Biochimica et Biophysica Acta, 311 (1973) 545–564

BBA 76369

PROPERTIES OF ATPase OF GASTRIC MUCOSA

V. PREPARATION OF MEMBRANES AND MITOCHONDRIA BY ZONAL CENTRIFUGATION

J. G. SPENNEY[a], A. STRYCH[a], A. H. PRICE[a], H. F. HELANDER[b] and G. SACHS[a,*]

[a] *University of Alabama in Birmingham, Birmingham, Ala. (U.S.A.) and* [b] *University of Umeå, Umeå (Sweden)*

(Received February 9th, 1973)

SUMMARY

Using zonal sucrose density gradient centrifugation methods to fractionate the subcellular components in gastric mucosal homogenates have been developed. Methods are described which give high yield or rapidity in fractionation. A procedure is described which enables large-scale preparations of smooth walled vesicular membranes containing HCO_3^--ATPase activity free from mitochondrial contamination as assessed by electron microscopic morphology and undetectable succinic dehydrogenase or monoamine oxidase activity. A method to purify gastric mitochondria is also described.

An HCO_3^--stimulated ATPase present in gastric mucosal homogenates has been implicated in proton transport by this epithelium[1,2]. The complexity of this tissue and the resulting homogenate necessitated isolation of the oxyntic (acid secreting) cells to confirm localization of HCO_3^--ATPase to these cells[3]. Further, the enzyme was localized in the oxyntic cell microsomes by differential centrifugation[3]. By sucrose density gradient centrifugation a fraction appearing as smooth walled vesicular membranes was also isolated from dog gastric mucosa[4]. These membranes contained no detectable glucose-6-phosphatase, succinic dehydrogenase, or monoamine oxidase activity[5]. A similar enzyme can be found in pancreas[6].

Further biochemical characterization necessitated large scale preparation in zonal rotors. This report describes preparative techniques utilizing zonal sucrose density gradient centrifugation to provide either rapid separation, high total yield, or high purity separation of gastric vesicular membranes or mitochondria. Our major concern has been with high purity separation of these particles.

METHODS

The stomach is removed from a fasted dog and immediately opened along the greater curvature, washed with water, and cooled in ice. After 3–5 min any remaining

* To whom correspondence should be sent.

esophagus and antrum is cut away. The stomach is layed flat on a cooled pyrex dish and with a microscope slide the mucosa is scraped as a sheet from the underlying muscular layer. The mucosa, suspended in 700 ml of buffer, is chopped into small fragments in a Waring blender with a dulled blade at medium speed. In a Parr "bomb" it is allowed to equilibrate with magnetic stirring for 20 min under 950 lb/inch^2 nitrogen. The mucosal fragments are disrupted on decompression. After 10 min standing at 0 °C the floating mucus layer is aspirated and discarded. The remaining homogenate is filtered through 4 layers of cheese cloth and subjected to differential and/or zonal sucrose density gradient centrifugation.

The buffer used in each instance is 40 mM Tris–HCl, pH 7.4, with sucrose. In methods designed for intact mitochondria 0.44 M sucrose is used while 0.25 M sucrose is used when gastric membranes are prepared. The addition of 3 mM $MgCl_2$ or EDTA or 1 mM dithiothreitol seemed to have no effect either on the levels of activity obtained or on the separations achieved, hence were omitted at later stages.

(1) Fractionation of the tissue by centrifugation

To ascertain the best methods of preparation of subcellular components of gastric mucosa we have studied zonal sucrose density fractionation of the total homogenate and at various stages of purification using differential centrifugation.

(A) Differential centrifugation (Fig. 1). The total homogenate in 40 mM Tris–HCl–0.25 M sucrose is centrifuged at 1000 × *g* for 30 min in a Sorvall RC-2 centrifuge. The pellet is washed once with the same buffer and the washing and original supernate are combined. This suspension is centrifuged at 8000 × *g* for 30 min and the pellet washed once. Washing and supernate are sedimented at 20000 × *g* for 30 min. The pellet is washed once and the supernate and washing are centrifuged at

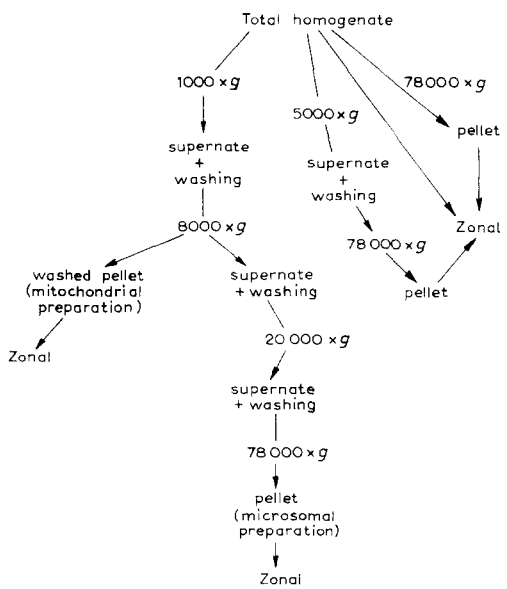


Fig. 1. Preparative steps in purification of material prior to zonal density gradient centrifugation.

78000×g* for 60 min in a No. 30 rotor in a Beckman L-2 preparative ultracentrifuge. The final 78000×g pellet is not washed. In preparations of gastric mitochondria 0.44 M sucrose is substituted for the 0.25 M sucrose.

(*B*) *Zonal centrifugation.* Three types of runs have been used: (1) Step gradients using 20, 24, 34, and 47% sucrose; (2) rate zonal centrifugation utilizing a 5–35% linear gradient; and (3) isopycnic density gradient centrifugation using 20–45 or 50% linear sucrose gradient.

Fractionation of the total homogenate presents a special problem since the volume of the sample approaches the volume of the rotor. When fractionating the total homogenate on a sucrose density gradient it is divided and dry sucrose added to raise the sucrose content to 20% and 50%, respectively, and the total volume of each to 325 ml. Total homogenate volume and volume to be raised to 20% sucrose (w/w) and 50% sucrose (w/w) can be estimated with sufficient accuracy that material is not wasted by the following formula:

$$V_I = V_F \cdot \frac{(1-S_F)}{(1-S_I)}$$

where V_I, starting volume; V_F, volume of homogenate to be used in forming gradient (usually 300–325 ml); D_F, density of final solution assuming it is a sucrose and water (w/w) solution; D_I, density of starting solution with same assumption; S_F, final desired % sucrose (w/w); S_I, % sucrose (w/w) of starting solution.

The sucrose to be added is estimated from:

$$V_F \cdot D_F - V_I \cdot D_I$$

The sucrose is dissolved by magnetic stirring in an ice water bath. After dissolution accuracy is checked on an Abbe refractometer. For zonal fractionation of all other material the gradient is formed from sucrose of the desired concentration dissolved in 40 mM Tris–HCl, pH 7.4.

(*C*) *Formation of the gradient.* Gradients are formed in a Beckman Ti-14 zonal rotor spinning at 3000 rev./min in a Beckman L-2 ultracentrifuge.

(1) Step gradients are formed using 100 ml 20% sucrose, 200 ml 24% sucrose, 150 ml 34% sucrose and 150 ml 47% sucrose. The solutions are sequentially pumped into the zonal rotor beginning with the 20% sucrose. The sample is added last, usually at the center of the rotor.

(2) Continuous gradients are formed from 5 and 35% sucrose or from 20 and 45 or 50% sucrose using a Beckman gradient maker calibrated to deliver a 600 ml gradient which is linear with volume delivered from the pump.

