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ULTRACENTRIFUGAL STUDIES OF MICROSOMES FROM STARVING, NON-PROLIFERATING AND PROLIFERATING YEAST

James K. Ashikawa

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ULTRACENTRIFUGAL STUDIES OF MICROSOMES FROM STARVING, NON-PROLIFERATING AND PROLIFERATING YEAST

James K. Ashikawa

Donner Laboratory of Biophysics and Medical Physics, University of California, Berkeley, California

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INTRODUCTION

In the decade following Claude's successful isolation of microsomes from liver and other tissue homogenates by differential centrifugation (1, 2), considerable progress has been made toward elucidating their biochemical and morphological characteristics. The microsomal "ribonucleoprotein" particles isolated from bacterial cells and from plant and animal tissues are rich in ribonucleic acid (3-10). Electron microscopic observations of these microsomal particles, either isolated or in intact plant and animal cells, appear as spherical particles with diameters ranging from 100-400 Å. These particles occur either bound to the endoplasmic reticulum or freely dispersed in the cytoplasmic matrix (7-14).

There are interesting studies indicating that microsomes may be actively involved in protein and lipid synthesis (5, 15, 16). In vivo (17-20) and in vitro studies (15, 20, 21) have shown that labelled amino acids are preferentially incorporated into the microsomal proteins which may be suggestive of synthetic activity.

Since these microsome particles appear to be functional organelle in both plant and animal cells, it is highly probable that the physicochemical properties of these particles will be altered by varying the physiological state of the cell. Preliminary studies of microsomes isolated from starving, non-proliferating and proliferating yeast cells have shown that several new

ribonucleoprotein particles appear during cell division (22, 23). This paper presents further evidence of this correlation.

EXPERIMENTAL PROCEDURE AND RESULTS

Haploid yeast cells (Saccharomyces cerevisiae strain S. C. 7) aerobically cultured for 24 hours at 30°C in yeast extract and dextrose (YED 1:2%) were harvested and washed twice with sterile distilled water. Aliquots of cells were then suspended in nitrogen deficient medium (4% dextrose:M/60 KH_2PO_4 3/60 M NaH_2PO_4) and aerated for 48 to 72 hours at 30°C. During this nitrogen starvation, the buffered dextrose medium in some of the cultures was changed every 12 hours. At the end of starvation, the cells were centrifuged and re-washed twice with sterile distilled water. Approximately 6 gram aliquots of wet cells were suspended in 2-liter flasks containing 1500 ml YED (1:2%) and induced to grow aerobically at 30°C for varying time intervals. The growth curve of the cells and the corresponding bud counts for the first 26 hours is given in figure 1. The yeast cells were then harvested at different times and microsomal particles isolated according to Wolfe (23). Cells hand ground with 100 grid carborundum in mortar and pestle at 0°C were extracted with several volumes of solvent (0.00125M KH_2PO_4 - K_2HPO_4 3:7, 0.001M $MgCl_2$, and 0.01M KCl). After vacuum filteration through celite filter-aid, the microsomes were sedimented from the filtrate by ultracentrifugation in the preparative ultracentrifuge (Spinco Model L) at 114,000 x g (40,000 rpm) for one hour. The translucent microsomal pellets isolated from yeast cells in different growth stages were carefully redissolved in the buffered

solvent and analyzed in the analytical untracentrifuge (Spinco Model E) at 200,000 x g. Figure 2 shows the changes in the sedimentation pattern which indicates the appearance of new microsomal particles (22, 23). During the lag phase when less than three percent of the cells have visible buds, only the 80 S component (α peak in Fig. 3) is present in the ultracentrifuge pattern of cytoplasmic extract. As the cells begin to proliferate and enter the log phase of growth (40-50% visible buds), four new microsomal components (β, γ, δ and ϵ peaks in figure 3) appear in the cell extracts. From log growth phase through stationary phase, the only change in the sedimentation pattern appears to be in the ratio of these components.

Cell starvation is first evidenced by a decrease in the stability of the microsomes which is indicated by the presence of polymerized material that sediments as a rapidly spreading fore peak (23) before the centrifuge is up to speed (UTS). The growing cell components disappear as starvation progresses.

The stability of microsome components isolated from log phase, stationary phase and starving cells and kept at 4°C or at room temperature in buffered solvent was also studied. There was no detectable difference in stability between particles isolated from log phase and from stationary phase. Figure 3 shows the degradation of microsomes isolated from 48 hour stationary phase cells and suspended in phosphate buffer at 4°C.

