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BICARBONATE ION-ATPase IN RAT LIVER CELL FRACTIONS

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SUMMARY

The distribution of HCO_3^--ATPase activity was studied in cell fractions prepared from homogenates of rat liver. The level of mitochondrial contamination in the microsomal fraction depended on the fractionation procedure and on the method of homogenization. With proper care, microsomes with undetectable mitochondrial contamination could be prepared. These microsomes had no detectable HCO_3^--ATPase activity. Approximately 85 % of the total HCO_3^--ATPase activity of the post $6000 \times g \cdot$ min supernatant was recovered in the mitochondrial fraction. The properties of this mitochondrial HCO_3^--ATPase were not distinguishable from those of the various microsomal HCO_3^--ATPases previously described by other investigators.

INTRODUCTION

There is currently a great deal of interest in enzyme systems that may be capable of affecting transmembrane pH gradients. Studies of these systems fall into two general categories defined by the subcellular location of the enzymes. The first consists of the mitochondrial enzymes and these are concerned with oxidative phosphorylation [1] and citrulline metabolism [2]. The second consists of the microsomal enzymes that are evidently concerned with transcellular fluxes of hydrogen ions and/or bicarbonate ions [3–5]. Both of these systems can be characterized as Mg^{2+}-ATPases that are stimulated by the presence of the bicarbonate ion. It was recently suggested [6–8] that the microsomal enzymes may be artifacts arising from mitochondrial contamination. One reason for the uncertainty on this matter is that the tissues studied to date could not be fractionated in a definitive manner using differential centrifugation techniques. Since methods for the fractionation of the rat liver have been extensively developed, this problem can be avoided by studying the distribution of HCO_3^--ATPase activity in cell fractions prepared from rat liver.

Both the microsomal [3, 5] and mitochondrial [9, 10] HCO_3^--ATPases have been characterized in some detail. In addition, these enzymes have been studied with [9, 7] and without [10] equilibrating the components of the carbonic acid system.

However, all of the previous studies on the mitochondrial enzymes were performed on intact mitochondria, and it would be expected that any mitochondrial contamination in microsomal fractions would involve mitochondrial fragments [11]. Since differences in ATPase properties of intact and fragmented mitochondria have been reported [12], we investigated the properties of the HCO_3^--ATPase in fragmented rat liver mitochondria. The present results should aid in evaluating the premise that the microsomal HCO_3^--ATPases in all tissues arise from mitochondrial contamination.

METHODS

Fed, adult male Sprague–Dawley rats were used in this study.

Two homogenization schemes were tested. The first was based on the fractionation procedure of Blobel and Potter [13] as modified by Smuckler [14] and utilized a hypertonic sucrose solution through the nuclear spin. The post nuclear supernatant was then diluted with distilled water to a final sucrose concentration of 0.25 M. Mitochondrial fractions were prepared by centrifuging these solutions in a Sorvall RC2B (SS34 rotor) at 17 000 × *g* for 30 min. The resulting pellets were suspended in a 1 mM Tris–EDTA, pH 7.4 solution. Microsomal fractions were prepared by centrifuging the post-mitochondrial supernatant at 105 000 × *g* for 1 h in a Beckman 40 rotor and suspending the resulting pellet in 1 mM Tris–EDTA.

The second fractionation procedure was based on another method by Smuckler [15] which utilized a 0.25 M sucrose solution that also contained 5 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.4) and 5 mM KCl. Either of two teflon pestles, one conventional and the other modified, were used to homogenize the tissue. (The modified pestle was machined to a clearance of 0.2 mm.). After the first spin at 600 × *g* for 10 min, the pellet was washed by vortexing with two volumes of homogenization solution and recentrifuging. The supernatants from both spins were combined and the mitochondria were obtained by spinning at 10 000 × *g* for 20 min. This pellet was also washed and the mitochondria were suspended in 1 mM Tris–EDTA. The microsomal pellet was prepared from the initial postmitochondrial supernatant. This supernatant was recentrifuged twice at 10 000 × *g* for 20 min to remove traces of light mitochondria, and then centrifuged in a Beckman 60 Ti rotor at 255 800 × *g* for 1 h. The microsomes were suspended in 1 mM Tris–EDTA using a glass–glass homogenizer. Assays were performed on fresh material as well as material that had been frozen and thawed. Previous reports have established that either suspension in hypotonic solution, or glass–glass homogenization or freezing and thawing, results in fragmented mitochondria [12]. No differences were noted between the freshly prepared material suspended in 1 mM EDTA and the frozen and thawed material.