(*D*) *Preparation of the sample.* Preparation of the total homogenate is given above. All other samples are obtained in pellet form during centrifugation. The pellet may be prepared for injection at the periphery of the rotor by suspending it in the most dense sucrose solution. When the sample is injected into the center of the rotor it is suspended in 0.25 M sucrose for isopycnic runs and in 5% sucrose for rate zonal centrifugation. We most commonly use central injection. We examined the efficacy of injection into the center of the gradient. In this instance 200–350 ml of the gradient is allowed to form. The pump is stopped and 10–20 ml of gradient is pumped from

* g value at R_{max} = 105500.

the side arm of the delivery tube. The pellet is resuspended in this solution and re-injected into the rotor *via* the side arm of the delivery tube. The remainder of the gradient is then formed.

(*E*) *Zonal fractionation.* After loading, the rotor is closed and brought to 47000 rev./min. For rate zonal and step gradient separations the run is terminated after 30 min and 2–3 h, respectively. Isopycnic centrifugation, our usual practice, is allowed to continue for 6–8 h. The gradient is unloaded at 3000 rev./min by central displacement by 60% sucrose pumped into the periphery of the rotor. 33 20-ml fractions are collected for assays and further biochemical characterization.

(*2*) *Assays*

Sucrose concentration was measured with an Abbe refractometer. Mg^{2+} and HCO_3^--ATPase and 5'-nucleotidase were measured as previously described[5]. Succinate dehydrogenase activity was measured by the method of King and Howard[7], and monoamine oxidase according to the method of Tabor *et al.*[8]. Lactate dehydrogenase was measured as described by Reeves and Fimognari[9].

(*3*) *Electron microscopy*

A 1–3 ml portion of one of the fractions is diluted to 8.6% sucrose with 40 mM Tris–HCl, pH 7.4, placed in centrifuge tubes in which a flat agar cushion had been formed. The material is centrifuged at 39000 rev./min in a Beckman SW-39 rotor for 60 min. Supernate is decanted and buffered 1% osmium tetroxide is layered on the agar base for 12 h. The material is subsequently dehydrated, embedded in Vestopal W. Thin sections contrasted with lead hydroxide and uranyl acetate, were studied in a Phillips EM 200 and EM 300 electron microscope at 80 kV.

RESULTS

The total homogenate was fractionated in two ways. Firstly (Fig. 2) the gradient was actually formed from the total homogenate as described above. By this technique protein distribution on the gradient is a reflection of the soluble protein concentration as well as the very heavy components which usually sediment out at low *g* forces in differential centrifugation. The Mg^{2+}-ATPase activity is a low broad plateau from 23% sucrose to 46% sucrose while the HCO_3^--ATPase is clearly bimodal in distribution. The upper peak is not associated with a discernible protein peak. Even with the large protein load the lower HCO_3^--ATPase closely reflects that of protein and succinic dehydrogenase. Table I gives the relative enrichment of these peaks.

To avert the soluble protein accompanying the total homogenate, the total homogenate in a volume of 325 ml was layered on a linear 20–50% sucrose gradient in the other 325 ml of the rotor. Protein, Mg^{2+}-ATPase and HCO_3^--ATPase distribution were bimodal. The lower peak of Mg^{2+}- and HCO_3^--ATPase coincided with the lower protein peak. The upper peak of Mg^{2+}- and HCO_3^--ATPase at 31% sucrose was not associated with a clear cut peak of protein. The peaks of Mg^{2+}- and HCO_3^--ATPase activity are very close and undoubtedly overlap considerably.

We further examined fractionation of all particulates in the total homogenate by collecting the $78000 \times g$ for 60 min sediment of the total homogenate. This pellet was fractionated on a 20–50% linear sucrose gradient, Fig. 3. The protein is distri-

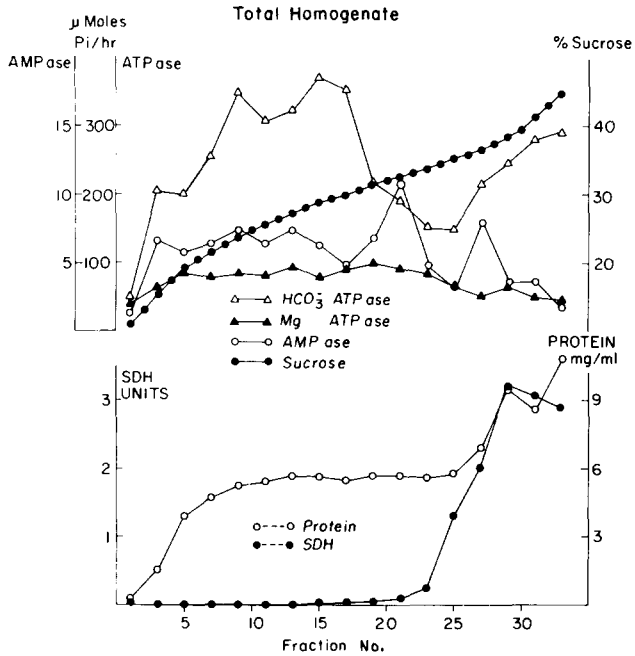


Fig. 2. Fractionation of protein and enzymes obtained from the total homogenate on a 20–45% linear sucrose gradient. SDH, succinate dehydrogenase.

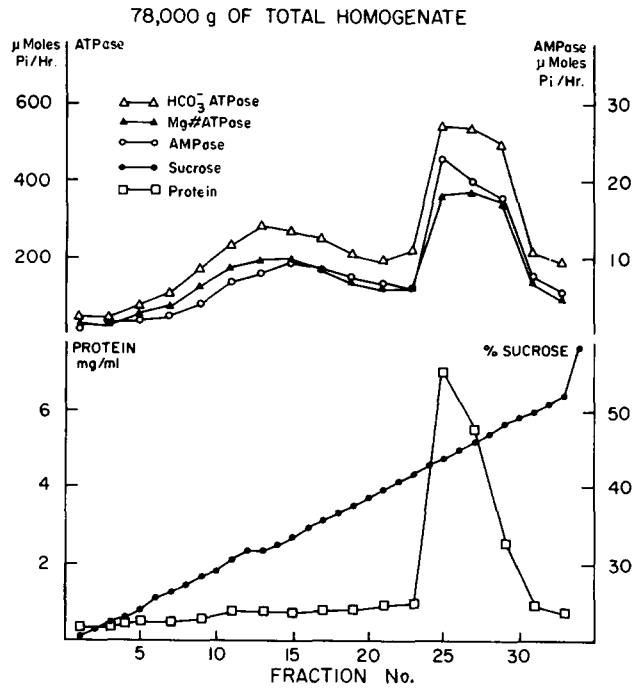


Fig. 3. Fractionation of the 78000 × *g* pellet from the total homogenate. A 20–50% sucrose gradient is used.

buted largely as a collection at 41–48%. On the other hand the distribution of AMPase, Mg^{2+}- and HCO_3^--ATPase is clearly bimodal with peaks at 29–33% and 43–48% sucrose, respectively. Further, this technique was examined by first removing the $5000 \times g$ sediment. Its washings and original supernate were sedimented at $78000 \times g$ for 60 min. The fractionation of this 5000–$78000 \times g$ pellet is shown in Fig. 4. By this technique distribution of protein is clearly bimodal at 28–32% and

TABLE I

C is the peak activity at 28–32% or 40–45% sucrose. C_0 is the activity if it had been spread evenly over the entire gradient.