Since only the 80 S component is present in old stationary phase cells and in starving cells, the stability of this component isolated from proliferating, non-proliferating and starving cells and dissolved

in buffered solvent was compared. Figure 4a shows the sedimentation pattern at 0, 2 and 4 minutes after UTS at 200,000 x g of the 80 S particles isolated from 24 hour stationary phase cells. The sedimentation pattern of this component from 72 hour N-starved cells is similar, except for the broad, rapidly spreading and sedimenting forepeak. As shown in figure 4b, the 80 S component isolated from log phase and stationary phase cells gave rise to the 60 S and 40 S component (24) when kept at room temperature for 2 days. On the other hand, the 80 S component isolated from cells nitrogen starved for 72 hours became degraded by forming rapidly sedimenting aggregates. See figures 4c and 5.

DISCUSSION

When starved cells are given utilizable nitrogen, the growth curve shows a characteristic lag phase corresponding to the degree of starvation. During this phase the 80 S component appears to be degraded and reconstituted. As shown in figure 2, a decrease in the 80 S component is noted 1.5 hours after starved cells are given utilizable nitrogen. Two hours after inoculation, the microsomal concentration appears approximately equal to that of the inoculum cells. At 3 hours new microsomal components appear in the cytoplasmic extract. The cells are now entering the log growth phase. During this phase the microsome concentration reaches a maximum. This is followed by a gradual quantitative and qualitative change in the microsomal components as the cells pass through the stationary phase.

Chao et al (8, 24) have shown in vitro that altering the ionic environment of the solvent will dissociate or aggregate the 80 S component. It would therefore be interesting to ascertain whether a similar mechanism is

responsible for changes in the microsomal components in respiring cells.

Since the 80 S particles from only starving cells of low viability are degraded by forming rapidly sedimenting aggregates (Figures 4c and 5), there appears to be a correlation between cell viability and the chemical state of this particle.

Furthermore, if microsomes are involved in the synthesis of proteins and lipids (5, 15, 16), the changes observed in the microsomal components with cell growth could be a mechanism that controls their synthetic activities.

SUMMARY

Ultracentrifugal studies of yeast cell extracts have shown a correlation between the physiological states of the cells and the stability and appearance or disappearance of microsomal particles from the cytoplasm.

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Fig. 1. Relationship of budding to growth of yeast cells.

a. Growth curve of 48 hour nitrogen starved yeast cells aerobically cultured at 30°C in YED (1:2%).

b. Curve correlating percent visible buds to corresponding growth stages.

Fig. 2. Sedimentation photographs of microsome particles from yeast cell extracts 6 minutes after up to speed (UTS) at 200,000 x g in the analytical ultracentrifuge (Spinco Model E).

a. Top: Cell extract from 48 hour nitrogen starved cells corresponding to the inoculum cells at time 0.

Bottom: Extract from N-starved cells 1.5 hours after cells given utilizable nitrogen.

b. Top: After three hours.

Bottom: After five hours.

c. Top: 10 x concentrated extract from N-starved cells 7 hours after cells given utilizable nitrogen.

Bottom: Extract from N-starved cells 10 hours after cells given utilizable nitrogen.

d. Top: After 26 hours.

Bottom: After 52 hours.

Fig. 3. Sedimentation photographs of microsomes isolated from 48 hour stationary phase cells six minutes after UTS at 200,000 x g.

a. Yeast extract suspended in phosphate buffer (0.00125M KH_2PO_4-K_2HPO_4 3:7, 0.001M $MgCl_2$ and 0.01M KCl) at 4°C for one day.

b. Yeast extract suspended in phosphate buffer at 4°C for 2 days.

c. After 7 days.

d. After 10 days.

e. After 13 days.

f. After 27 days.

Fig. 4. Sedimentation photographs of isolated 80 S particles taken at 0, 2 and 4 minutes after UTS at 200,000 x g in the analytical ultracentrifuge. Particles moving from left to right.

a. 80 S particles from 24 hour stationary phase cells observed immediately after isolation.

b. 80 S particles, isolated from 24 hour stationary cells, suspended in phosphate buffer and kept at room temperature for 2 days.

c. 80 S particles isolated from cells nitrogen starved for 72 hours suspended in phosphate buffer and kept at room temperature for 2 days.