The ATPase and succinate dehydrogenase assays were performed as was previously described in detail [7]. The Fiske–Subbarow procedure was used to measure the inorganic phosphate liberated through ATP hydrolysis, and the Lowry procedure was used for protein determination. The carbonic acid system was not equilibrated in these experiments since this is the usual procedure in studies of the microsomal HCO_3^--ATPase. The pH dependence of the ATPases was measured as was previously described [7] except that Tris–H_2SO_4 and piperazine-*N*,*N*′-bis-2-ethane sulphonic acid–H_2SO_4 buffers were employed.

RESULTS

Comparison of fractionation procedures

The two fractionation procedures resulted in quantitatively dissimilar microsomal fractions. The procedure based on the method of Blobel and Potter [13] which utilized hypertonic sucrose solutions during the isolation of the nuclear fractions consistently yielded microsomes which were approx. 20 % contaminated with mitochondria as measured by succinate dehydrogenase activities (Fig. 1). The microsomes prepared according to the second scheme which utilized the 0.25 M sucrose solution and the unaltered teflon pestle had approximately half as much mitochondrial contamination as the preceding microsomes. If the altered, loose-fitting pestle were used in this latter procedure, then the succinate dehydrogenase activity in the microsomal fraction decreased to undetectable levels.

The HCO_3^--ATPase activities in the microsomal fractions were proportional to the succinate dehydrogenase activities (Fig. 1). In addition, although the slope of the line relating microsomal succinate dehydrogenase activity to microsomal HCO_3^--ATPase activity was not determined exactly, nonetheless it is clear that those microsomes with undetectable succinate dehydrogenase activities also had undetectable HCO_3^--ATPase activities (Fig. 1 and Table I).

Table I summarizes the ATPase properties of the microsomal fractions that had undetectable succinic dehydrogenase activities. The procedures for the assays are given in the figure legend. The ATPase activity in the presence of Mg^{2+} and HCO_3^- was within the range obtained with Mg^{2+} alone or in the presence of Mg^{2+} and NaCl. No (Na^+, K^+)-stimulated ATPase activity was detected in these microsomal fractions in agreement with the results of Emmelot and Bos [16]. On the other hand, the mitochondrial fractions from the same preparations demonstrated a HCO_3^--stimulated

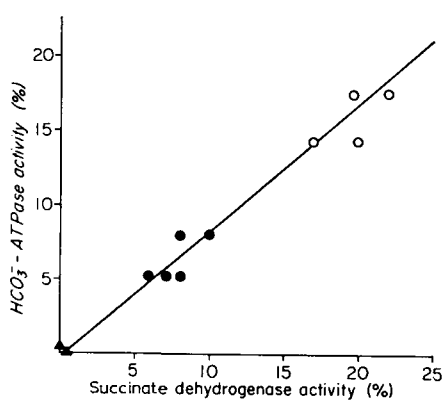


Fig. 1. The relationship between the HCO_3^--ATPase activities and the succinate dehydrogenase activities in the microsomal fractions (relative to the mitochondrial fractions) prepared from rat livers. Each point represents the average of duplicate determinations on different preparations. The open circles represent the results obtained with microsomes prepared according to the method based on the procedure of Blobel and Potter [13]. The closed circles represent the results obtained with microsomes prepared according to the method of Smuckler [15] using the unmodified pestle, and the triangles represent the results obtained with the modified pestle. The line through the points was drawn by eye.

TABLE I

ATPase ACTIVITIES IN MICROSOMAL AND MITOCHONDRIAL FRACTIONS FROM RAT LIVER

Rat liver microsomal and mitochondrial fractions were prepared as described in the text. The basic incubation medium contained 50 mM Tris-H_2SO_4 buffer (pH 7.5 at 37.5 °C) and 5 mM Tris-ATP (pH 7.5). Further additions of $MgSO_4$ (5 mM) and $NaHCO_3$ (25 mM) or NaCl (25 mM), and $MgSO_4$ (5 mM) plus NaCl (150 mM) and KCl (5 mM) with and without ouabain (10^{-4} M) were made depending on the ATPase to be measured. All incubations were done in 2.0 ml total volume for one hour. Either 0.07 mg protein (Lowry) of the microsomal fractions or 0.02 mg protein of the mitochondrial fraction was added per tube. The resulting changes in absorbance (A) for the Mg^{2+}-ATPases corresponded to specific activities, of 2.4 μM P_i · mg protein^{-1} · h^{-1} and 31.3 μM P_i · mg protein^{-1} · h^{-1} respectively, for the microsomal and mitochondrial fractions. The absorbance values have been corrected for the non-enzymatic release of P_i from ATP during the incubation period. The readings were done in duplicate and the experiment was repeated several times. Typical values from a representative experiments are given here.