	C/C_0	
	28–32%	*40–45%*
HCO_3^--ATPase	1.52	1.23
Mg^{2+}-ATPase	1.35	0.68
AMPase	1.3	1.5
Succinate dehydrogenase	—	4.25

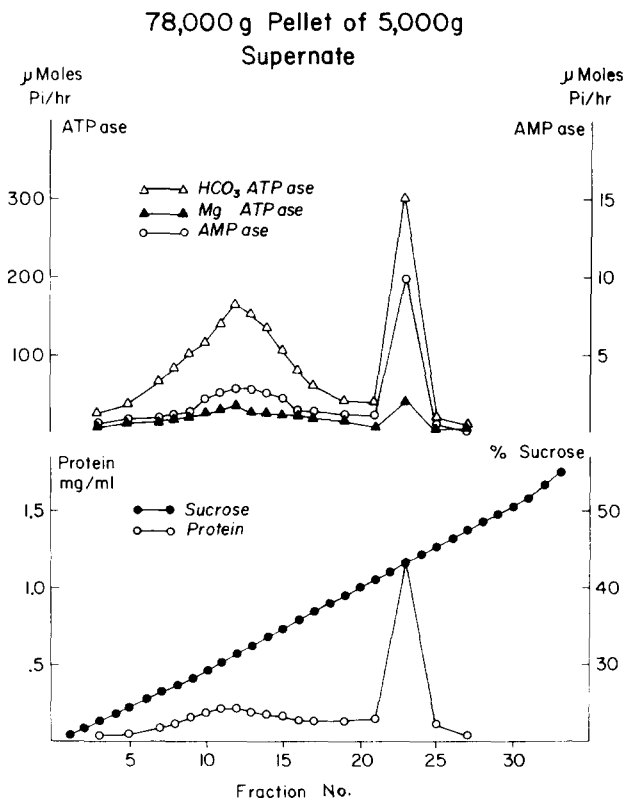


Fig. 4. Fractionation of the $78000 \times g$ pellet from the post $5000 \times g$ supernate. A 20–50% linear sucrose gradient is used.

41–45% sucrose. Mg^{2+}-ATPase shows two small peaks of activity while AMPase and HCO_3^--ATPase clearly show a bimodal distribution corresponding with the protein peaks.

TABLE II

	Total membrane HCO_3^--ATPase ($\mu M\ P_i/h$)
Total homogenate	3050
$7.8 \cdot 10^4 \times g$ pellet of total homogenates	1560
$7.8 \cdot 10^4 \times g$ pellet of $5 \cdot 10^3 \times g$ supernate	919

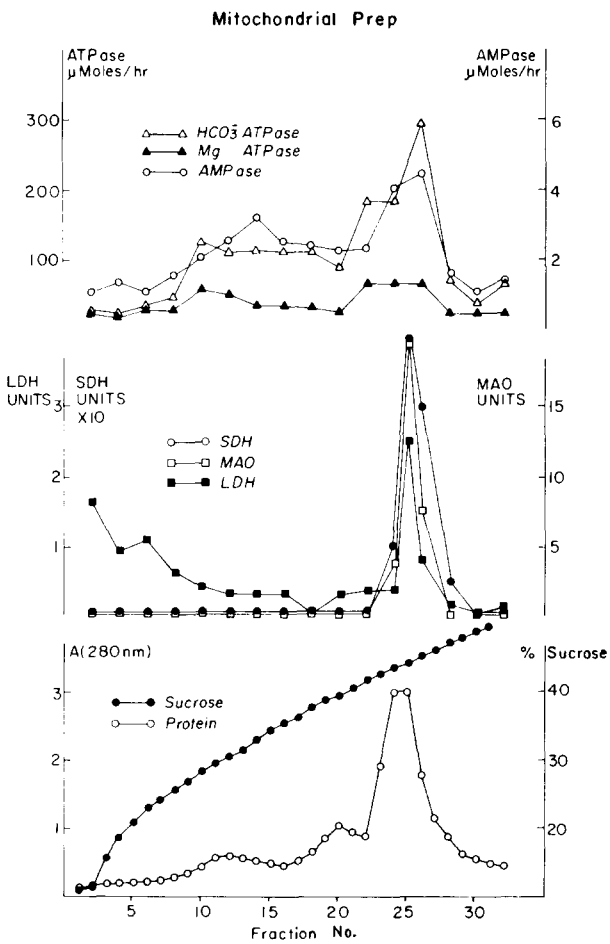


Fig. 5. Fractionation of the washed $8000 \times g$ pellet on a 20–50% linear sucrose gradient. MAO, monoamine oxidase; SDH, succinate dehydrogenase; LDH, lactate dehydrogenase.

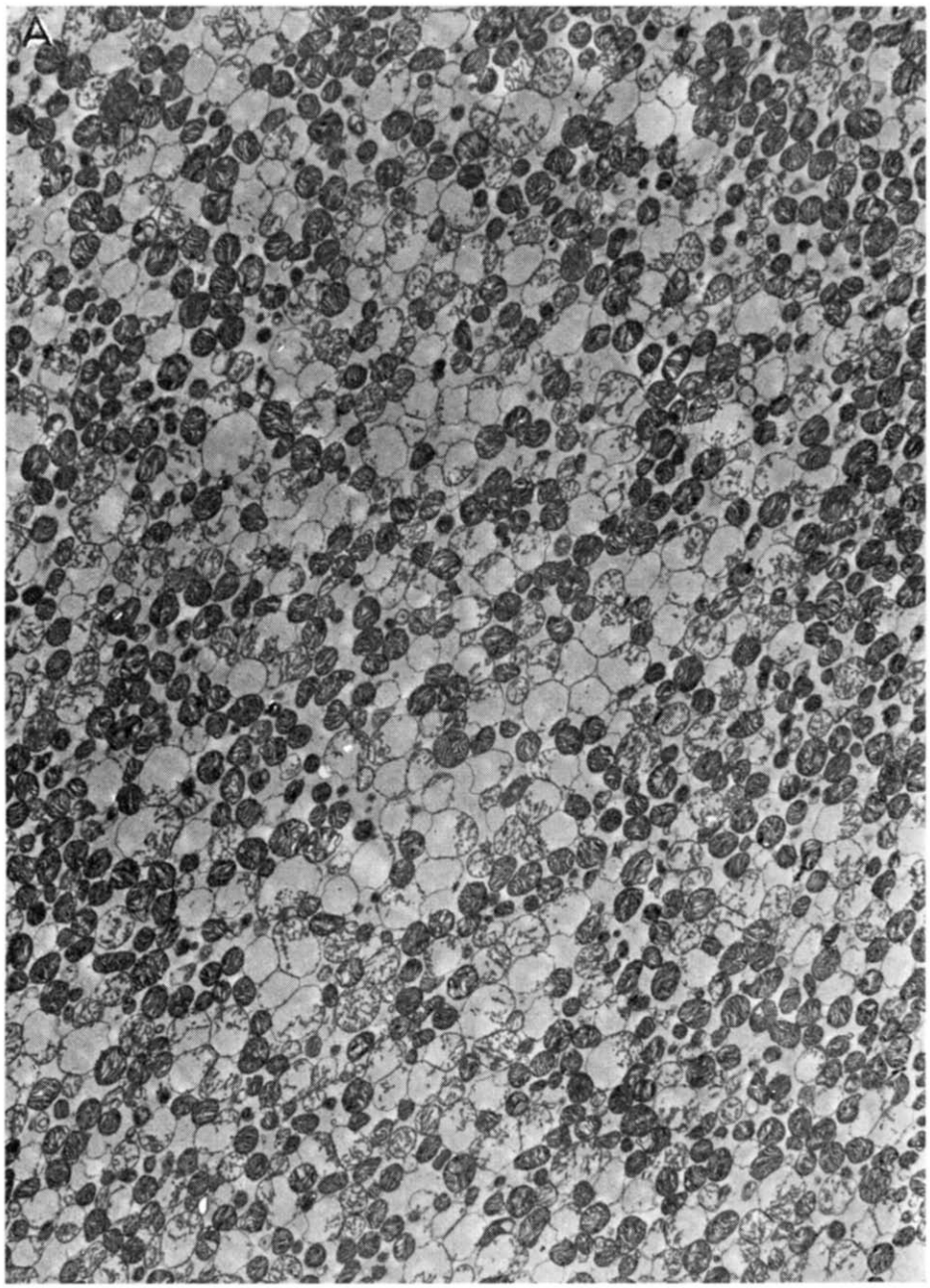


Fig. 6. (A) Electron microscopic morphology (magnification ×3100) of the fraction at 41–45% sucrose obtained from the washed 8000 × *g* pellet. (B) Mitochondrial morphology (magnification ×31000) from the 41–45% sucrose fraction of the washed 8000 × *g* pellet.