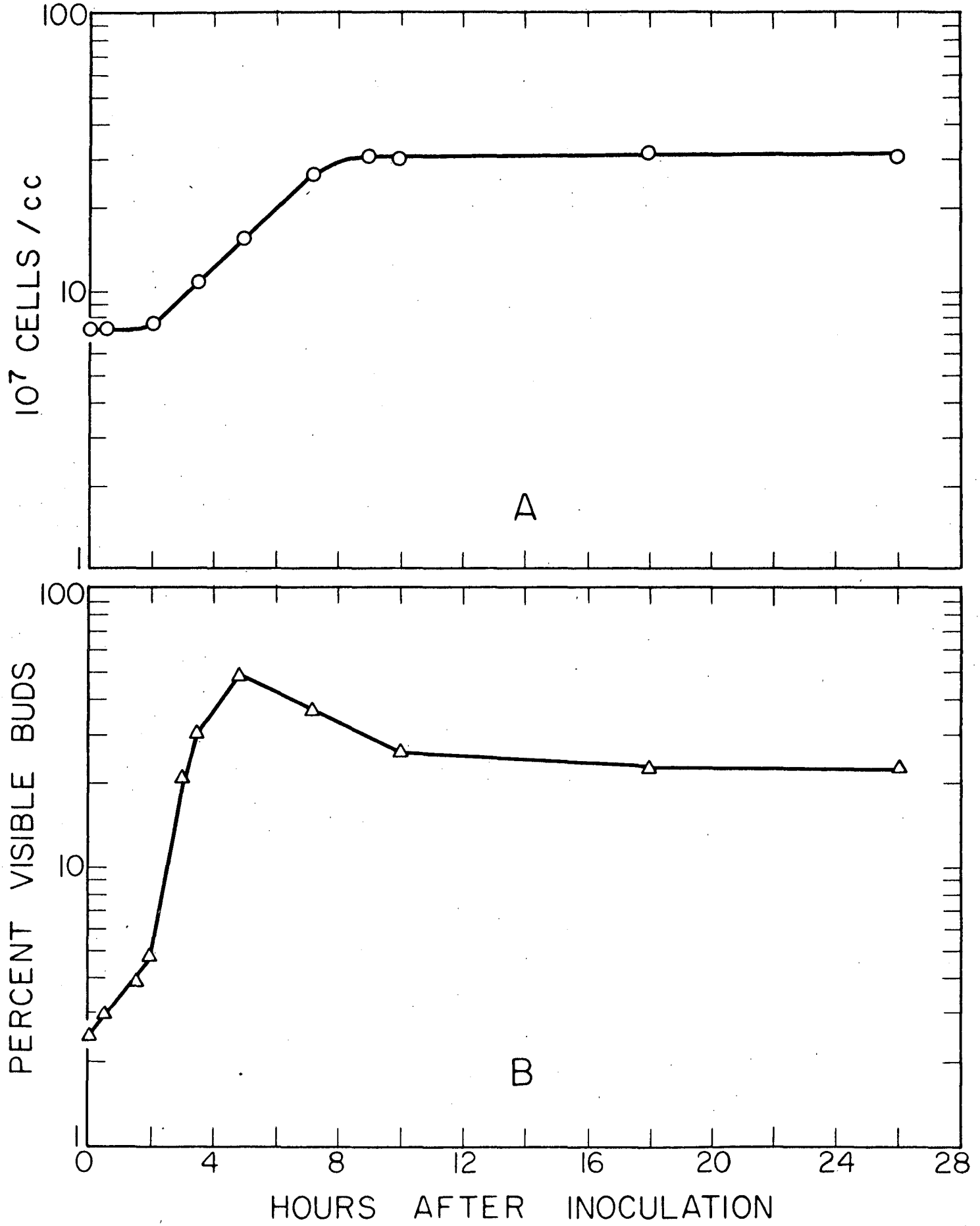
Fig. 5. Sedimentation photographs at UTS (200,000 x g) of 80 S particles isolated from cells nitrogen starved for 72 hours and suspended in phosphate buffer at 4°C.

a. After 1 day.

b. After 2 days.

c. After 7 days.

d. After 11 days.