Additions	P_i split from ATP (A)	
	Microsomes	Mitochondria
Mg^{2+}	0.250	0.480
Mg^{2+} + HCO_3^-	0.255	0.600
Mg^{2+} + Cl^-	0.250	0.415
Mg^{2+} + Na^+ + K^+	0.250	0.334
Mg^{2+} + Na^+ + K^+ + ouabain	0.248	0.325

ATPase and an inhibition in the presence of NaCl and KCl (Table I). Ulrich [17] has reported that this inhibition is due to an ionic strength effect.

Enzymatic activity recovery measurements indicated that there was no serious degradation of the HCO_3^--ATPase during the fractionation procedure. In a representative experiment utilizing the loose-fitting teflon pestle procedure, 20 % of the HCO_3^--ATPase activity in the original homogenate was recovered in the post 6 000 · g · min supernatant while the normally discarded pellet accounted for the balance of the activity. Of the 20 % remaining activity, 78 % was recovered in the mitochondrial fraction, 7 % was recovered in the post-mitochondrial supernatant, and there was undetectable HCO_3^--ATPase activity in the microsomal fraction. Thus, approx. 97 % of the original activity could be accounted for in this procedure. After it was established that the HCO_3^--ATPase activity in rat liver cell fractions was limited to the mitochondrial fraction, the properties of this ATPase were further investigated.

Properties of the mitochondrial HCO_3^--ATPase

HCO_3^- and Mg^{2+} requirement. The mitochondrial ATPase was found to be stimulated by the addition of HCO_3^- (Table II). $NaHCO_3$ and $KHCO_3$ were equally effective. Mg^{2+} was required in order to obtain this HCO_3^- stimulation.

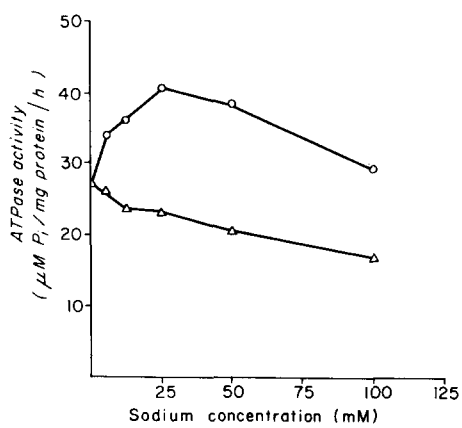
Activation by HCO_3^-. The effect of the HCO_3^- concentration on the mitochondrial ATPase activity is shown in Figs 2 and 3. Since increasing ionic strength is known to inhibit the mitochondrial ATPase activity [17], the HCO_3^- stimulation was measured as the difference between the activities in the presence of Mg^{2+} and HCO_3^- and in the presence of Mg^{2+} and Cl^-. The total ATPase activities (Mg^{2+}

TABLE II

Mg^{2+} REQUIREMENT FOR HCO_3^--ATPase ACTIVITY

ATPase activity in a mitochondrial fraction prepared from rat liver was measured in a 2 ml reaction volume which contained 50 mM Tris–H_2SO_4 buffer (pH 7.5 at 37 °C), 5 mM Tris–ATP and approx. 20μg of mitochondria in addition to the ions listed. The activities are the means from two experiments. All incubations were for 1 h.

