

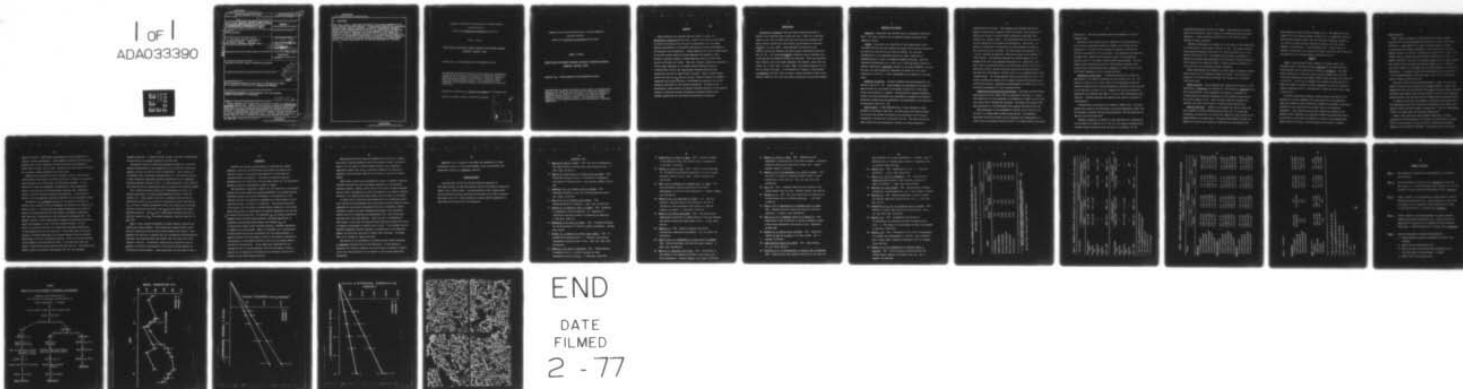
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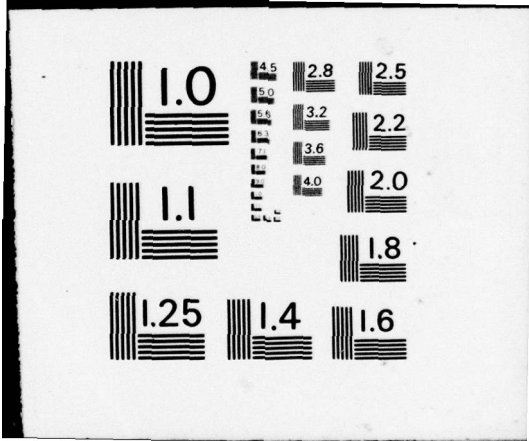
ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/1
ISOLATION AND PARTIAL CHARACTERIZATION OF PLASMA MEMBRANES FROM--ETC(U)
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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER 6		2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) ISOLATION AND PARTIAL CHARACTERIZATION OF PLASMA MEMBRANES FROM THE LIVERS OF CONTROL AND STREPTOCOCCUS PNEUMONIAE-INFECTED RATS; Plasma Membranes After Pneumococcal Sepsis,		5. TYPE OF REPORT & PERIOD COVERED Interim	
7. AUTHOR(s) James S. Little		8. CONTRACT OR GRANT NUMBER(s)	
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases SGRD-U1B-A Fort Detrick, Frederick, Maryland 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102 3M161102BS03/00/015	
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE 29 November 1976	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		13. NUMBER OF PAGES 28	
		15. SECURITY CLASS. (of this report) UNCLASSIFIED	
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited			
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)			
18. SUPPLEMENTARY NOTES Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in <u>Infection and Immunity</u>.			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Streptococcus pneumoniae; Plasma membranes; Cell fractionation; Alkaline phosphatase; 5'-Nucleotidase			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Plasma membranes were isolated from the livers of control and Streptococcus pneumoniae-infected rats. Purity of the fractions was judged by electron microscopy and by the use of enzyme markers for microsomes (glucose-6-phosphatase), mitochondria (glutamate and malate dehydrogenase), and lysosomes (acid phosphatase). Plasma membranes from infected cells banded at the same sucrose density as plasma membranes from uninfected cells. Moreover, equivalent amounts of plasma membranes could be isolated from control and infected rat livers.			

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Isolation and Partial Characterization of Plasma Membranes
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Control and Streptococcus pneumoniae-Infected Rats

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Running Title: PLASMA MEMBRANES AFTER PNEUMOCOCCAL SEPSIS

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Submitted for publication to Infection and Immunity on 29 November 1976.

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ABSTRACT

Plasma membranes were isolated from the livers of control and Streptococcus pneumoniae-infected rats. Purity of the fractions was judged by electron microscopy and by the use of enzyme markers for microsomes (glucose-6-phosphatase), mitochondria (glutamate and malate dehydrogenase), and lysosomes (acid phosphatase). Plasma membranes from infected cells banded at the same sucrose density as plasma membranes from uninfected cells. Moreover, equivalent amounts of plasma membranes could be isolated from control and infected rat livers. There were, however, significant alterations in the enzyme complement of the plasma membrane following infection. 5'-Nucleotidase activity was significantly decreased while alkaline phosphatase activity was significantly increased. Kinetic analysis demonstrated that only the V_{\max} and not the K_m of these two enzymes was changed, suggesting that altered affinity of the enzymes for substrate was not the mechanism responsible for the observed alterations. No change in the mitochondrial enzyme markers was observed following infection but the specific activity of microsomal glucose-6-phosphatase decreased significantly. Possible explanations for the observed alterations are discussed.