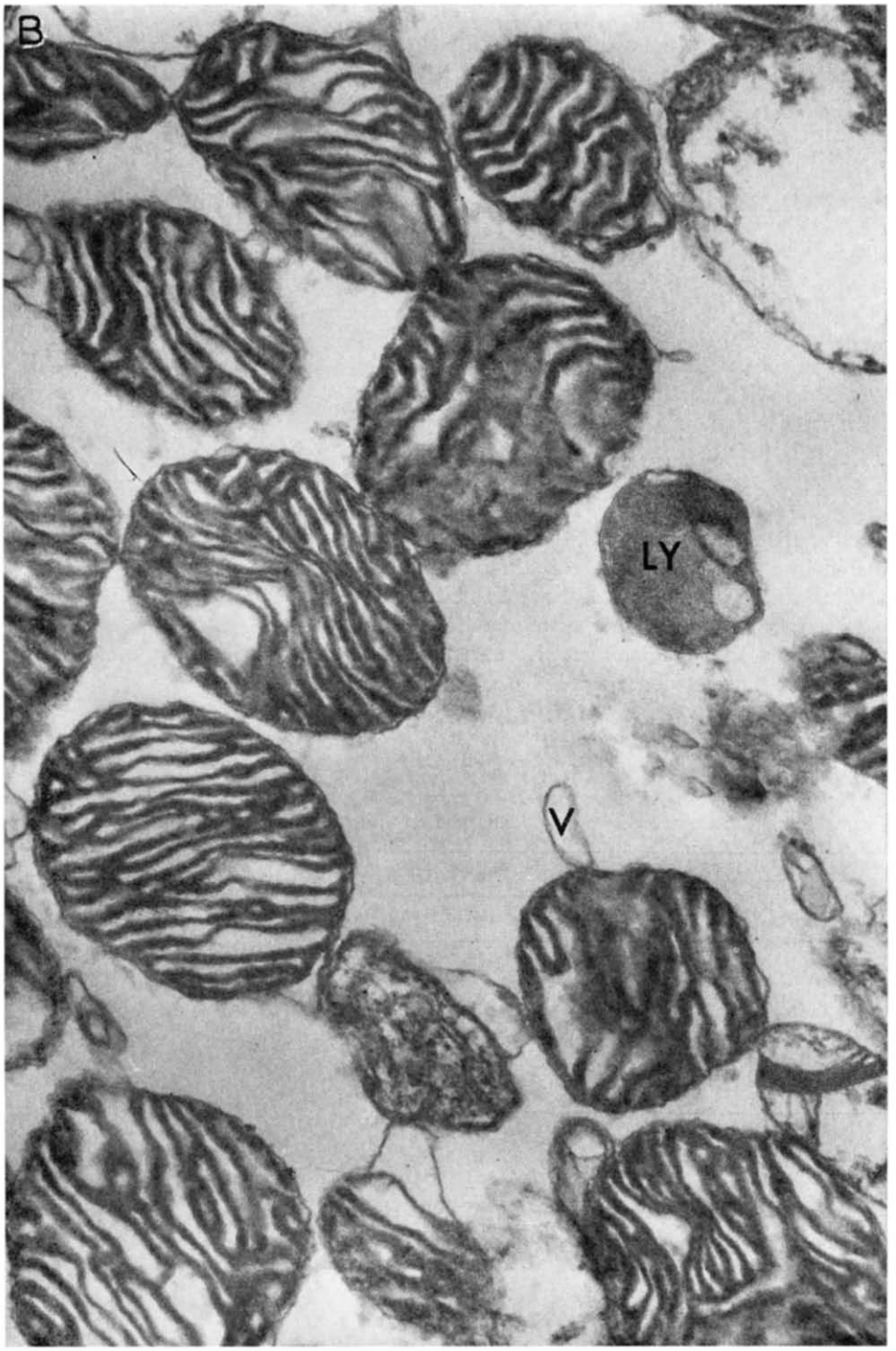

B
LY
V

Table II gives the total ATPase activity recovered from the upper peak of HCO_3^--ATPase which would be usable for further biochemical characterization.

To further examine the losses of ATPase activity during differential centrifugation, we have subjected the washed $8000 \times g$ pellet of the $1000 \times g$ supernate to zonal fractionation.

Fig. 5 shows the analysis of a representative gradient. Protein distribution expressed as absorbance at 280 nm is clearly trimodal while the distribution of AMPase, Mg^{2+}-ATPase, and HCO_3^--ATPase is bimodal. The upper peak of Mg^{2+}- and HCO_3^--ATPase coincides with the protein peak at 30% sucrose while the AMPase peak is at slightly greater sucrose concentration, 33%. The lower peak of ATPase and AMPase activity at 42–45% sucrose correspond to the third protein peak. Succinic dehydrogenase and monoamine oxidase activity are uni-modal with peaks at 44% sucrose. Lactate dehydrogenase on the other hand has one peak at 44% sucrose, but appears to have a second peak at the point of application to the gradient. The middle protein peak at 39% sucrose is a constant finding when measured at 280 nm but is not associated with ATPase, AMPase, succinic or lactic dehydrogenase or monoamine oxidase activity.

Electron microscopy (Fig. 6) of the peak at 41–45% sucrose was obtained to further clarify the presence of the HCO_3^--ATPase and AMPase. The picture is dominated by intact gastric mitochondria; some, however, are burst or have some distortion of the cristae. In addition, a small number of lysosomes and smooth walled vesicular membrane structure are seen.

Table III shows the relative enrichment of the upper and lower peaks with respect to the enzymes assayed.

TABLE III

C is peak total activity in the region 28–32% or 40–44% sucrose. C_0 is the hypothetical total activity/sample if activity were evenly distributed on the gradient.

	C/C_0	
	28–32%	*40–44%*
Mg^{2+}-ATPase	1.6	2.0
HCO_3^--ATPase	1.2	2.8
AMPase	1.5	2.1
Succinate dehydrogenase	—	5.0
Monoamine oxidase	—	7.7

Lastly, fractionation of the microsomal preparation, the $78000 \times g$ pellet from the $20000 \times g$ supernate, was assessed. In the example (Fig. 7) the sample was injected into the center of the rotor but in other runs the sample was injected at about 30% in the gradient. By this preparative technique a protein peak (280 nm absorbance) at 28–31% sucrose is about equal to a peak at the periphery of the rotor. In addition a peak usually appears at 36–39% sucrose. HCO_3^-- and Mg^{2+}-ATPase distribution is trimodal with major peaks at 28–31% sucrose and at the periphery of the rotor.