Added ions (concentration)	ATPase activity (μM P_i/mg protein per h)
$MgSO_4$ (5 mM)	27.0
$MgSO_4$ (5 mM)+$NaHCO_3$ (25 mM)	37.3
$MgSO_4$ (5 mM)+$KHCO_3$ (25 mM)	38.0
$MgSO_4$ (5 mM)+NaCl (25 mM)	22.8
$NaHCO_3$ (25 mM)	0.4
$KHCO_3$ (25 mM)	0.8
NaCl (25 mM)	0.4



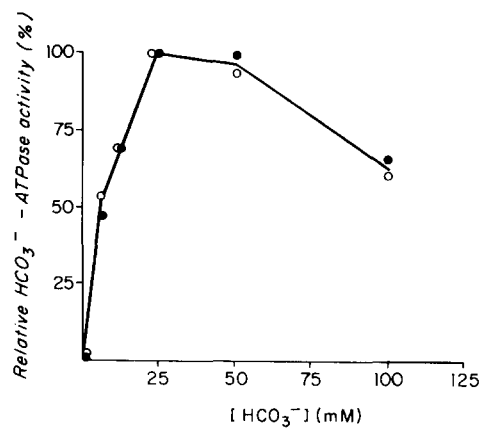


Fig. 2. Rat liver mitochondrial ATPase activity in the presence of $MgSO_4$ and $NaHCO_3$ or NaCl. ATPase activity was measured as described in the legend to Table I except that all solutions contained 5 mM $MgSO_4$ and varying concentrations of either $NaHCO_3$ (○ – ○) or NaCl (△ – △). The activities presented are from a representative experiment. Similar results were obtained in two other experiments.

Fig. 3. The effect of varying the HCO_3^- concentration on the HCO_3^--stimulated ATPase activity. ATPase activity was measured as described in the legend to Fig. 1. The HCO_3^--stimulated ATPase activity was taken as the difference between the activity in the presence of the stock solution plus $NaHCO_3$ and that obtained with NaCl. The results are expressed relative to the activity obtained in the presence of 25 mM $NaHCO_3$. The filled and open circles represent the results from two different experiments.

$+HCO_3^-$ and $Mg^{2+}+Cl^-$) are given in Fig. 2 while the stimulation due to HCO_3^- is given in Fig. 3. A HCO_3^- concentration of approx. 6 mM yielded half-maximal stimulation and by 25 mM the stimulation was maximal. Further increases in the HCO_3^- concentration resulted in decreased activity.

pH dependence of the HCO_3^--ATPase activity. The pH dependence of the HCO_3^--ATPase activity is given in Fig. 4., The ATPase activity in the presence of

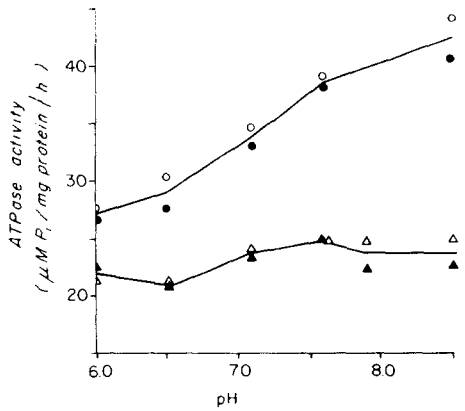


Fig. 4. pH Dependence of the ATPase activities in a rat liver mitochondrial fraction. ATPase activities were measured in the presence of 5 mM $MgSO_4$, 5 mM Tris–ATP and a 50 mM Tris–piperazine-*N*,*N*′-bis-2-ethane sulphonic acid –H_2SO_4 buffer with (circles) or without (triangles) 25 mM $NaHCO_3$. The solid and open symbols represent the results obtained in two experiments.

Mg^{2+} + HCO_3^- increased with increasing pH and reached a maximum at pH 8.5. The Mg^{2+}-ATPase activity was less affected by variations in pH.

Substrate specificity. The ability of the mitochondrial ATPase to utilize different nucleoside triphosphates is given in Table III. The Mg^{2+}-dependent enzyme was able to utilize all of these triphosphates to some degree. On the other hand, the HCO_3^--ATPase was not able to utilize CTP or UTP and ITP was utilized half as well as GTP.

Activation by different anions. The effects of different anions on the mitochondrial Mg^{2+}-ATPase are summarized in Table IV. The presence of arsenate in the incubation medium appeared to yield a stimulation of the Mg^{2+}-ATPase activity that was five times greater than that obtained with HCO_3^-. However, further examination indicated that this result was an artifact resulting from an effect of arsenate on the Fiske–Subbarow assay. Because of this interference, the true ability of arsenate to

TABLE III

SUBSTRATE SPECIFICITY OF THE ATPase IN A RAT LIVER MITOCHONDRIAL FRACTION

Nucleoside triphosphatase activity was measured in the basic incubation medium described in the legend to Table I except that all solutions contained the sodium salt of one of the nucleotide triphosphates (5 mM) given below, plus the additional ions listed in the first column. The activities are the averages from two experiments.