INTRODUCTION

Streptococcus pneumoniae infection induces marked alterations in systemic host metabolism which involve the liver. There is an increased uptake of zinc (18) and amino acids (30) into liver and increased acute phase globulin synthesis by liver (Beisel, In CRC Handbook of Nutrition and Foods, in press, 1976). Plasma glucagon and insulin are also elevated during this infection and affect hepatic carbohydrate metabolism (21, 22, 33). The plasma membrane may, therefore, play an important role in altered hepatic metabolism during this infection. There are indications that infection alters the enzyme complement (33, Canonico, Ayala, Rill and Little, Am. J. Clin. Nutr., in press, 1976), and surface properties (9, 13, 27) of several cell types. It was therefore of interest to study whether S. pneumoniae infection, which is known to induce decreases in other intra-hepatic organelles (3), would also effect alterations in the plasma membrane.

MATERIALS AND METHODS

Chemicals. Nucleotides were obtained from P-L Biochemicals (Milwaukee, Wis.). All other chemicals were of analytical grade and obtained from commercial sources.

Animals. Male albino rats (150-200 g) of the Sprague-Dawley strain (Cr1:COBS^R CD^R (SD) BR) were obtained from Charles River and were maintained on stock Purina Lab Chow and tap water ad libitum. All rats were acclimatized to a 12-h day-night cycle for at least 7 days prior to experimentation in order to eliminate circadian variations. Rats were inoculated subcutaneously (s.c.) with 3 to 6 x 10⁵ heat-killed (control) or virulent (infected) *S. pneumoniae*, serotype I, A5 strain (USAMRIID) organisms. All rats were fasted during the experiment and killed 20 or 40 h after inoculation at 8:30 A.M., a time corresponding to the midpoint of the night cycle.

Analytical procedures. Inorganic-phosphate (Pi) was determined by the method of Chen et al. (4). Total phosphate was determined by the method of Ames and Dubin (1) and protein by the method of Lowry et al. (15) using bovine serum albumin as a standard. Concentrations of glucose-6-phosphate (G-6-P) and β -glycerolphosphate were determined by assaying total phosphate. Nucleotide concentration was determined by the measurement of its absorbance as described by Bock et al. (2).

Cell fractions. Plasma membranes (Fig. 1) were isolated by a modification of the method of Ray (19). For both control and infected animals 4 g of liver were excised and placed in ice-cold buffer (0.25 M sucrose containing 0.5 mM CaCl₂ and 1 mM Tris-Cl, pH 7.5). The livers were minced with scissors and then homogenized 8 strokes in a Dounce homogenizer

containing 40 ml of buffer. This homogenate was filtered through four layers of sterile gauze, diluted to 80 ml with buffer, and centrifuged at $1000 \times g$ for 10 min in a Sorvall RC2-B refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant from this step was used for the preparation of microsomes and mitochondria. (See below). The pellet was washed twice by suspension and recentrifugation at $1000 \times g$ for 10 min in 40 ml of 1 mM EGTA. The final pellet was suspended in 2 to 4 ml of 1 mM EGTA and adjusted to 48% sucrose (wt/wt) by the addition of 2.45 M sucrose. This sucrose suspension was divided equally into two Beckman SW 27 rotor tubes (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) and layered with 10 ml of 45% sucrose (wt/wt), then 10 ml of 41% sucrose (wt/wt), and the tubes were filled with 37% sucrose (wt/wt). The tubes were centrifuged at $131,000 \times g$ for 2 h in a Beckman Model L2-65B ultracentrifuge. The plasma membrane fraction, which bands at the 37 - 41% sucrose interface was removed with a syringe, diluted with deionized water to 8% sucrose (wt/wt), sedimented by centrifugation at $10,000 \times g$ for 10 min and finally suspended in 2.0 ml of deionized water.

Preparation of microsomes and mitochondria. Microsomes and mitochondria were prepared from the initial $1000 \times g$ supernatant (see Fig. 1). Twenty-five milliliters of this supernatant was sedimented at $10,000 \times g$ for 10 min in a Sorvall RC2-B refrigerated centrifuge. Microsomes were prepared from this supernatant (a) and mitochondria were prepared from this pellet (b).

This supernatant (a) was sedimented at $205,000 \times g$ for 30 min in the 50 rotor of a Beckman Model L2-65B ultracentrifuge. The sedimented microsomal fraction was washed once by suspension and resedimentation in 0.25 M sucrose containing 20 mM Tris-Cl pH 7.5 to remove adsorbed cytoplasmic

proteins (5). The final microsomal pellet was suspended in 2.0 ml of deionized water.

The pellet (b) was washed twice by suspension and resedimentation in 0.25 M sucrose containing 1 mM EGTA at 10,000 x g for 10 min and then suspended in 1 to 3 ml of 0.25 M sucrose and adjusted to 48% sucrose (wt/wt) by the addition of 2.45 M sucrose. This suspension was placed into two SW27 tubes, layered with 40% sucrose (wt/wt) and centrifuged at 131,000 x g for 2 h in a Beckman Model L2-65B ultracentrifuge. The mitochondria were recovered from the 48 to 40% interface, adjusted to 8% sucrose (wt/wt) with deionized water, sedimented at 10,000 x g for 10 min and finally suspended in 2.0 ml of deionized water.

Biochemical enzyme assays. Glucose-6-phosphatase was assayed by a modification of the method of Swanson (26) and de Duve et al. (7). The assay was carried out in a total volume of 1-ml containing 10 mM G-6-P, 100 mM Tris-malate pH 6.6, and enough protein to hydrolyze no more than 30% of the substrate in 20 min at 37 C. Samples of 0.2 ml were withdrawn at appropriate time intervals and added to 0.2 ml of 20% trichloroacetic acid (vol/vol). Inorganic phosphate was analyzed after removal of protein by centrifugation.

5'-Nucleotidase was assayed by the method of Widnell (32). Acid phosphatase was assayed in the same manner as glucose-6-phosphatase except that the incubation contained 100 mM β -glycerolphosphate, 200 mM sodium acetate (pH 5.0) and 0.2% Triton X-100.

Alkaline phosphatase was assayed at room temperature by a modification of the method of De Chatelet et al. (6) in a 3-ml system containing 620 mM 2-amino-2-methyl-1-propanol buffer (pH 10.2), 0.1 mM MgCl₂, 15.2 mM

p-nitrophenol-phosphate and 100 μ l of sample. The production of p-nitrophenol was followed for 3 min at 420 nm in a Beckman DU recording spectrophotometer. A molar extinction coefficient of 1.62×10^4 was used to determine specific activities.