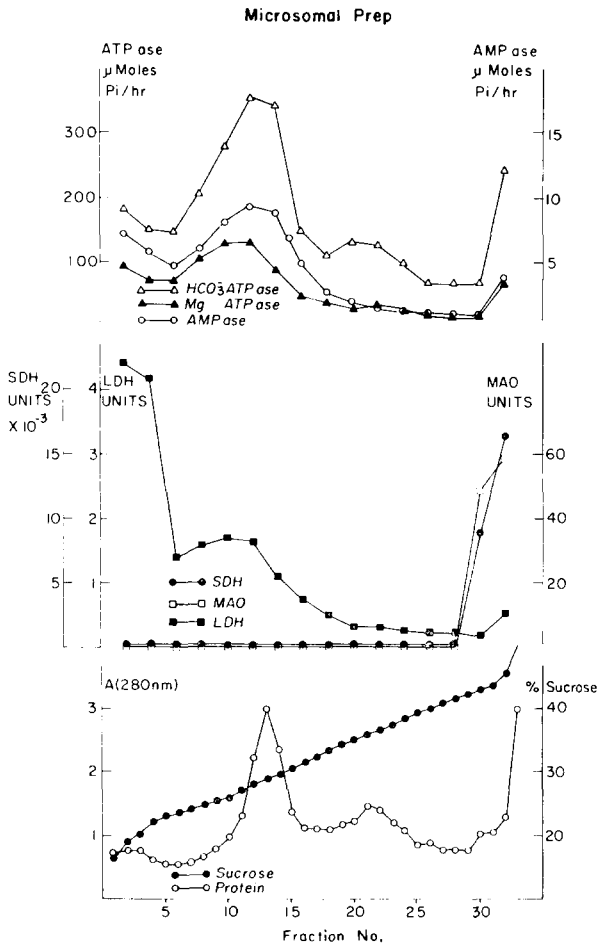


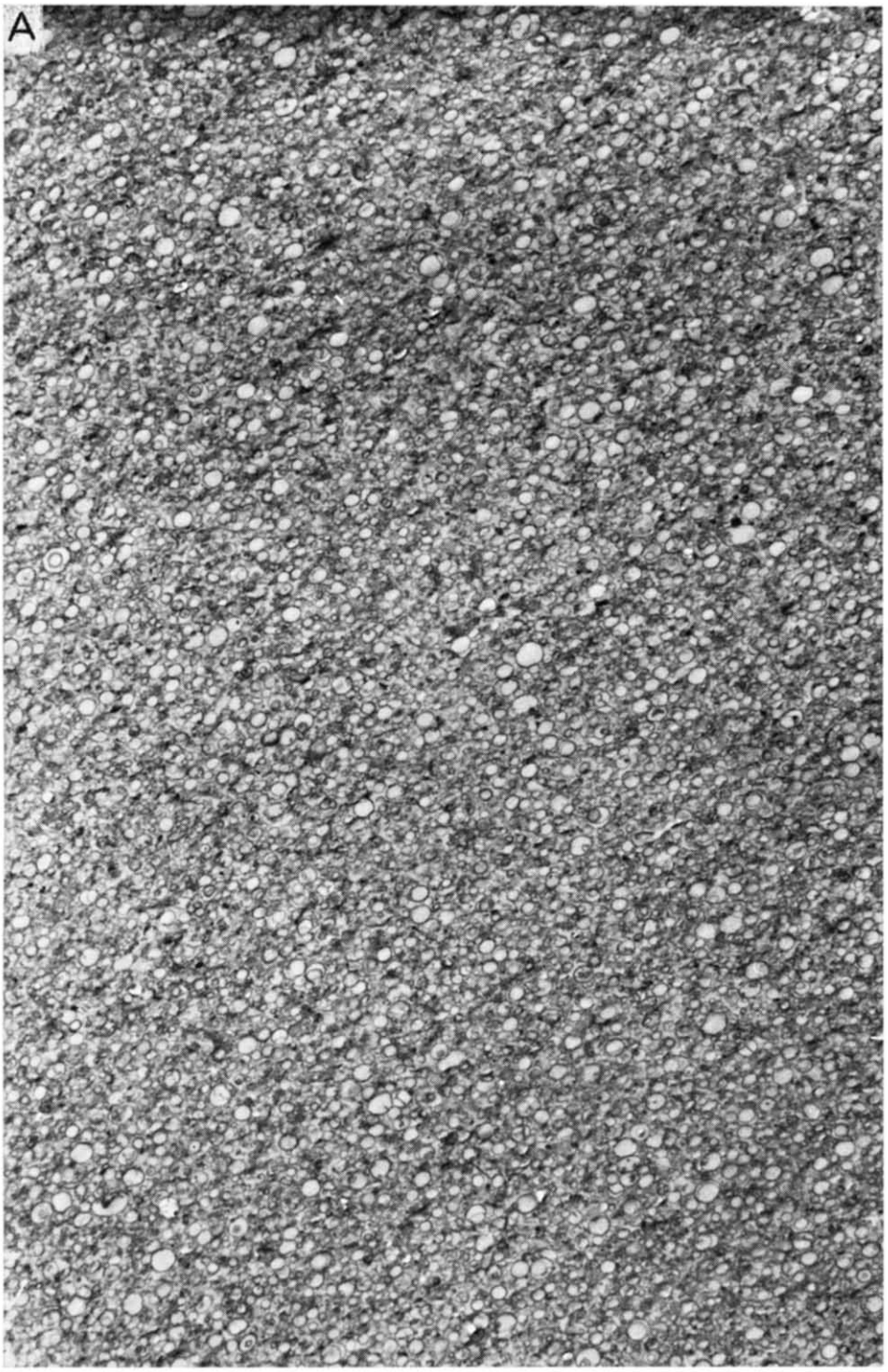
Fig. 7. Fractionation of the microsomal fraction on a 20–45% sucrose gradient. MAO, monoamine oxidase; LDH, lactate dehydrogenase; SDH, succinate dehydrogenase.

A small tertiary peak occurs at 35–38% sucrose. AMPase activity is bimodal coinciding with the two major ATPase peaks. Succinic dehydrogenase and monoamine oxidase are undetectable below 41.5% and peak at the periphery of the rotor. Lactic dehydrogenase is most active at the site of sample application, but a peak coincides with the lighter density HCO_3^--ATPase and a lesser peak at the periphery of the rotor.

Table IV shows the enrichment of the two major protein peaks with respect to these enzymes.

Electron microscopic morphology of the material recovered at 28–32% sucrose is shown (Fig. 8). In five experiments the fractions at 28–32% sucrose displayed smooth surfaced vesicular membrane structures of very high purity.

We further examined the feasibility of performing fractionation of this fraction on step gradients and by rate zonal centrifugation to achieve more rapid separation of particles. Fig. 9 shows fractionation achieved by rate zonal fractionation. There