100
10
1
10^7 CELLS / cc
A
100
10
1
PERCENT VISIBLE BUDS
B
0
4
8
12
16
20
24
28
HOURS AFTER INOCULATION

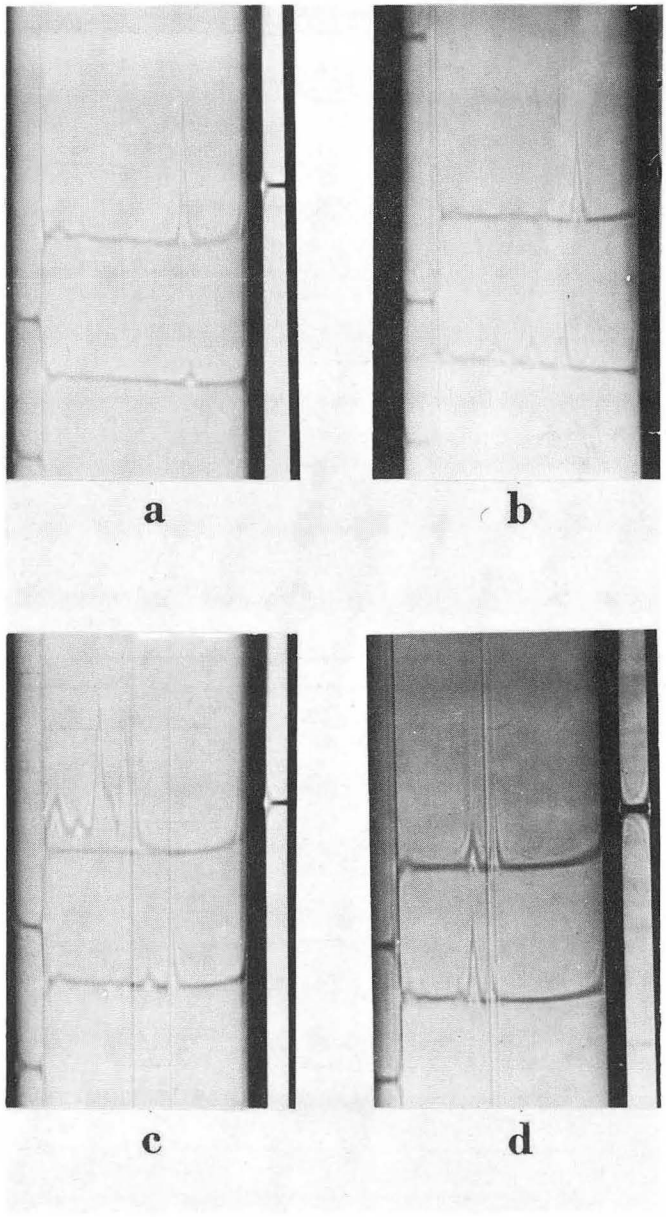


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Fig. 2.