Glutamate dehydrogenase was assayed in a 3-ml system at room temperature by a modification of the method of Hoek et al. (11). The system contained 50 mM potassium phosphate buffer (pH 7.4), 20 mM L-glutamic acid, 0.4 mM NAD and 0.2% Triton X-100. The oxidation of glutamate was measured on a Beckman DU recording spectrophotometer by the increase of optical density at 340 nm caused by the reduction of NAD. A molar extinction coefficient of 6.20×10^3 was used to determine specific activities.

Malate dehydrogenase was assayed in the same manner as glutamate dehydrogenase except that the oxidation of NADH was followed. The assay was as described in the Sigma Technical Bulletin (24) except that it contained 0.2% Triton X-100.

Kinetic analysis. Plasma membranes were isolated from control (3×10^5 heat-killed *S. pneumoniae*) and infected (3×10^5 virulent *S. pneumoniae*) rats 40 h postinoculation. Each preparation was assayed as described for 5'-nucleotidase and alkaline phosphatase at five substrate concentrations for various lengths of time. Under the assay conditions employed both reactions were linear with respect to time and enzyme concentration.

Electron microscopy. Samples to be prepared for electron microscopy were suspended in 100 mM sodium-cacodylate buffer (pH 7.2) containing 10 mM $MgCl_2$ and fixed for 2 h on ice by the addition of equal volumes of 4% glutaraldehyde in the same buffer. Following glutaraldehyde fixation the samples were washed once in buffer by suspension and centrifugation and

then suspended in buffer and left overnight at 0 C. The samples were then fixed on ice in 1% osmium tetroxide (vol/vol) for 3 h, sedimented, washed in 50 mM sodium-cacodylate buffer (pH 7.2) containing 5 mM MgCl₂, dehydrated in a series of graded ethanols followed by propylene oxide and finally embedded in Epon-Araldite. Sections prepared with a diamond knife on a Reichert ultramicrotome were stained with uranyl acetate and lead citrate (20) and examined with an Hitachi electron microscope at 75 kv.

RESULTS

Figure 2 shows the mean febrile response of 6 fasted control rats inoculated with 3×10^5 heat-killed S. pneumoniae and 12 fasted infected rats inoculated with the same dose of virulent S. pneumoniae. The normal rectal temperature of these rats varied slightly depending upon the time of the day; temperatures were slightly higher during the night cycle (2:00 A.M. to 2:00 P.M.). Two of the 12 infected rats survived this dose and both of these rats showed an increase of rectal temperature at 40 h and a return to control temperature by 95 h. The mean time to death was 66.1 h with a range of 40 to 95.5 h.

In initial experiments attempted to define optimal conditions for the preparation of plasma membranes with a high yield and low contamination from other organelles, Ray's method (19), modified as described, consistently provided the best results and was therefore used in all subsequent experiments. Since identical results were obtained in perfused or non-perfused livers, membranes were routinely isolated from nonperfused livers. As previously reported (19) the addition of calcium to the homogenization medium resulted in nearly a two-fold increase in the yield of isolated

plasma membranes.

Table 1 shows the percent of total activity for selected enzymes recovered with the plasma membranes from control and infected rats fasted 20 and 40 h. Approximately 10 to 30% of the plasma membrane markers, 5'-nucleotidase and alkaline phosphatase, were recovered with the plasma membrane fraction for control or infected rats fasted for 20 or 40 h. This recovery value is higher than most previously reported figures (8). It appears from Table 1 that more of the marker enzymes for the plasma membrane was recovered from the control and infected animals at 40 h. This may be the result, however, of the higher yield of plasma membranes for these 40-h preparations. It should be noted that plasma membrane yields varied from 1 to 2 mg plasma membrane protein per gram of liver for both control and infected animals at both 20 and 40 h.

Less than 0.433% of the glucose-6-phosphatase, a marker for the endoplasmic reticulum, was recovered with the plasma membranes. Table 1 also demonstrates that less than 0.255% of both markers for mitochondria, glutamate and malate dehydrogenase, and less than 0.103% of the marker for lysosomes, acid phosphatase, were recovered with the isolated plasma membrane fraction.

It should be noted that the stability of glutamate dehydrogenase under our homogenization and assay conditions was determined since reports have appeared stating that this enzyme is unstable (23). Glutamate dehydrogenase activity was stable under the conditions employed here.

Table 2 shows that these plasma membrane preparations contained approximately 12% contamination by microsomes and mitochondria, as judged from the enzyme markers for these fractions. Washing isolated plasma membranes in 150 mM NaCl, 10 mM EGTA, or 20 mM Tris-Cl (pH 7.5) did not

improve the purity. Differential centrifugation was also employed in an attempt to remove contamination. Plasma membranes isolated from the sucrose gradient (Fig. 1) were adjusted to 8% sucrose (wt/wt) and sedimented at either 1000 or 10,000 x g for 10 min. Essentially the same amount of activity for all marker enzymes sedimented under both forces.

Slightly purer preparations could be obtained, however, when isolated plasma membranes were washed in 20 mM Tris-Cl pH 7.5, layered on a continuous sucrose gradient (31 to 41% wt/wt), and centrifuged overnight. It was difficult to assess the biochemical contamination of these preparations, however, since certain of the enzyme markers employed lost a significant amount of activity following overnight centrifugation. It was therefore decided to use the membranes prepared as described for all subsequent studies.