Added ions (concentration)	Nucleoside triphosphatase activity (μM P_i/mg protein per h)				
	ATP	GTP	ITP	CTP	UTP
$MgSO_4$ (5 mM)	28.9	24.9	17.9	10.1	3.2
$MgSO_4$ (5 mM) + $NaHCO_3$ (25 mM)	37.6	28.3	19.9	9.2	2.3
$MgSO_4$ (5 mM) + NaCl (25 mM)	24.0	24.0	16.8	9.5	2.9

TABLE IV

ATPase ACTIVITY IN A RAT LIVER MITOCHONDRIAL FRACTION IN THE PRESENCE OF VARIOUS ANIONS

ATPase activity was measured as described in the legend to Fig. 2 except that various anions were added to the incubation solutions, and all solutions contained 5 mM $MgSO_4$. The values listed are from a representative experiment. Similar results were obtained in 2 other experiments.

Added anions	ATPase activity (μM P_i/mg protein/h)	
	anion concentration	
	2.5 mM	25 mM
none	25.0	25.0
bicarbonate	26.8	30.0
chloride	24.3	20.8
arsenate*	130.0	155.0
borate	23.0	26.0
sulfite	—	41.3
nitrate	—	13.0
chromate	41.8	—

* The arsenate activity is an artifact. See text.

stimulate the Mg^{2+}-ATPase could not be estimated here. On the other hand, sulphite and chromate clearly stimulated the Mg^{2+}-ATPase while borate was without effect and chloride and nitrate inhibited the enzyme.

Effect of miscellaneous agents (Table V)

Ouabain. Neither the Mg^{2+}-ATPase nor the HCO_3^--ATPase was affected by the presence of 10^{-4} M ouabain.

2,4-*Dinitrophenol.* The presence of 10^{-5} M 2,4-dinitrophenol in the incubation

TABLE V

EFFECT OF VARIOUS INHIBITORS ON THE ATPase ACTIVITY IN A RAT LIVER MITOCHONDRIAL FRACTION

ATPase activity was measured in the basic incubation medium described in Table I except that the incubation solutions contained the inhibitors listed below. The activities are relative to the activity in the presence of 5 mM $MgSO_4$. The results are from a representative experiment. Similar results were obtained in two other experiments.

Added Ions	Relative ATPase activity in the presence of					
	no inhibitor	2,4-dinitro-phenol ($5 \cdot 10^{-4}$ M)	ouabain (10^{-4} M)	SCN^- (10^{-2} M)	SCN^- (10^{-3} M)	diamox (10^{-3} M)
$MgSO_4$ (5 mM)	1.00	1.10	1.02	0.36	0.61	0.96
$MgSO_4$ (5 mM)+ $NaHCO_3$ (25 mM)	1.41	1.43	1.41	0.50	1.10	1.35
$MgSO_4$ (5 mM)+ NaCl (25 mM)	0.81	1.07	0.85	0.37	—	0.79

medium was without effect on the ATPase activities. However, 2,4-dinitrophenol at $5 \cdot 10^{-4}$ M yielded inconsistent results. The Mg^{2+}-ATPase was stimulated by about 10 % while the (Mg^{2+} · HCO_3^-)-ATPase activity was unaffected. In addition, $5 \cdot 10^{-4}$ M 2,4-dinitrophenol removed the inhibition observed in the presence of Cl^-.

SCN^-. Both the Mg^{2+}-ATPase and the HCO_3^--ATPase activities were inhibited in the presence of SCN^- which was the only substance in this group that was capable of inhibiting these enzymes.

Diamox. Acetazolamide at a concentration of 1 mM had no effect on the ATPase activities.

DISCUSSION

The present results describing the distribution of HCO_3^--ATPase activity in the various cell fractions prepared from rat liver (Table 1) are consistent with the premise that there is no microsomal HCO_3^--ATPase activity in this tissue. On the other hand, since only approx. 97 % of the total HCO_3^--ATPase activity could be accounted for in the recovery studies, it is conceivable that there is a microsomal HCO_3^--ATPase that is selectively degraded during the fractionation procedures. However, the fact that appreciable HCO_3^--ATPase activity can be recovered in the microsomal fraction (Fig. 1) under a more vigorous fractionation procedure (as judged by the release of succinate dehydrogenase activity) makes this premise unlikely. Thus the favored interpretation of these results is that there is no HCO_3^--ATPase activity in microsomal fractions prepared from rat liver.