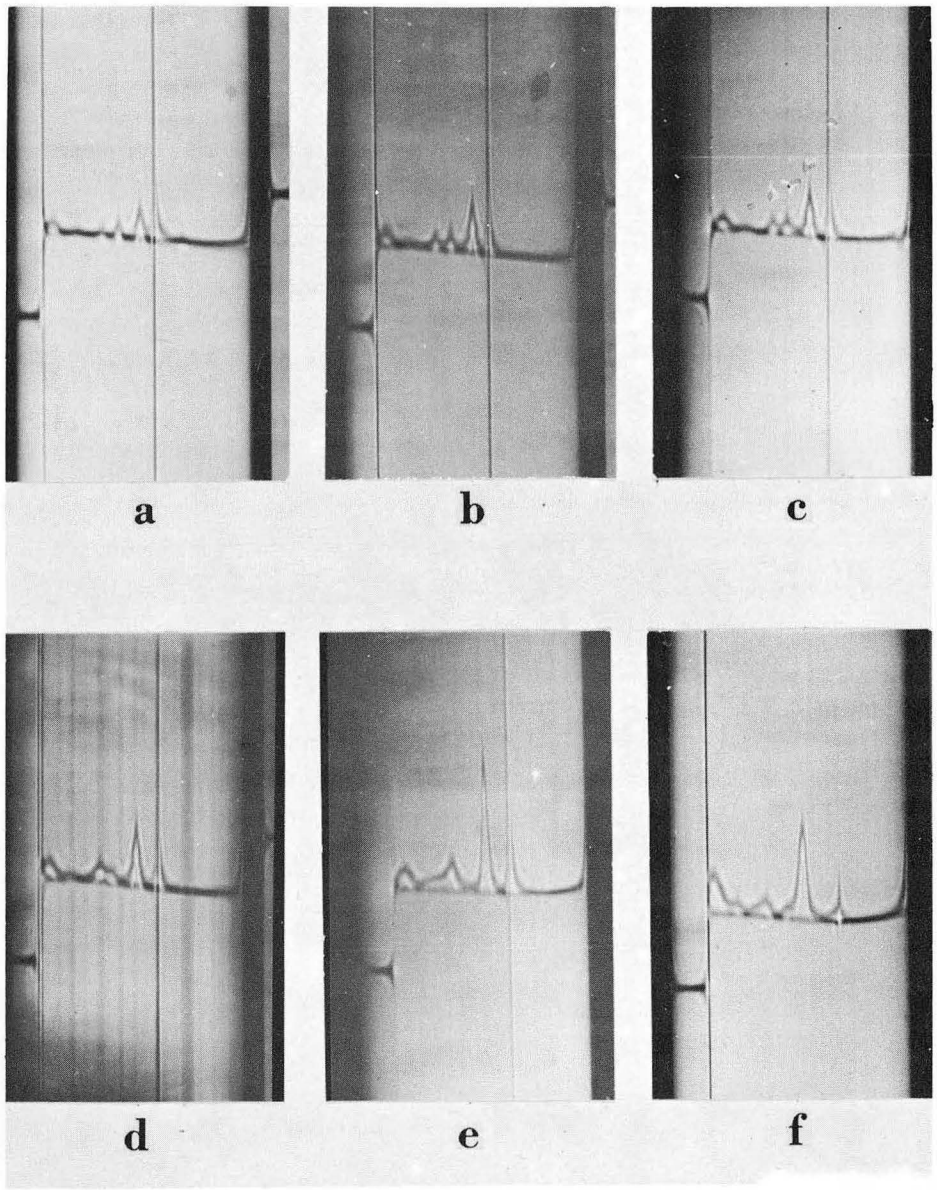

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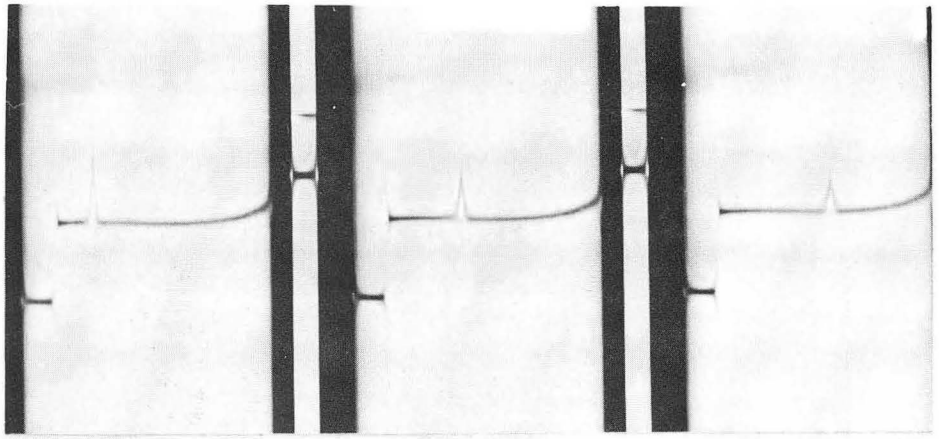
Fig. 3.



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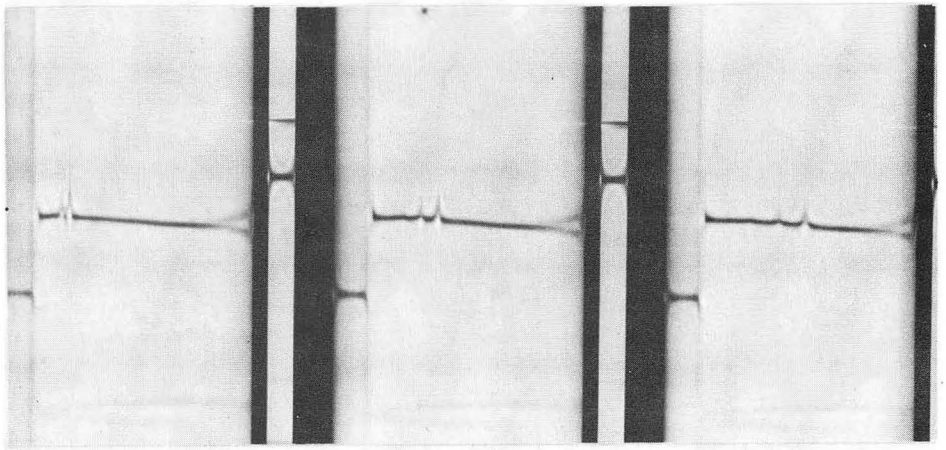