The effects of S. pneumoniae infection on the specific activities of selected liver enzymes in both the homogenate and isolated cell fractions are shown in Table 3. 5'-Nucleotidase and alkaline phosphatase were chosen as markers for plasma membranes (10, 28); however, cytochemical experiments have shown that 5'-nucleotidase is also localized in microsomes (31). The specific activity of this enzyme showed no change 20 h following infection in either the homogenate or any of the cell fractions studied. At 40 h, however, the specific activity of 5'-nucleotidase decreased significantly in both homogenate and all cell fractions. On the other hand, the specific activity of alkaline phosphatase in the plasma membrane fraction increased significantly 20 and 40 h following infection. At 40 h it was also increased in the homogenate and microsomal fractions. It has been shown that this enzyme is inducible following bacterial infections (14, 29). The mitochondrial markers, malate and glutamate dehydrogenase (16, 25), were unchanged

following infection. It should be noted, however, that malate dehydrogenase is also found in the soluble fraction of the cell (16).

The specific activity of glucose-6-phosphatase (endoplasmic reticulum) decreased significantly in the homogenate and in the microsomal and plasma membrane fractions at both 20 and 40 h postinfection. These results are in agreement with a previously published report (3). The specific activity of acid phosphatase (lysosomes) decreased significantly in the homogenate and plasma membrane fraction at 40 h. It is also important to note (Table 3) that infection did not significantly alter the yields of any fraction studied.

Fig. 3 shows the kinetic analysis of 5'-nucleotidase and Fig. 4 of alkaline phosphatase in isolated plasma membranes from both control and infected rats. These results show that *S. pneumoniae* infection did not significantly alter the K_m of either 5'-nucleotidase (control and infected $K_m = 1.29$ mM) or alkaline phosphatase (control and infected $K_m = 0.330$ mM). As could be predicted from Table 3 the V_{max} for both enzymes was altered following infection. The V_{max} for 5'-nucleotidase decreased from 13.9 to 10.5 μmol whereas the V_{max} for alkaline phosphatase increased from 30.1 to 80.0 nmol.

Figure 5 shows electron micrographs of plasma membranes isolated from control and infected animals. Both preparations appeared similar except that the preparations from the latter seemed slightly more compact. From scanning thin sections, it appeared that the contamination from mitochondria, lysosomes, and rough microsomes was minimal; only very rarely were these organelles observed. As mentioned, slightly purer preparations could be obtained by overnight centrifugation of the isolated plasma membranes in a continuous sucrose gradient. These preparations are also shown in Fig. 5.

DISCUSSION

Isolation and partial characterization of relatively pure plasma membranes, microsomes and mitochondria from control and infected rats revealed evidence for decreased plasma membrane 5'-nucleotidase but increased alkaline phosphatase. A decrease in microsomal glucose-6-phosphatase was also observed but the two marker enzymes for the mitochondria, glutamate and malate dehydrogenase, showed no change.

The mechanism(s) responsible, however, for the alteration in the specific activity of 5'-nucleotidase and alkaline phosphatase in the plasma membrane following infection have not been determined. One possibility is that infection may alter the synthesis or degradation of these enzymes or other molecules serving as activators or inhibitors. The observed alterations do not appear to be the result of altered kinetic properties of the enzymes since the K_m of both 5'-nucleotidase and alkaline phosphatase were the same in control and infected rats. The increased V_{max} for alkaline phosphatase suggests either increased enzyme synthesis, decreased degradation, or activation of preexisting enzyme whereas the decreased V_{max} for 5'-nucleotidase suggest either decreased synthesis, increased degradation, or masking of existing enzyme. While it is possible that 5'-nucleotidase is being synthesized but not incorporated into the plasma membrane, it appears unlikely since the specific activity of the enzyme is decreased in the homogenate and microsomal fraction as well as in the plasma membrane fraction 40 h postinfection. If the enzyme were synthesized but not incorporated into the plasma membrane, then one would expect to find an increase in the homogenate and possibly in the microsomal fraction but a decrease in the plasma membrane fraction.

The observed decrease in glucose-6-phosphatase activity is not merely the result of glycogen depletion which occurs during fasting. Nor does it appear to be the result of altered kinetic behavior since preliminary experiments suggest that the K_m of glucose-6-phosphatase for glucose-6-phosphate in the microsomal fraction is the same for control and infected animals.

These results could also be explained by a change in membrane properties following infection such that they behave differently in the isolation procedure employed. However, since both the total yield of plasma membranes and the percent contamination by other cell organelles was similar following infection, it is doubtful that these results are an artifact of the method.

It should also be noted that these preparations are heterogeneous since liver contains both parenchymal cells and reticuloendothelial (Kupffer) cells. Suspensions of pure hepatic parenchymal cells may be preferable; however, techniques required to produce homogeneous parenchymal cell suspensions involve the use of collagenase and hyaluronidase (12), both of which may seriously alter the plasma membrane. Since the liver is heterogeneous with respect to cell type, the observed enzyme alterations could then be due to changes in parenchymal or Kupffer cells, or both. It is possible that the alkaline phosphatase increase represents a proliferation in the phagocytic Kupffer cell which may then serve to enhance host resistance. At present there is no evidence to support this idea.

The reasons for the alterations in organelle enzyme profile following S. pneumoniae infection have not been determined. It may represent a purposeful and selective synthesis of certain proteins (enzymes) of benefit to the host during infection at the expense of other enzymes momentarily dispensable.

Experiments are in progress to determine the significance of these alterations and what role the plasma membrane plays in maintaining cell homeostasis following S. pneumoniae infection.

ACKNOWLEDGEMENTS

I am sincerely grateful for the fine technical assistance of SP/4 Carol Williams and SP/5 Paul Merrill, and for the excellent secretarial support of Ms. Patricia Kemp. I am also grateful to Mrs. Frances Shirey for the excellent technical assistance she provided in the area of electron microscopy, and to Dr. Michael Powanda for several helpful suggestions he made during the preparation of the manuscript.