The above finding may not be unexpected since the liver does not show a marked transepithelial active hydrogen ion or bicarbonate ion transport as is found in gastric, pancreatic or salivary gland tissues. Nonetheless, it must be remembered that in hepatic cells [18] as well as in other tissues [19–22], hydrogen ions are not passively distributed across the surface membrane, and that an active hydrogen ion or bicarbonate ion pump is necessary to explain the observed hydrogen ion gradient. It is attractive to hypothesize that the enzymatic basis for this transmembrane transport is the same as that found in the hydrogen ion and bicarbonate ion secreting tissues, just as was found to be the case for the (Na^+, K^+)-ATPase.

The above result is also in accord with a growing list of results that indicates that the microsomal HCO_3^--ATPase is not as ubiquitous as was first believed. We previously suggested because of the correlation between succinate dehydrogenase activity and HCO_3^--ATPase activity that the microsomal HCO_3^--ATPase prepared from dog submandibular gland arose from mitochondrial contamination [7]. However this conclusion was not definitive because NaI treatment was used to decrease the microsomal succinate dehydrogenase activity, and it was possible that the NaI affected both enzymes nonspecifically. Similarly, Katz and Epstein [6] concluded that the HCO_3^--ATPase in rat kidney microsomes arose from mitochondrial contamination. However their results were not definitive because they could not reduce the level of mitochondrial contamination in their microsomal fractions below 9 %. In addition, Epstein and Whittam (cited in Katz and Epstein [6]) have reported the absence of a HCO_3^--ATPase in red cell membranes. Also, Kimelberg and Bourke [10] concluded that the HCO_3^--ATPase activity in rat brain homogenates was of mitochondrial origin even though they obtained a slight HCO_3^- stimulation in the

microsomal fraction following freezing and thawing. Finally, Soumarmon et al. [8] used isopycnic equilibration centrifugation procedures to conclude that the HCO_3^--ATPase in rat fundus mucosa microsomes also arose from mitochondrial contamination. This latter result is in direct contrast to the results of Sachs et al. [3] who used similar procedures to conclude that a HCO_3^--ATPase of microsomal origin did exist in dog gastric mucosa. This difference is unlikely to be due to a species variation if the enzyme is responsible for acid secretion in the stomach as has been suggested by Sachs et al. [4]. Thus artifacts arising from differences in the preparatory procedures remain as the most likely explanation for the conflicting results obtained with the gastric mucosae. These previous results and the present findings in rat liver require that conclusions concerning the physiological significance of the microsomal HCO_3^--ATPase be withheld.

Comparison with previous studies of the mitochondrial HCO_3^--ATPase

The present results confirm and extend the results of Fanestil et al. [9], Racker [23], Grisolia et al. [2] and Kimelberg and Bourke [10], who previously studied the mitochondrial HCO_3^--ATPase. Of these, the report by Racker [23] was in abstract form; and HCO_3^--ATPase activity was reported incidentally to citrulline synthesis, the main topic of concern, in the studies by Grisolia and his colleagues [2, 24]. Thus, the studies by Fanestil et al. [9] and by Kimelberg and Bourke [10] contain the bulk of the previous data on the mitochondrial HCO_3^--ATPase. Of these, the measurements by Fanestil et al. [9] were made using rat liver mitochondria incubated in solutions in which the components of the carbonic acid system had been equilibrated. Since they chose to use total CO_2 in solution rather than the HCO_3^- concentration as the experimental variable, comparisons between their study and the more recent studies using nonequilibrated solutions are difficult. However, the following similarities and differences between their results and the present results are noted. The present results relating ATPase activity to HCO_3^- concentration (Fig. 2) and pH (Fig. 4) are in general agreement with the results of Fanestil et al. [9]. On the other hand, there are two areas in which seemingly conflicting results were obtained. The first involves nucleoside specificity and the second involves the Mg^{2+} requirement. Fanestil et al. [9] found that the HCO_3^--ATPase could not utilize GTP while the opposite was found in the present study. This difference may be due to the longer incubation times used here (1 h vs 12 min), or it may be due to the fact that we used disrupted mitochondria. Also, Mg^{2+} was required to obtain the HCO_3^--stimulated ATPase activity in the present study (Table II) and in the study the Kimelberg and Bourke [10], but not in the study by Fanestil et al. [9]. This conflicting result was probably due to differences in the preparatory procedures or to differences in the ionic composition of the assay solutions.