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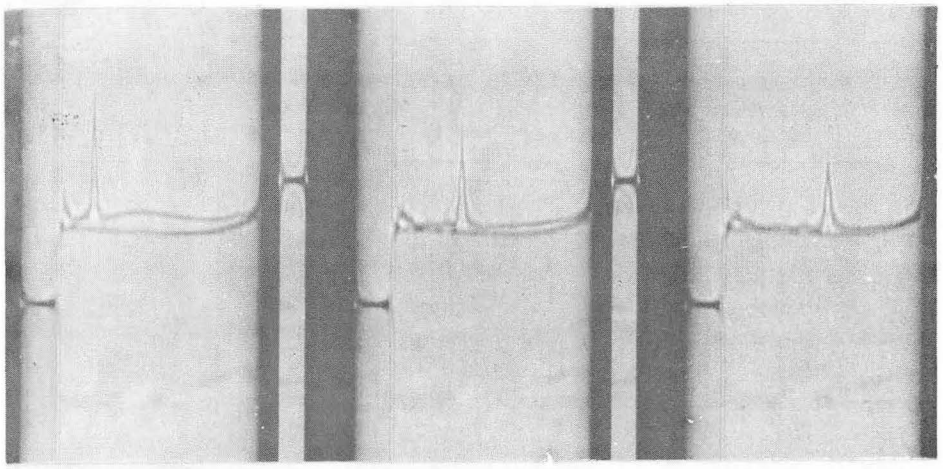
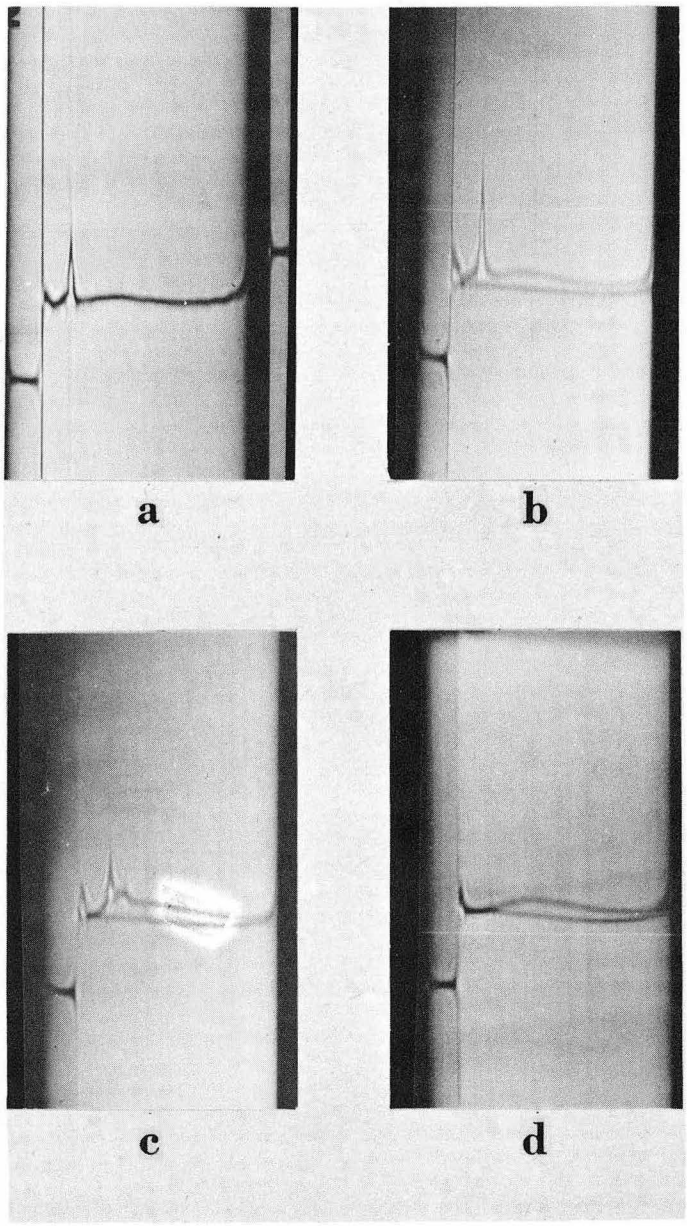


c

Fig. 4.

ZN-1926



ZN-1927

Fig. 5