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TABLE 1. TOTAL HOMOGENATE ACTIVITY OF SELECTED ENZYMES RECOVERED WITH PLASMA MEMBRANES ISOLATED

FROM CONTROL AND INFECTED RATS FASTED 20 AND 40 h

Enzyme	% Activity ^a			
	20 h		40 h	
	Control	Infected	Control	Infected
5'-Nucleotidase	10.7	13.2	20.2	30.1
Alkaline phosphatase	19.8	29.7	19.0	25.4
Glucose-6-phosphatase	0.263	0.316	0.433	0.424
Glutamate dehydrogenase	0.194	0.071	0.255	0.351
Malate dehydrogenase	0.133	0.141	0.200	0.260
Acid phosphatase	0.103	0.074	0.100	0.093

^a % = $\frac{\text{specific activity of enzyme in plasma membranes X plasma membrane yield}}{\text{specific activity of enzyme in homogenate X homogenate yield}} \times 100$

Data from Table 3 were used to obtain these values.

TABLE 2. BIOCHEMICAL ASSESSMENT OF CONTAMINATION OF ISOLATED PLASMA MEMBRANES BY OTHER CELL ORGANELLES.

Cell Fraction	Specific Activity ^a			
	Glucose-6-phosphatase ^b		Glutamate dehydrogenase ^c	
	Control	Infected	Control	Infected
Plasma membrane (PM)	1.00 ± 0.080	0.600 ± 0.050	0.004 ± 0.002	0.005 ± 0.002
Microsomes (M)	8.30 ± 0.197	4.99 ± 0.395	--	--
Mitochondria (MC)	--	--	0.032 ± 0.003	0.031 ± 0.002
Lysosomes (L)	--	--	--	----
Ratio x 100Z	PM/M = 12.0	PM/M = 12.0	PM/MC = 12.5	PM/MC = 16.1
20				
	Malate dehydrogenase ^c		Acid phosphatase ^b	
Plasma membrane (PM)	0.280 ± 0.044	0.285 ± 0.022	0.117 ± 0.022	0.065 ± 0.006
Microsomes (M)	--	--	--	--
Mitochondria (MC)	2.20 ± 0.138	1.97 ± 0.047	--	--
Lysosomes (L)	--	--	11.1 ^d	ND ^e
Ratio x 100Z	PM/MC = 12.7	PM/MC = 14.5	PM/L = 1.10	--

^a Mean of 6 rats ± standard error of the mean

^b μmol P_i released/20 min per mg protein.

^c μmol formed/min per mg.

^d (value from ref. 17).

^e ND = not determined.

TABLE 3. SPECIFIC ACTIVITY OF SELECTED LIVER ENZYMES IN HOMOGENATE AND ISOLATED CELL FRACTIONS OF INFECTED AND CONTROL RATS

Enzyme	Specific Activity ^a			
	20 h		40 h	
	Controls	Infected	Controls	Infected
HOMOGENATE				
5'-Nucleotidase ^b	1.60 ± 0.113	1.42 ± 0.060	1.40 ± 0.079	0.868 ± 0.103 ^f
Alkaline phosphatase ^c	0.666 ± 0.055	0.853 ± 0.200	1.13 ± 0.124	2.14 ± 0.438 ^f
Malate dehydrogenase ^d	1.25 ± 0.056	1.04 ± 0.016 ^f	1.29 ± 0.055	1.15 ± 0.048
Glutamate dehydrogenase ^d	0.009 ± 0.0005	0.008 ± 0.0004	0.016 ± 0.0005	0.015 ± 0.0007
Glucose-6-phosphatase ^b	2.24 ± 0.090	1.79 ± 0.079 ^g	2.16 ± 0.067	1.49 ± 0.070 ^g
Acid phosphatase ^b	0.706 ± 0.142	0.744 ± 0.148	1.05 ± 0.072	0.735 ± 0.050 ^f
PLASMA MEMBRANES				
5'-Nucleotidase ^b	29.2 ± 2.12	32.8 ± 2.60	30.3 ± 1.48	24.8 ± 1.68 ^f
Alkaline phosphatase ^c	22.5 ± 1.30	44.5 ± 6.34 ^f	23.9 ± 2.38	51.6 ± 10.0 ^f
Malate dehydrogenase ^d	0.285 ± 0.026	0.258 ± 0.020	0.286 ± 0.044	0.285 ± 0.022
Glutamate dehydrogenase ^d	0.003 ± 0.000	0.001 ± 0.000	0.004 ± 0.002	0.005 ± 0.002
Glucose-6-phosphatase ^b	1.46 ± 0.048	0.993 ± 0.052 ^g	1.00 ± 0.080	0.600 ± 0.050 ^g
Acid phosphatase ^b	0.124 ± 0.012	0.097 ± 0.012	0.117 ± 0.022	0.065 ± 0.006 ^f
MICROSOMES				
5'-Nucleotidase ^b	2.79 ± 0.134	2.63 ± 0.095	3.23 ± 0.207	2.01 ± 0.127 ^g
Alkaline phosphatase ^c	1.41 ± 0.231	1.99 ± 0.321	1.58 ± 0.243	3.27 ± 0.531 ^f
Malate dehydrogenase ^d	0.053 ± 0.009	0.048 ± 0.007	0.069 ± 0.007	0.061 ± 0.008
Glutamate dehydrogenase ^d	0.001 ± 0.0002	0.001 ± 0.0001	0.002 ± 0.0002	0.001 ± 0.0005 ^f
Glucose-6-phosphatase ^b	7.57 ± 0.292	5.70 ± 0.369 ^g	8.30 ± 0.197	4.99 ± 0.395 ^g

TABLE 3. Continued ...

MITOCHONDRIA

5'-Nucleotidase ^b	0.286 ± 0.055	N.D. ^e	0.286 ± 0.055	0.137 ± 0.010 ^f
Alkaline phosphatase ^c	Not detectable	Not detectable	Not detectable	Not detectable
Malate dehydrogenase ^d	N.D.	N.D.	2.20 ± 0.138	1.97 ± 0.047
Glutamate dehydrogenase ^d	N.D.	N.D.	0.032 ± 0.003	0.031 ± 0.002
Glucose-6-phosphatase ^b	N.D.	N.D.	0.401 ± 0.064	0.458 ± 0.033

YIELD mg/g liver

Homogenate	170 ± 6.43	175 ± 1.20	185 ± 12.6	173 ± 19.1
Plasma membranes	0.994 ± 0.067	0.997 ± 0.88	1.73 ± 0.250	1.82 ± 0.240
Microsomes	15.5 ± 0.840	15.5 ± 0.48	17.7 ± 0.910	16.5 ± 0.570
Mitochondria	N.D.	N.D.	6.56 ± 0.660	8.58 ± 0.980

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^a Mean of 6 rats ± standard error of the mean.