A

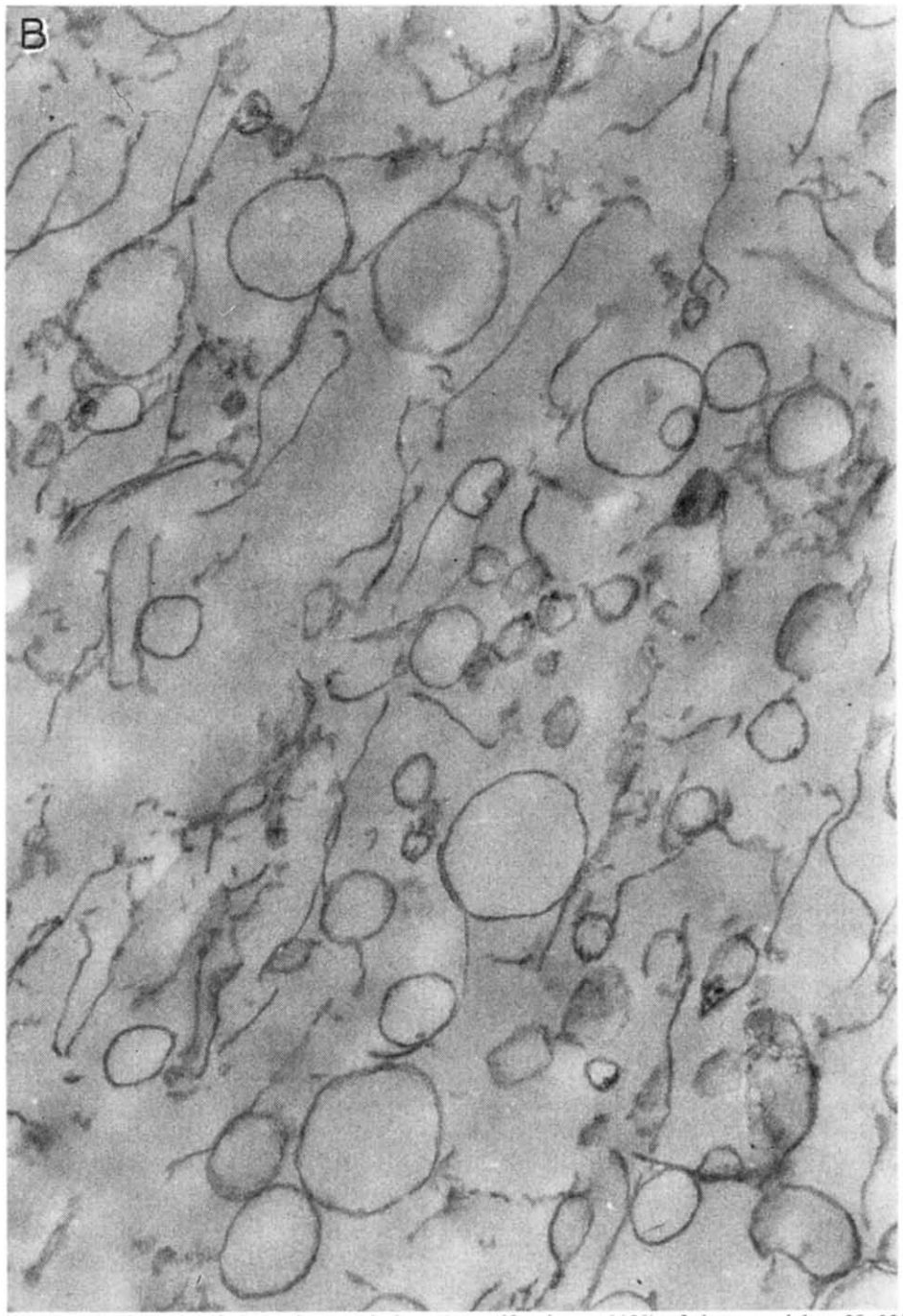


Fig. 8. (A) Electron microscopic morphology (magnification ×3100) of the material at 28–32% sucrose obtained from fractionation of the microsomal fraction. (B) Morphology of the membrane structures (magnification ×31000) isolated from the microsomal preparation at 28–32% sucrose on linear gradients.

is bimodal distribution of Mg^{2+}- and HCO_3^--ATPase. Protein is widely distributed but greatest near entry into the gradients. Mg^{2+}- and HCO_3^--ATPase and AMPase peak at 10% sucrose and again at 24–26%; the activity is, however, widely distributed.

Step gradient fractionation of the gastric mucosa has been investigated. Fig. 10

TABLE IV

C is the peak total activity in the region 28–32% or 40–44% sucrose. C_0 is the hypothetical total activity/sample if activity were evenly distributed on the gradient.

	C/C_0	
	28–32%	*40–44%*
Mg^{2+}-ATPase	2.2	1.1
HCO_3^--ATPase	2.1	1.4
AMPase	2.2	0.9
Succinate dehydrogenase	—	8.5
Monoamine oxidase	—	11.0

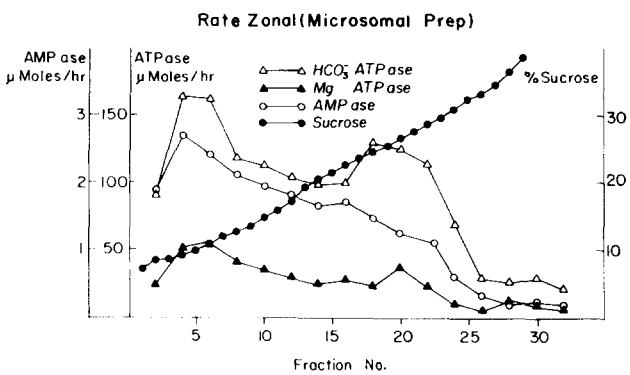


Fig. 9. Fractionation of the microsomal pellet by rate zonal centrifugation on 10–35% sucrose gradient.

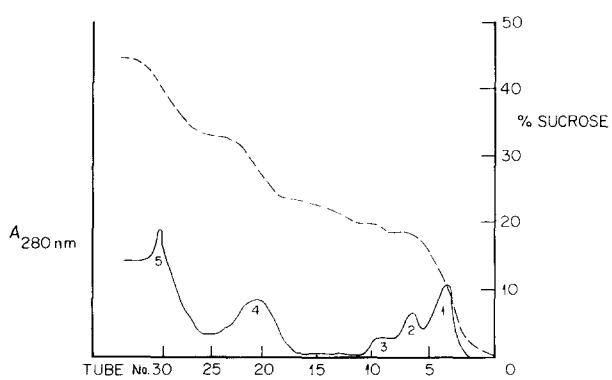


Fig. 10. Fractionation of the microsomal pellet on a sucrose step gradient.

shows the protein fractionation and Table V the ATPase activities obtained in these protein peaks. Protein is accumulated at each step in the gradient, and HCO_3^--ATPase preferentially in the fourth protein peak at about 28% sucrose. Succinic dehydrogenase and monoamine oxidase activity were not assessed in either the rate zonal or the step gradient.

On many linear sucrose gradients of the microsomal fraction there appeared to be a separation of one or two tubes between the peak HCO_3^--ATPase and AMPase activities. Gradients consisting of 7.5% Ficoll–0.25 M sucrose to 0% Ficoll–37.5% sucrose were utilized to separate the ATPase and AMPase activities. Fig. 11 shows

TABLE V

ACTIVITY OF BANDS FROM ZONAL ROTOR

Band	*HCO_3^--ATPase specific activity (μmoles $P_i \cdot mg^{-1}$)*
Total homogenate	3.20
1	0.25
2	3.73
3	4.50
4	26.55
5	11.91

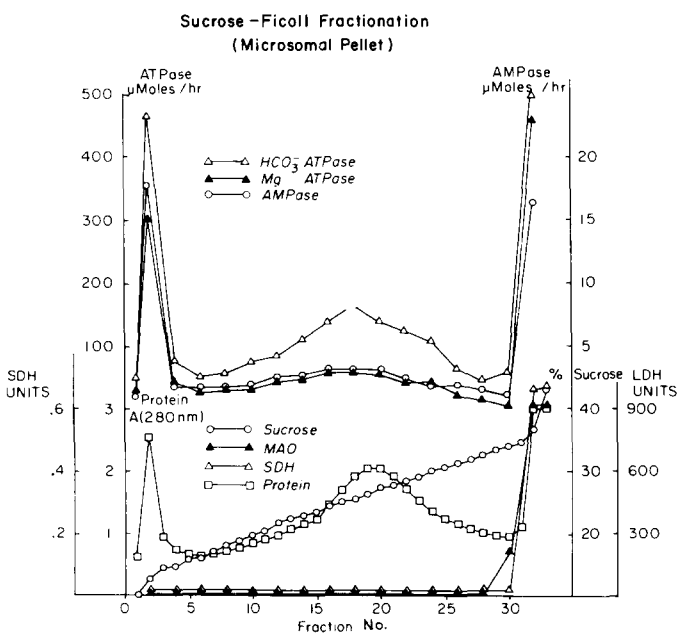


Fig. 11. Fractionation of the microsomal preparation on a linear Ficoll–sucrose gradient. Refraction index expressed as percent sucrose. MAO, monoamine oxidase; SDH, succinate dehydrogenase; LDH, lactate dehydrogenase

