inhibit acid secretion but its mechanism of action remains unclear. Hogben (1965) suggested that it inhibits chloride transport and the accompanying decrease in spontaneous electrical potential inhibits acid secretion in the same way as current application. This idea is interesting but untenable in view of the rather small potential changes usually produced by the drug (Heinz & Durbin 1957; Hersey & High 1971). A former suggestion (Janowitz, Dreiling, Robbin & Hollander 1957; Rehm *et al.* 1961) holds that Diamox inhibits carbonic anhydrase resulting in a decrease in the intracellular hydrogen-ion concentration available to the transport mechanism. Since the pH of the bathing medium is known to influence several characteristics of isolated mitochondria including cytochrome redox levels (Chance & Conrad 1958; Chance, Lee & Mela 1967; Packer 1970), we tested the effect of Diamox on intracellular pH and cytochromes in the gastric mucosa. Intracellular pH was measured using the indicator dye, bromcresol purple (Chance & Mela 1966), which was incorporated into the cells by incubating the tissue in the presence of the dye for 1 to 2 h. The major difficulty of this method is that because the intracellular pK of the dye is unknown (due to uncertain protein binding) it can only yield qualitative information. While the lack of quantitation limits the interpretation, the qualitative results are themselves significant.

Figure 8 shows the response of the intracellular pH to the addition of Diamox at a necessarily high concentration (Hogben 1965). Diamox produces a rather rapid and substantial alkaline response which tends to support the view that the drug acts via carbonic anhydrase or at least acts to reduce the hydrogen-ion concentration available to the transport mechanism. Figure 9 demonstrates that cytochrome a_3 becomes more reduced in response to Diamox addition. The time course of the cytochrome reduction indicates that it follows the pH change with regard to both onset and the time to reach steady state. This interpretation is tenuous, since the comparable results were necessarily obtained on different tissues or on the same tissue at different times. However, even taking tissue differences into account, the pH response appears to precede

the inhibition of acid secretion which requires 5 to 10 min to reach a new steady value (Hersey & High 1971). The reduction of cytochromes associated with an alkaline pH shift is consistent with the available observations on isolated mitochondria and suggest that the redox changes associated with the other agents tested may also be due to intracellular pH changes.

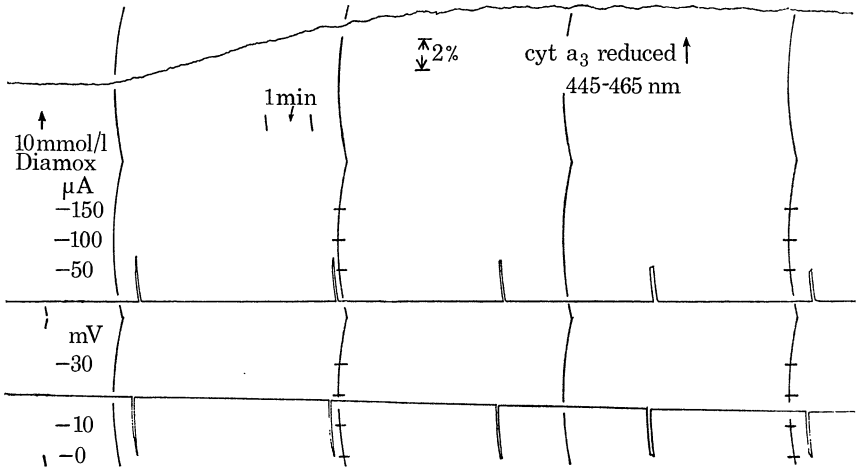

FIGURE 9. Response of cytochrome a_3 and electrical parameters to Diamox. Upper trace, cytochrome a_3; middle trace, short-circuit current; lower trace, potential difference. Diamox added to serosal medium at arrow, upward deflexion indicates cytochrome reduction. Open circuit potential was brought to zero by applying a brief current (spikes) to measure short-circuit current. Potential values refer to serosal positive. Note small decrease in potential and short-circuit current accompanying cytochrome change. Tissue stimulated with histamine prior to record, 18 °C. (Hersey & High 1971).