^b μmol Pi released/20 min per mg protein at 37 C.

^c nmol formed/min per mg protein.

^d μmol formed/min per mg protein.

^e N.D. = not determined.

^f P < 0.05

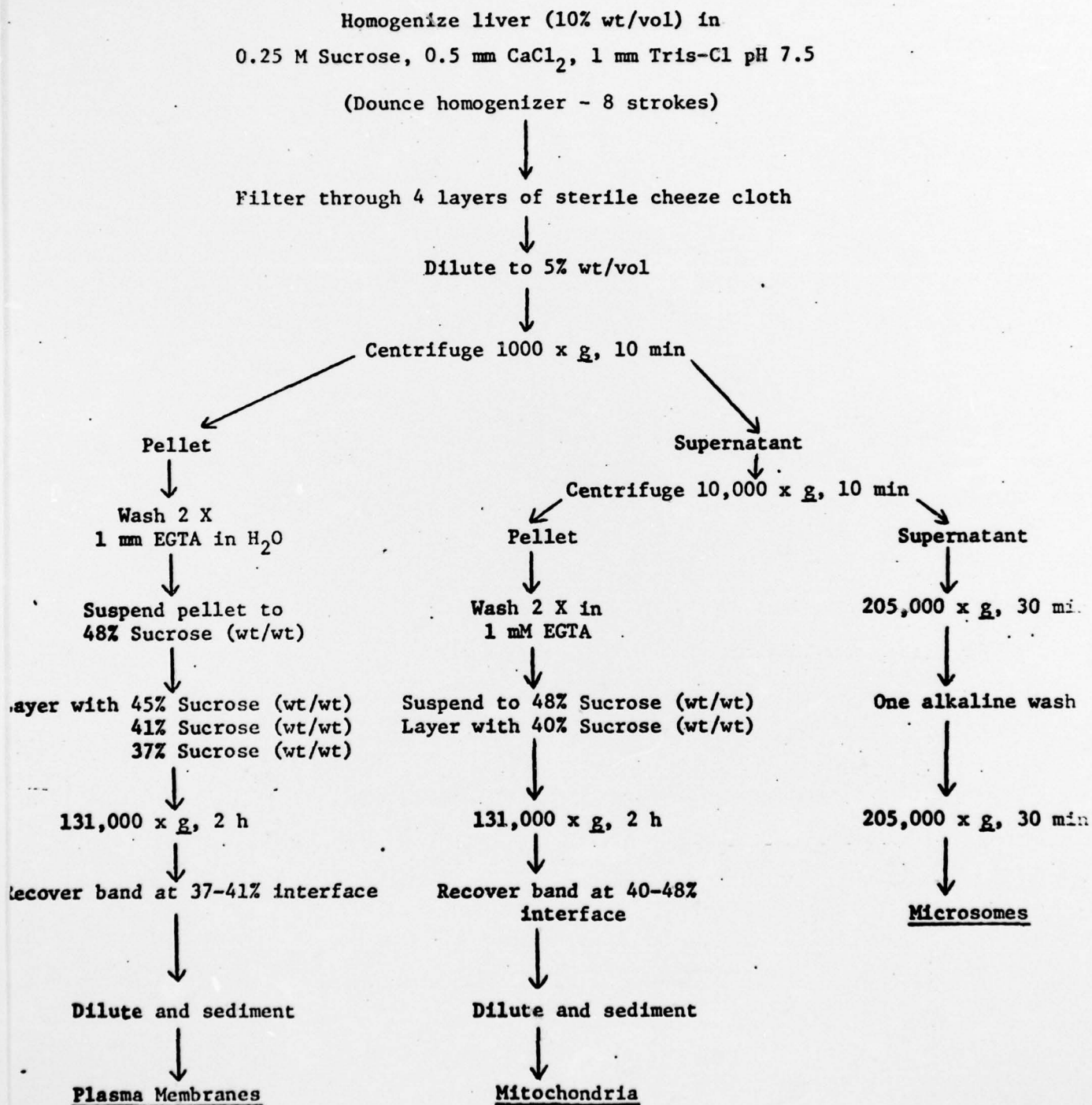
^g P < 0.005

LEGENDS TO FIGURES

- Fig. 1. Flow diagram for preparation of plasma membranes, mitochondria, and microsomes.
- Fig. 2. Rectal temperatures of control and S. pneumoniae-infected rats. Six control (Δ - 3×10^5 heat-killed) and 12 infected (O - 3×10^5 virulent) rats were inoculated subcutaneously with S. pneumoniae
- Fig. 3. Kinetic analysis of 5'-nucleotidase in plasma membranes isolated from control and infected rats. Values represent the mean \pm SEM of 6 control (Δ) or 6 infected (O) rats inoculated with either 3×10^5 heat-killed (Δ) or virulent (O) S. pneumoniae.
- Fig. 4. Kinetic analysis of alkaline phosphatase in plasma membranes isolated from control and infected rats. Values represent the mean \pm SEM of 6 control (Δ) or 6 infected (O) rats inoculated with either 3×10^5 heat-killed (Δ) or virulent (O) S. pneumoniae.
- Fig. 5. Electron micrographs of purified plasma membranes.
- A. Plasma membranes isolated as described from control rats.
X 18,000.
 - B. Same as (A) but from infected rats.
 - C. Same as (A) but further purified by centrifugation in a 31 to 41% (wt/wt) sucrose gradient. X 18,000.
 - D. Same as (C) but for infected rats.