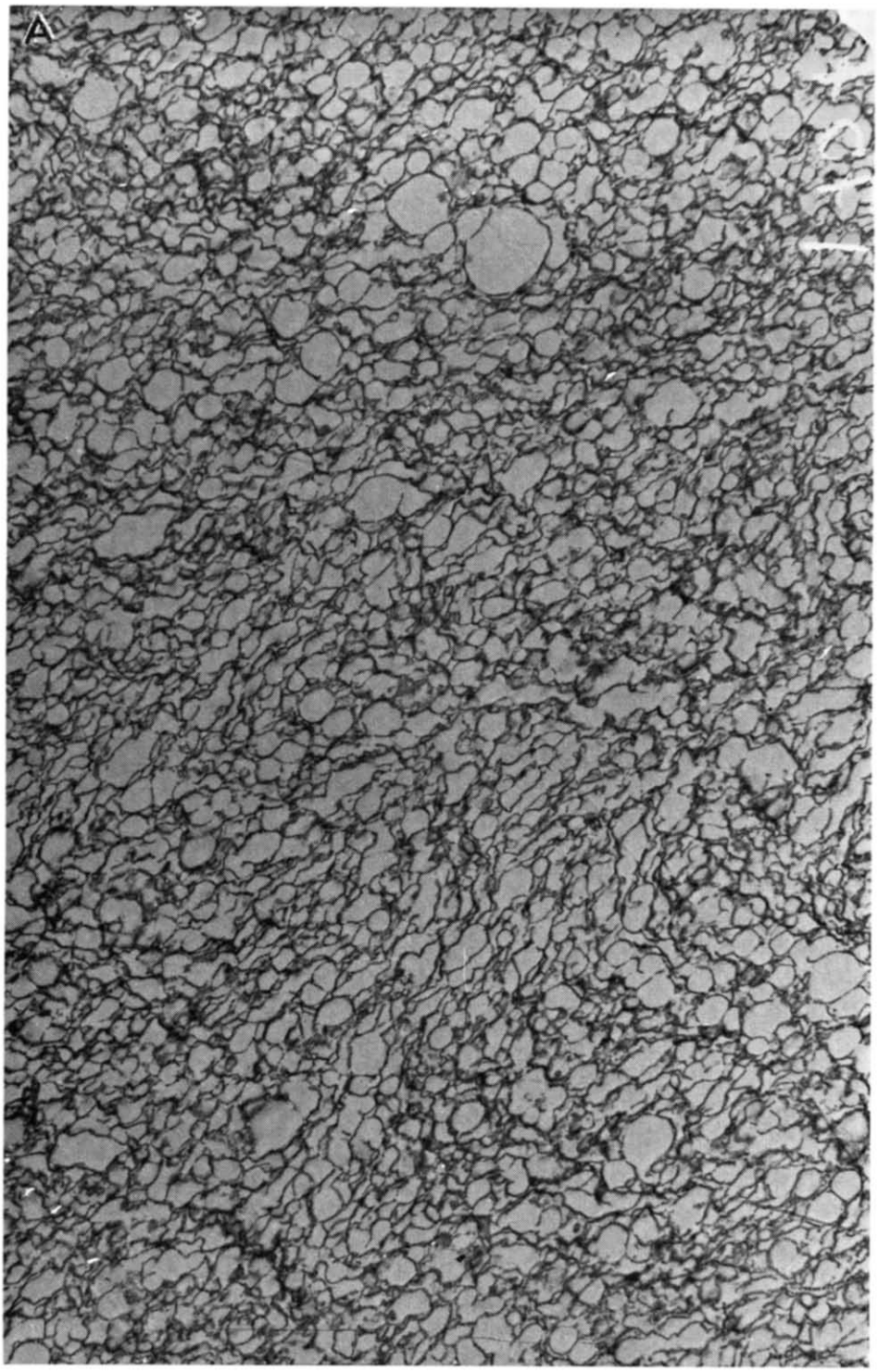


Fig. 12. (A) Morphology (magnification $\times 8500$) of the fraction at 13% on the Ficoll–sucrose gradient.

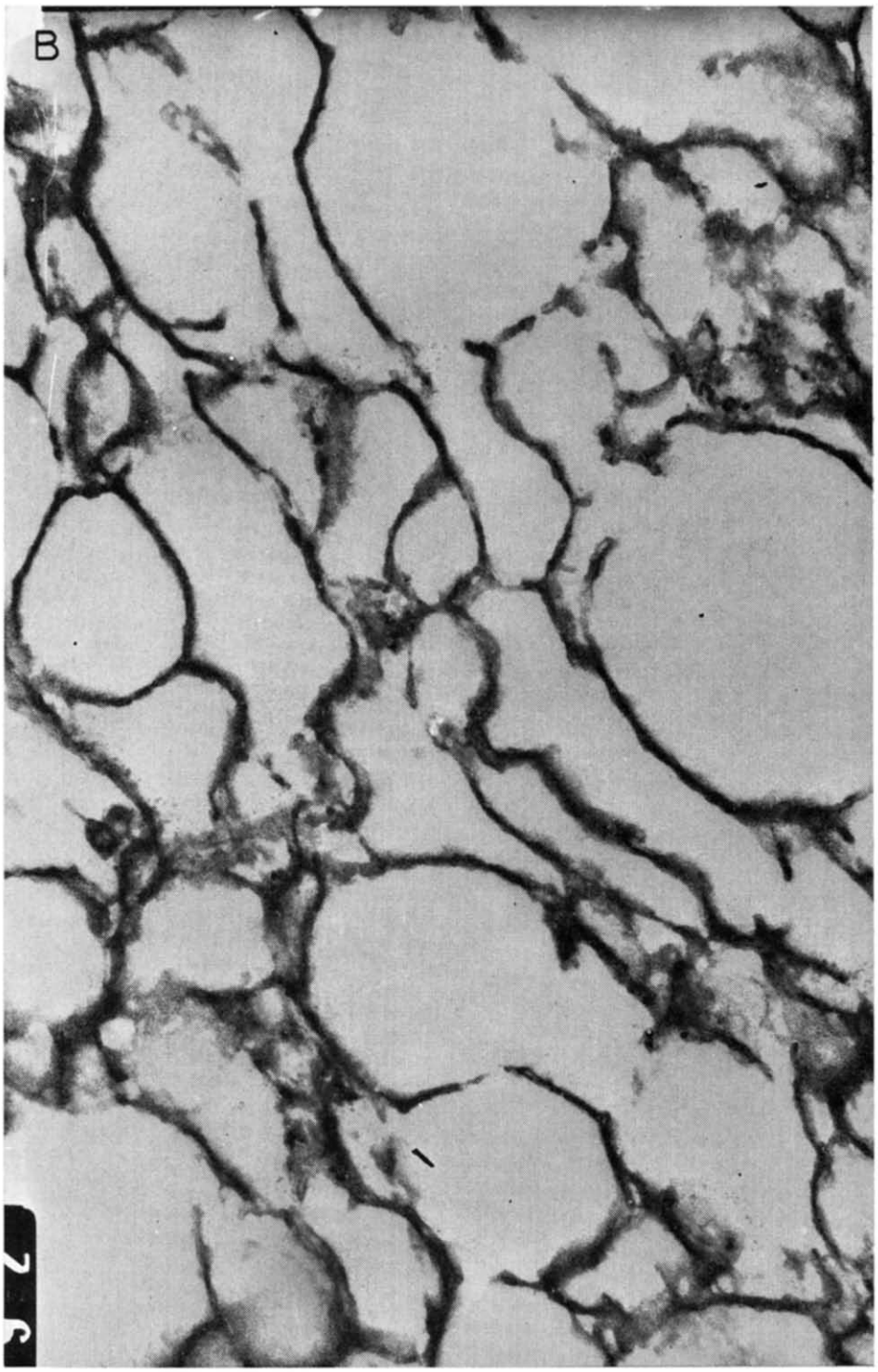


Fig. 12. (B) Detailed structure (magnification $\times 68\,200$) of the membranes isolated at 13% on the Ficoll–sucrose gradient.