The present study supplements the study by Kimelberg and Bourke [10] since there was a tissue difference and since they did not study substrate specificity, effects of other anions, nor the effect of 2,4-dinitrophenol. Our results with different anions are in agreement with the results of Mitchell and Moyle [1] who studied the Mg^{2+}-ATPase in sonicated particles prepared from rat liver mitochondria. The present result on the effect of 2,4-dinitrophenol on the Mg^{2+}-ATPase is in agreement with that of Meyers and Slater [12] who found that the Mg^{2+}-ATPase in mitochondria that were frozen and thawed or exposed to hypotonic solutions were only slightly

(if at all) activated by 2,4-dinitrophenol. They attributed this decreased stimulation to the fact that the two procedures had disrupted the mitochondria.

Finally, it should be noted that there may be two HCO_3^--ATPase in rat liver mitochondria. The first is evidently associated with the Mg^{2+}-ATPase of the inner membrane [1] and the second is an apparent HCO_3^--ATPase activity associated with citrulline synthesis [2]. However, synthesis of citrulline cannot be used to differentiate between the two as was previously suggested [9] since the citrulline synthesis system can hydrolyze ATP without synthesizing citrulline [24].

Comparison with microsomal HCO_3^--ATPases

There are currently two HCO_3^--ATPases that are thought to be microsomal in origin. One is isolated from the gastric mucosae of various species [3, 25] and the other from the pancreas [5]. A comparison of these enzymes with the present enzyme follows.

(1) All of the enzymes require Mg^{2+} in order to elicit the HCO_3^- stimulated ATPase activity.

(2) The relationship between HCO_3^- concentration and ATPase stimulation as measured in the present study (Figs 2 and 3) is nearly identical to the relationship obtained in the mammalian pancreas [5]. The rising face of this curve is also similar to that obtained with bullfrog gastric mucosa microsomes [25].

(3) The pH dependence of the (HCO_3^- + Mg^{2+})-ATPase and the Mg^{2+}-ATPase activities obtained in the present study is also similar to that obtained in the mammalian pancreas [5]. In contrast, the HCO_3^--ATPase in microsomes prepared from dog gastric mucosa [26] has a sharp maximum at about pH 7.5 that was not detected in the present study. However, this difference may not be significant since Meyers and Slater [12] have shown that disrupted mitochondria can have a variable pH dependence.

(4) The substrate (nucleoside triphosphate) specificity of the present mitochondrial enzyme is also in the same order as that reported in the pancreas [5] and gastric mucosa [25].

(5) The stimulation of the mitochondrial Mg^{2+}-ATPase by the arsenate, sulfate and borate anions (Ref. 1 and Table III) are in the same order as that observed in the gastric mucosa [26].

(6) Ouabain at 10^{-4} M and acetazolamide at 10^{-3} M had no effect on the present enzyme (Table V) nor on the enzyme from the bullfrog gastric mucosa [25].

(7) SCN^- at 10^{-2} M inhibited the Mg^{2+}-ATPase and the HCO_3^--ATPase in the present instance (Table V) as well as in gastric mucosa [27] and in the mammalian pancreas [28].

(8) 2,4-Dinitrophenol yielded a variable small stimulation in the present study (Table V) as would be expected for disrupted mitochondria [12]. Kasbekar and Durbin [25] reported a small variable stimulation of their bullfrog gastric mucosa enzyme that is reminiscent of the present results. Sachs et al. [29] report no stimulation with 2,4-dinitrophenol in the mammalian gastric mucosa.

In a previous comparison of the mitochondrial and microsomal HCO_3^--ATPases only two differences were noted [29]. The one was that the microsomal enzyme was not stimulated by 2,4-dinitrophenol and the second was that the mitochondrial enzyme was inhibited by the borate anion. The present results indicate that

neither of these points can be used to definitively distinguish between the two enzymes. Instread, the many similarities between the microsomal HCO_3^--ATPase and the HCO_3^--ATPase in disrupted mitochondria strengthens the suspicion that the microsomal activity arises from mitochondrial contamination as was recently suggested [6–8].

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