FIGURE 1

PREPARATION OF PLASMA MEMBRANES, MITOCHONDRIA, AND MICROSOMES



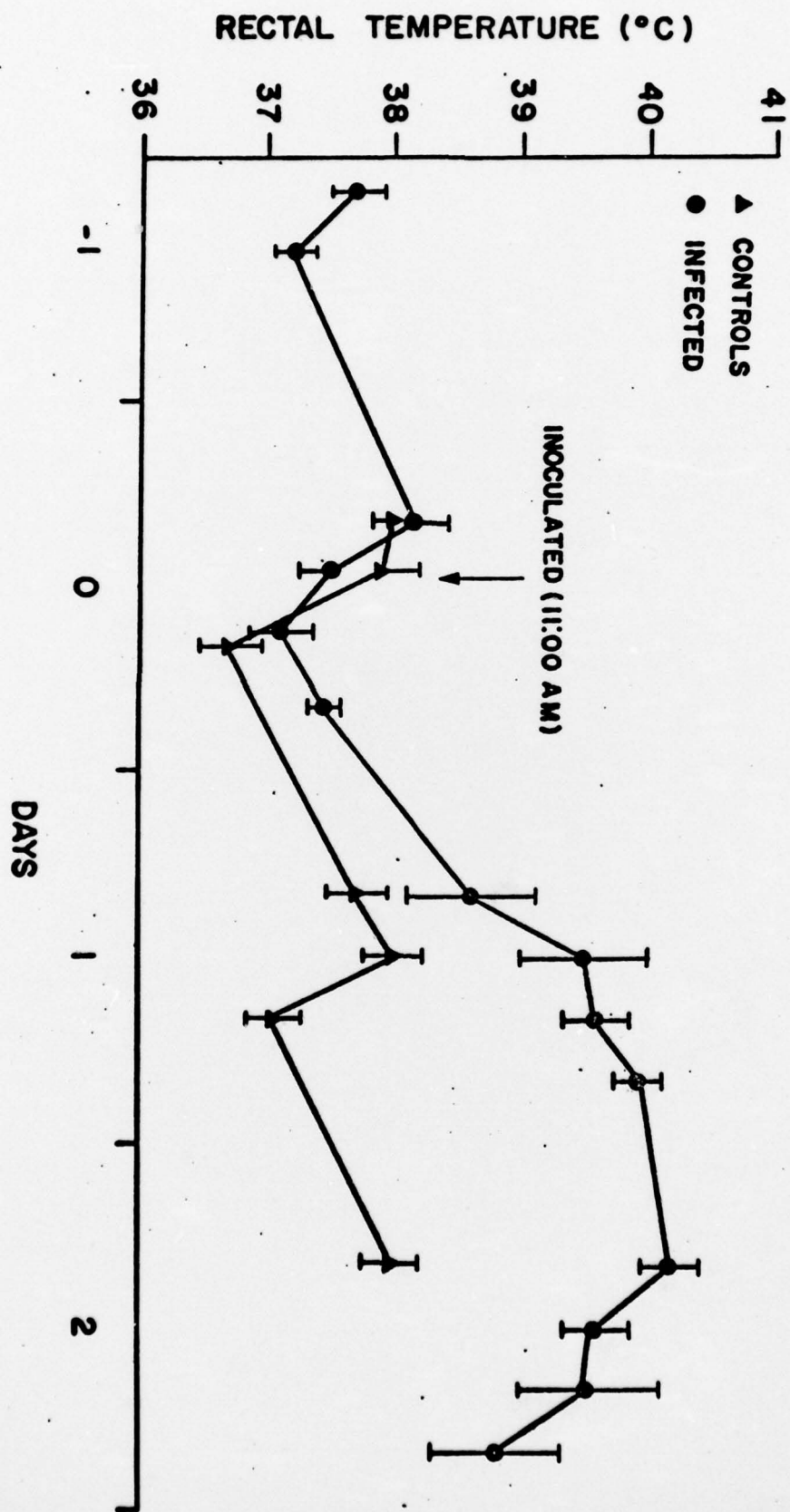