one such gradient. Mg^{2+}-, HCO_3^--ATPase and AMPase show a trimodal distribution. The least dense fraction at 13% sucrose is greatly enriched in AMPase and ATPase activity. Morphologically this fraction (Fig. 12) is virtually homogeneous membranes interconnected in a syncytium. During centrifugation lasting as long as 18 h in a Beckman SW-25 rotor, this membrane fraction fails to leave the top of the gradient. A sharp protein peak coincides with the lowest density ATPase and AMPase peak. A broad protein peak from 23.5 to 29% sucrose is also associated with a peak of Mg^{2+}-, HCO_3^--ATPase and AMPase activity. At the edge of the rotor a third protein peak is located. Not only ATPase and AMPase activity, but also succinic dehydrogenase and monoamine oxidase activities are associated with this protein peak.

DISCUSSION

Development of zonal rotors has made preparative scale density gradient centrifugation feasible. We have utilized this technique to develop methods of fractionation of gastric mucosal homogenates to achieve: (1) high total yield, or (2) rapidity, or (3) purity.

We first studied fractionation of the total homogenate or the $78000 \times g$ pellet of the $5000 \times g$ supernate to achieve a technique which was not only rapid but would provide a high yield. With the total homogenate rapidity was not achieved since isopycnic centrifugation was necessary to achieve satisfactory separation of the vesicular membrane fraction and mitochondrial fraction as indicated by succinic dehydrogenase activity. Soluble protein present also necessitates further centrifugation to free the vesicular membranes of this contamination. Nonetheless by this technique relatively high purity of a large quantity of vesicular membrane is achieved.

Overlaying the total homogenate over the gradient, each in 325 ml, did not allow sufficient volume for satisfactory separation. Larger rotors which are now available may make this a practical method to avoid soluble protein contamination and achieve relatively high purity.

We found that the $8000 \times g$ pellet yielded a relatively pure preparation of gastric mitochondria as indicated by electron microscopic assessment. A small amount of smooth vesicular membrane contamination is indicated by electron microscopic morphology and AMPase activity in the mitochondrial region, 40–44% sucrose, of the gradient. In an effort to free the mitochondrial region from AMPase and HCO_3^--ATPase activity associated with the vesicular membranes, we have treated the purified mitochondria with 1 M NaCl, sonication, and techniques to prepare inner and outer mitochondrial membranes. None of these treatments of the purified mitochondria resulted in accumulation of significant amounts of protein at 28–32% sucrose on subsequent isopycnic sucrose density gradient centrifugation in a SW-25 rotor. It would appear that gastric mitochondria prepared in this way, while not free of extra mitochondrial enzymes, are sufficiently pure to enable further studies of gastric mitochondrial metabolism.

Fractionation of the microsomal fraction by rate zonal or step gradient centrifugation has resulted in the most rapid purification of the vesicular membrane fraction free from soluble protein contamination. Our greatest interest has, however, been in the greater purity of the smooth vesicular membrane fraction obtained by

isopycnic density gradient centrifugation of the microsomal preparation. Membranes prepared in this manner are free from mitochondrial contamination as assessed by electron microscopic morphology and lack of detectable succinate dehydrogenase or monoamine oxidase activity. We do, however, routinely use SDH to monitor the freedom of these membranes from mitochondrial contamination since mitochondrial ATPase also shows HCO_3^- stimulation.

It is this preparative technique which we routinely use to prepare gastric membranes for solubilization with Triton X-100 and further characterization of transport properties. In addition to the HCO_3^--ATPase these solubilized membranes have yielded at least 3 proteins, separable by analytical polyacrylamide gel electrophoresis, which induce step increments in the conductance of lipid bilayers. Characterization of this effect has shown one to be anion, another cation, and the last non-selective.

The observation that AMPase activity frequently peaks one to two tubes after HCO_3^--ATPase led to efforts to resolve these two activities. Rate zonal centrifugation, however, failed to resolve these into two peaks of activity. On Ficoll-sucrose gradients a third peak of ATPase activity was resolved, while AMPase activity accompanies each peak there is enrichment in the peak at 13% sucrose (Table VI). The very light isopycnic density of these membranes is certainly not expected for plasma membranes. In long runs high frictional resistance should be overcome, but these membranes are found at the same density after 6- or 10-h zonal runs or after 18 h of centrifugation in an SW-25 rotor. We have felt that osmotic intactness would best explain the failure of these membranes to sediment. It is consistent that on sucrose gradients this very light peak is non existent or quite small and is greatly increased when Ficoll is used in the gradient.

TABLE VI

C is the peak total activity in the regions of 13%, 26–27% or 35% sucrose. C_0 is the hypothetical total activity/sample if activity were evenly distributed on the gradient.

	C/C_0		
	13%	*26–27%*	*35%*
Mg^{2+}-ATPase	3.8	0.73	5.8
HCO_3^--ATPase	3.3	1.1	3.4
AMPase	4.7	0.72	4.2

Using hypotonic lysis of isolated oxyntic cells from Necturus we are able to prepare large vesicular "ghost-like" structures which float on 5% Ficoll. On sucrose gradients these structures are reduced to tiny granules when viewed under the phase microscope. Thus the possibility remains that the upper peak of HCO_3^--ATPase activity resides in osmotically intact membrane structures.

ACKNOWLEDGEMENTS

This work was supported by NIH Grants AM08541 and AM15878 and NSF Grant GB31075. A.S. is a trainee on NIH Grant TIAM05286. Credit is given to Project No. 8059-01, VA Hospital in Birmingham, Ala.

REFERENCES

1 Kasbekar, D. K. and Durbin, R. P. (1965) *Biochim. Biophys. Acta* 105, 472–482
2 Sachs, G., Mitch, W. E. and Hirschowitz, B. I. (1965) *Proc. Soc. Exp. Biol. Med.* 119, 1023–27
3 Wiebelhaus, V. D., Sung, C. P., Helander, H. F., Shah, G., Blum, A. L. and Sachs, G. (1971) *Biochim. Biophys. Acta* 241, 49–56
4 Blum, A. L., Shah, G., St. Pierre, T., Sung, C. P., Wiebelhaus, V. D. and Sachs, G. (1971) *Biochim. Biophys. Acta* 249, 101–113
5 Sachs, G., Shah, G., Strych, A., Cline, G. and Hirschowitz, B. I. (1972) *Biochim. Biophys. Acta* 266, 625–638
6 Simon, B., Kinne, R. and Sachs, G. (1972) *Biochim. Biophys. Acta* 282, 293–300
7 King, T. E. and Howard, R. L. (1967) *Methods in Enzymology*, Vol. 10, pp. 275–294, Academic Press, New York
8 Tabor, C. W., Tabor, H. and Rosenthal, S. M. (1954) *J. Biol. Chem.* 208, 645–661
9 Reeves, W. J. and Fimognari, G. M. (1966) *Methods in Enzymology*, Vol. 9, pp. 288–294, Academic Press, New York