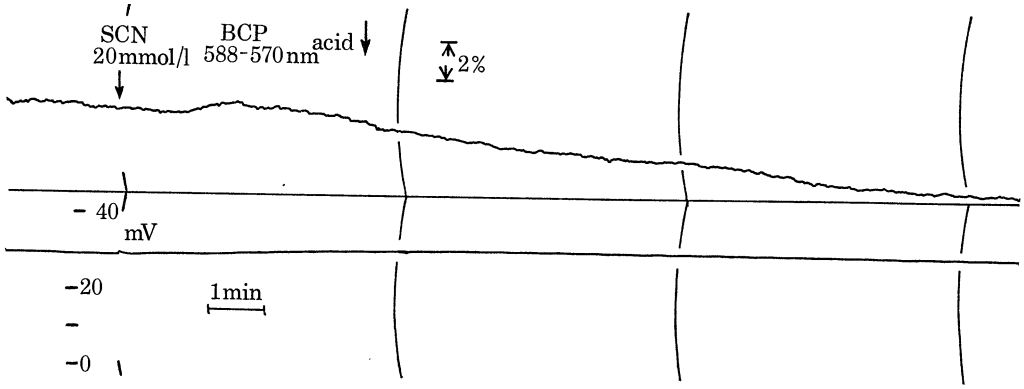

FIGURE 10. Response of bromcresol purple and potential to thiocyanate addition. Upper trace, bromcresol purple; lower trace, potential. Downward change indicates acidification. Note initial alkaline transient. Potential refers to serosal positive. 18 °C.

The association of cytochrome responses with intracellular pH changes is clearly demonstrated in figure 10 and figure 11. The effect of thiocyanate on intracellular pH as indicated by the response of bromcresol purple is seen to be an initial alkaline transient followed by a slow acidification. These changes correspond closely in time with the reduction and oxidation phases of the cytochrome responses (figure 4). The bromcresol purple response to applied current also corresponds with remarkable closeness to the cytochrome response (figure 7). The application of a potential large enough to completely inhibit acid secretion results in a rapid, large alkaline shift after a lag of about 5 s. Upon turning off the external current the pH change continues for a few seconds before reversing and returning to about the original level. Moreover,

application of a potential so as to stimulate secretion failed to produce a change in pH (Jobsis *et al.* 1971). In support of these correlations histamine produces a slow and small but measurable alkaline shift (S. J. Hersey, unpublished results). In summary, all of the agents tested result in bromcresol purple changes which correlate closely with the cytochrome responses, indicating that reduction is associated with alkaline changes while acidification of the intracellular space corresponds to cytochrome oxidation.

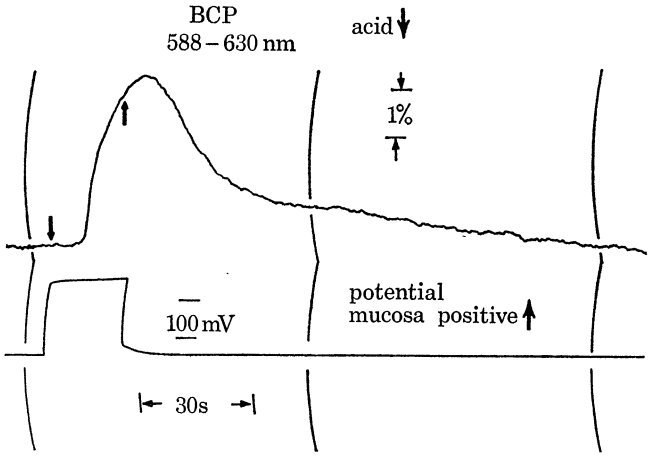

FIGURE 11. Bromcresol purple response to applied potential. Arrows indicate points at which potential is applied and removed. Upward deflexion indicates alkaline shift. 18 °C.

The excellent correlation between intracellular pH and cytochrome oxidation–reduction level strongly suggests a cause and effect relation, although this has not been directly demonstrated. Assuming that the intracellular pH change is the primary effect of the agents employed and this in turn produces respiratory chain redox changes, we are then faced with answering three crucial questions: (1) How do the various agents alter the intracellular pH? (2) How do pH changes produce cytochrome redox changes? and (3) To what extent do the pH and cytochrome changes represent a mechanism for coupling between acid secretion and oxidative metabolism? Obviously, the answers to these questions are not available as yet. We can only provide a few isolated facts surrounded by conjecture and hypothesis. However, we feel that obtaining answers to these questions represents a positive and important orientation for future experimentation.