Fig. 2

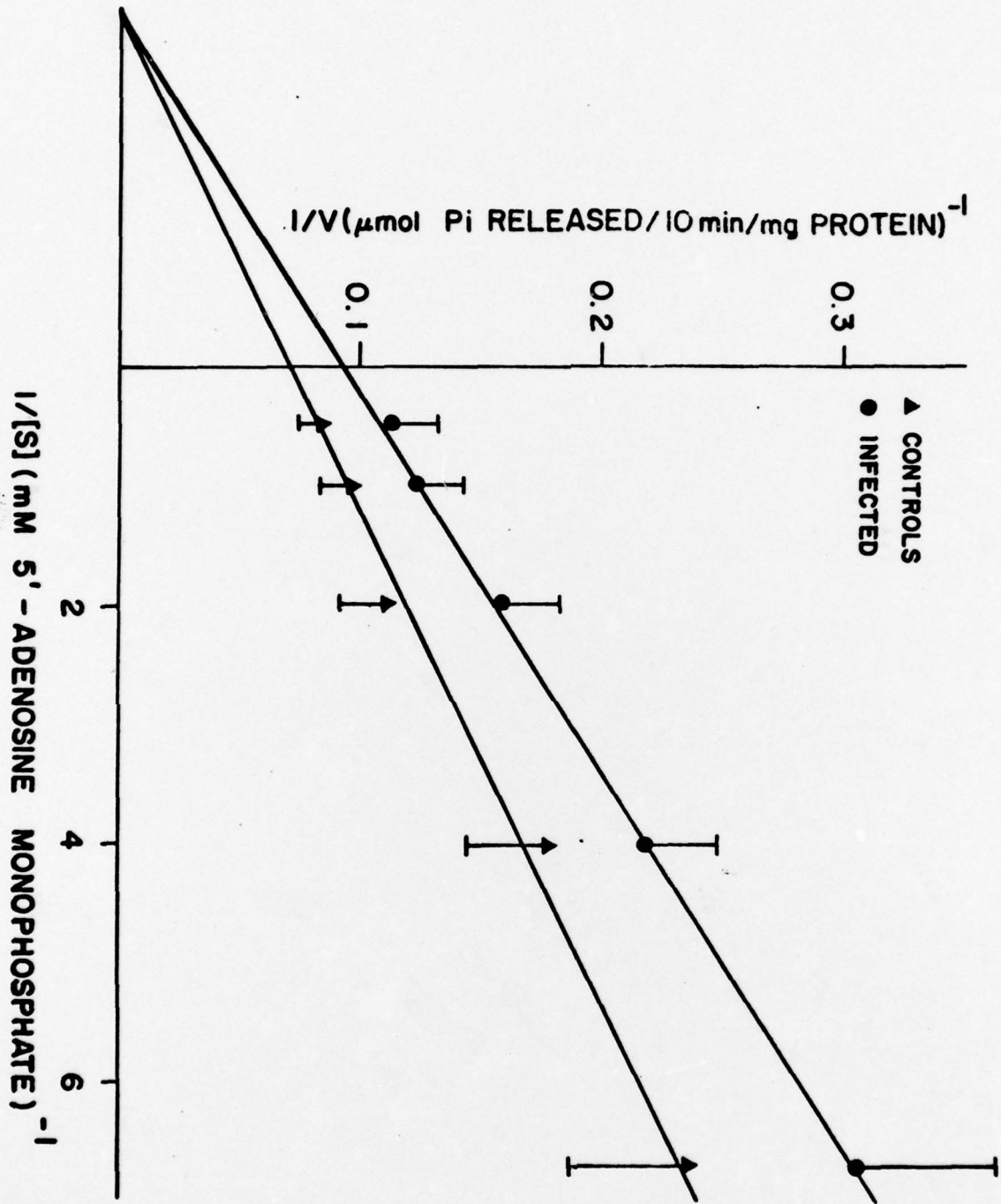


Fig. 3.

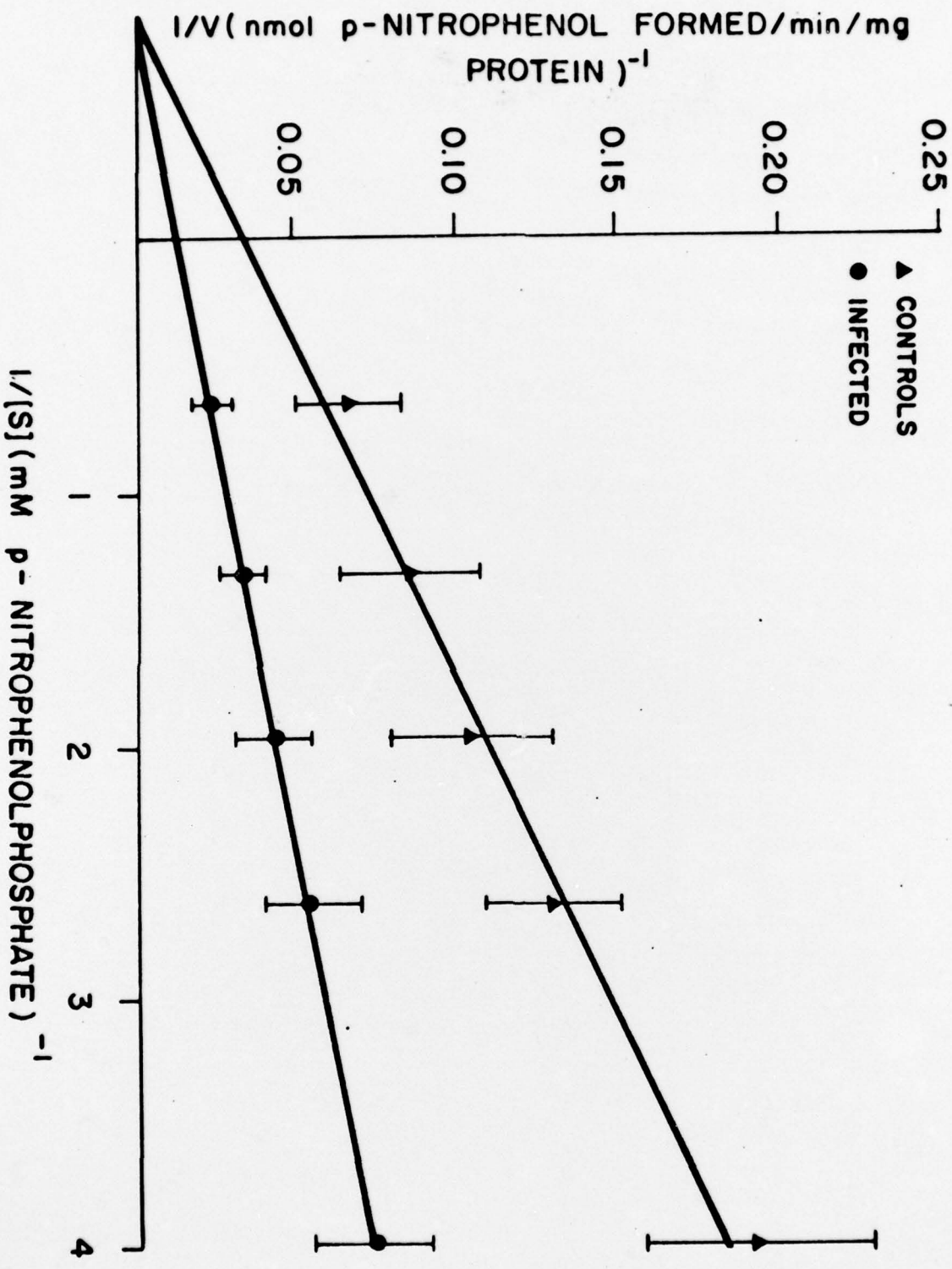


Fig. 4