For the present we are working with a rather simple, and perhaps naïve, model (figure 12) which represents nothing new, since the various aspects of it have been postulated by many previous investigators. This model is based on three primary postulates. First, the hydrogen ions liberated during substrate oxidation (presumably at the level of cytochrome *b*) are not directly available to the cytochrome a_3–oxygen reaction but rather enter into a cellular hydrogen-ion pool. This would be required since if the substrate hydrogen ions were directly donated to reduced oxygen, changes in intracellular pH should not affect cytochrome a_3. Secondly, the hydrogen-ion transport mechanism competes for hydrogen ions in the pool with the cytochrome a_3–oxygen reaction and the subsequent neutralization of the hydroxyl product of that reaction. Thirdly, the hydrogen-ion pool receives a substantial contribution from carbonic acid and for reasons of volume regulation and electroneutrality this requires a steady removal of the concomitantly formed bicarbonate as the hydrogen ions are removed by one process or another.

Thus, there are three reactions which donate hydrogen ion to the pool and four reactions which remove it. According to the model, the intracellular pH will depend on the dynamic balance between these reactions, a consideration of which is too difficult to undertake without the use of computer techniques. It is to be emphasized that this model is nothing more than a working hypothesis and should not be considered as final. With this in mind, we may ask how it might contribute to answering our proposed questions.

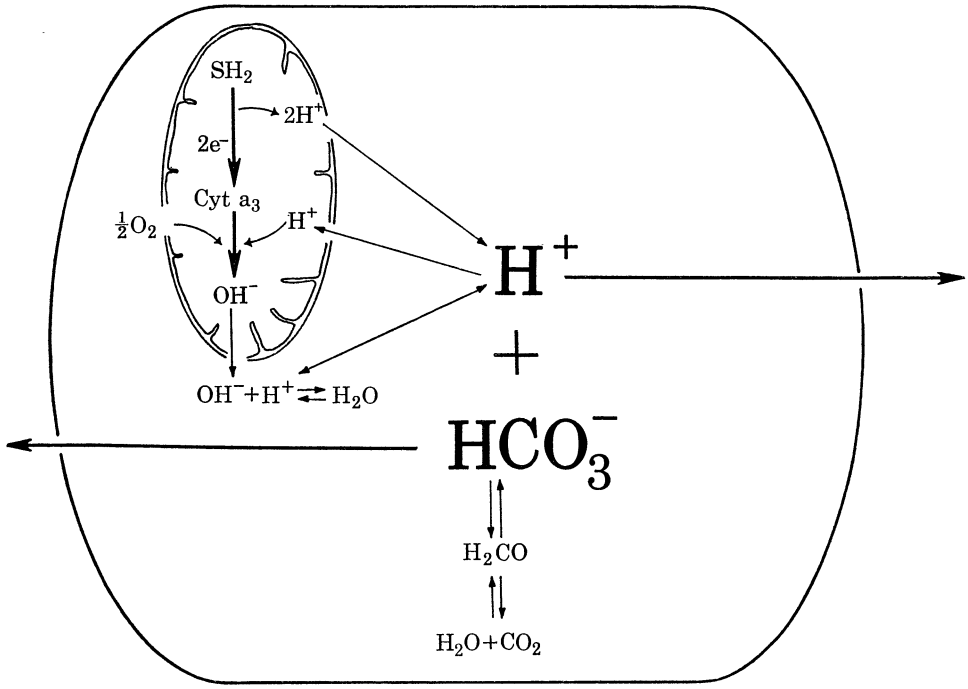

FIGURE 12. Hypothetical scheme for reactions controlling intracellular pH in gastric mucosa. Large symbols indicate cellular ion pools. Thick arrows indicate major paths for removal of hydrogen and bicarbonate.

The various agents which we have tested could alter the intracellular pH, represented by the hydrogen-ion pool, by acting on one or more of the reactions communicating with that pool. In this scheme histamine would act to stimulate hydrogen production by the substrate oxidation reactions and also stimulate hydrogen removal via the transport process. The latter effect appears to dominate since the pH is observed to rise. Thiocyanate presumably acts primarily by decreasing the removal of hydrogen ion via transport. An alternative possibility is that this compound increases the permeability of the mucosal surface to hydrogen ion (Moody & Davis 1970) allowing hydrogen to enter the cell from the lumen. In either case an acid shift would be expected under the conditions of our experiments. The alkaline shift observed with Diamox might be due to a decrease in the contribution of carbonic acid to the hydrogen-ion pool. The effect of applied potential is somewhat more difficult to explain in terms of the model. If the potential acts only to inhibit hydrogen ion removal by the transport process one would expect an acidification similar to that observed with thiocyanate. Since the opposite pH change occurs, the potential must be producing another effect in addition to or instead of inhibiting the transport mechanism directly. One possibility is that a potential which inhibits acid secretion will be oriented so as to also inhibit the efflux of bicarbonate through the serosal membrane or indeed

to drive bicarbonate into the cell from the serosal medium. If the influx of bicarbonate exceeds the decrease in hydrogen ion removal a net alkaline change would occur. This would be consistent with the known effect of respiratory alkalosis on secretion (Byers, Jordan & Maren 1962). This mechanism in itself would not account for the lag time nor for the rectification observed in these experiments. However, little is known of the mechanism of bicarbonate movements and it is possible that a delay and rectification are properties of that process. The mechanisms by which the various agents could alter intracellular pH presented above do not constitute the only possibilities and it would be very surprising if they act by such simple means. These mechanisms do however offer possibilities which can be tested in future experiments.

The mechanisms by which intracellular pH changes can give rise to oxidation–reduction changes in the respiratory chain remains speculative. The results require that the action at least be at the level of cytochrome a_3. An effect on this component might then be reflected back along the respiratory chain in agreement with the observation that the entire chain appears to respond as a unit. The effect of hydrogen ion on cytochrome a_3 might simply be a consequence of the mass law effect since hydrogen ion is a component of the reaction of cytochrome a_3 and oxygen to form hydroxyl ions;

$$2a_3^{2+} + \tfrac{1}{2}O_2 + H^+ = 2a_3^{3+} + OH^-,$$

where a_3^{2+} and a_3^{3+} are respectively the reduced and oxidized forms of cytochrome a_3. Assuming that this reaction is always near eqilibrium, it may be calculated that a pH change from 7.0 to 9.0 would result in approximately a 50 % reduction of cytochrome a_3. A test of this mechanism obviously requires knowledge of the exact pH changes accompanying a given cytochrome change, information which we cannot as yet obtain. Of course, other mechanisms such as allosteric effects of hydrogen ion could be operative and we offer this mechanism as only one possibility.

A more important, and more difficult, question is that of the role of pH in coupling secretion and oxidative metabolism. The potential for coupling exists since hydrogen ion is common to both processes although there is no unique cycle of production and utilization. This question then resolves itself into two consideration; does pH control respiration? and, does pH control the secretory rate? The present results show that intracellular pH is not uniquely controlling respiration since an increase in pH may be accompanied by an increase in respiration (e.g. with histamine) or a decrease in respiration (e.g. with applied potential). However, the intracellular hydrogen ion concentration may serve to set an upper limit to respiration associated with acid secretion. At very high rates of secretion the increase in intracellular pH may be sufficient to limit respiration (Chance & Conrad 1958). Respiration below an upper limit could then be controlled primarily by the level of ADP, although the cytochrome changes produced by ADP variation would be masked by pH dependent redox changes.

Control of the acid secretory rate by intracellular pH could occur by at least two mechanisms. Since the transport mechanism presumably requires the interaction of both an energy source such as ATP and hydrogen ion, limited availability of either component could control the secretory rate. Limitation by hydrogen ion availability is conceptually the simpler mechanism. However, little is known of the relationship between hydrogen-ion concentration and secretory rate, i.e. the affinity of the 'pump'. It is possible that the 'pump' can extract maximal amounts of hydrogen ion from solutions of very high pH. As discussed above, intracellular pH can limit respiration and therefore can limit the energy available to the 'pump'. Such a mechanism

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would also serve to limit the transport dependent pH increase, affording some protection for alkaline sensitive components of the cell.

To summarize briefly, measurement of respiratory chain components and intracellular pH in the intact gastric mucosa reveals substantial changes in both parameters in response to various agents which alter acid secretion. The results indicate that the respiratory chain redox changes are due to the changes in intracellular pH. The effects of the various agents employed provide important clues as to the action of these agents but do not uniquely identify their mechanism of action. The role of intracellular pH and cytochrome oxidation–reduction levels in controlling respiration and acid secretion likewise remain speculative. Perhaps the most important aspect of the present work is that it offers a fresh approach to the study of gastric secretion, using non-destructive optical techniques. The results presented here represent a limited view of the potential of these techniques and future experiments hold great promise. In addition, it is hoped that this work will provide direction and stimulate other investigators to join in attempting to answer some of the questions raised here.

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