Conclusion

Our observations indicate that, in *Streptococcus pyogenes*, new cell wall is not diffusely intercalated with the old, but its formation is instead initiated equatorially along a circumference which is the site of the next cross-wall formation. These findings, made by labeling of living cells, confirm the conclusions expressed by Bisset (*13*) concerning the site of the main growth of the cell wall in septate bacteria. The new cell wall growth, in actively dividing cultures, is well under way, and the cross-wall may have progressed centripetally halfway, before there is complete separation by cell wall of the two previously formed cocci; as a result, the predominant forms in a chain appear as diplococci. The method at present does not indicate the presence or absence of cytoplasmic membrane septa, and does not therefore confirm or deny the activity of any part of such a membrane in secreting cell wall. It is obvious, however, that there are at least two sites of simultaneous activity—membranous or other—within the bounds of any one coccus as defined by cell wall furrowing: at one (*I*, Fig. 1) cross-wall to complete the previous division is still being formed, and at the other (*II*, Fig. 1) peripheral cell wall and cross-wall for the current division are being initiated.

Our findings and interpretations apply only to *S. pyogenes*. The methods described, however, should be widely applicable to any microorganism, with antigenic cell wall components, which can be grown in the presence of homologous antibody (*14*).

References and Notes

1. M. J. R. Salton, in *Symposium Soc. Gen. Microbiol. 6th* (1956), p. 81.
2. C. F. Robinow, in R. J. Dubos, *The Bacterial Cell* (Harvard Univ. Press, Cambridge, Mass., 1945), Addendum; K. A. Bisset, *The Cytology and Life History of Bacteria* (Williams and Wilkins, Baltimore, ed. 2, 1955); G. Knaysi, *Elements of Bacterial Cytology* (Comstock, Ithaca, N.Y., ed. 2, 1951).
3. G. B. Chapman and J. Hillier, *J. Bacteriol.* **66**, 362 (1953); G. B. Chapman, *ibid.* **78**, 96 (1959); *ibid.* **79**, 132 (1960); J. L. Roth, C. W. Lewis, Jr., R. P. Williams, *ibid.* **80**, 772 (1960).
4. I. M. Dawson and H. Stern, *Biochim. et Biophys. Acta* **13**, 31 (1954).
5. B. A. D. Stocker, in *Symposium Soc. Gen. Microbiol. 6th* (1956), p. 19; M. A. Williams, *J. Bacteriol.* **78**, 374 (1959).
6. S. Rothbard, *J. Exptl. Med.* **82**, 107 (1945).
7. J. D. Marshall, Jr., W. C. Eveland, W. C. Smith, *Proc. Soc. Exptl. Biol. Med.* **98**, 898 (1958).
8. R. C. Lancefield, *J. Exptl. Med.* **47**, 91 (1928).
9. Baumann Photo-Chemical Corp., 125 W. Hubbard St., Chicago, Ill.
10. H. F. Swift, A. T. Wilson, R. C. Lancefield, *J. Exptl. Med.* **78**, 127 (1943).
11. R. C. Lancefield and G. E. Perlmann, *ibid.* **96**, 71 (1952).
12. M. Goldman, *Am. J. Trop. Med. Hyg.* **5**, 375 (1956).
13. K. A. Bisset, in *Symposium Soc. Gen. Microbiol. 6th* (1956), p. 1.

14. The technical assistance of Richard S. Whitt is gratefully acknowledged.

10 October 1961

Index for Measurement of Synchronization of Cell Populations

Abstract. An index for the estimation of synchronization in a microbial population is proposed and discussed. The index is equal to the fraction of cells that divide during a measured interval in excess of the fraction that would divide during random growth in the same interval.

In studying the effects of various treatments on the synchronization of cell populations, it is desirable to have a quantitative measure for comparing the amounts of synchrony obtained. Such an index should have the following characteristics. It should reach an upper limit if all the cells simultaneously carry out the particular reaction used as the criterion for synchrony. It should be proportional to the fraction of the cells undergoing the reaction and inversely proportional to the length of time in which the synchronized fraction of the culture carries out the reaction. It should permit positive identification of logarithmic growth even if the treatments have caused a lag or an acceleration of growth rate.

A number of indexes have appeared (*1*), but none seems to meet these requirements satisfactorily. We wish to propose an index that seems to be a reasonable compromise among these criteria. We have used cell division for the measurement of synchrony, but certain other parameters of growth could be used.

If the number of cells in a culture increases from N_0 to N in an interval t less than one generation time g, then

$$N/N_0 - 1$$

is the fraction of cells in the culture which divide during t, and

$$2^{t/g} - 1$$

is the fraction of cells in the culture which would have divided during t if the culture had been growing logarithmically. The quantity

$$F = (N/N_0 - 1) - (2^{t/g} - 1)$$
$$= N/N_0 - 2^{t/g}$$

measures the fraction of the population which divides during t in excess of that expected to divide during logarithmic growth in the same interval.

F has a maximum value of $+1$ if the entire population divides during an infinitely small time interval. It has positive values less than 1 if doubling takes a finite time (which, of course, it must), or if less than the entire population divides during the measured interval. After a synchronized burst of divisions, the population must increase at a rate less than that of normal logarithmic growth, and F falls to negative values. Thus the criterion for synchronized cell division is a positive value followed by a negative value. (The index should never fall below -1.) A logarithmically growing culture has an index of zero over any interval. An index of 0.8 (followed by a negative index in the next interval measured) would indicate excellent synchrony (*2*).

LINDA K. BLUMENTHAL
STANLEY A. ZAHLER
Division of Bacteriology,
Cornell University, Ithaca, New York

References and Notes

1. K. G. Lark and O. Maaløe, *Biochim. et Biophys. Acta* **15**, 345 (1954); O. Scherbaum, *J. Protozool.* **6** (suppl.), 17 (1959); E. Zeuthen, *Advances in Biol. and Med. Phys.* **6**, 37 (1958).

2. This work was supported by research grant No. E-3221 from the National Institutes of Health. The senior author held a Cornell University graduate fellowship during part of the time involved.

21 September 1961

Hydrostatic Pressure Has a Selective Effect on the Copepod Tigriopus

Abstract. High hydrostatic pressures have been found to be an agent in producing a shift in the sex ratio in populations of the marine copepod *Tigriopus.*

The study of the effect of hydrostatic pressure on the harpacticoid copepod *Tigriopus* was initiated in an attempt to obtain morphological mutations in this organism. These experiments revealed a definite shift in sex ratio of the pressure-treated organisms and are therefore being reported here.

The first experiments consisted of collecting adults from their natural habitat in the splash pools of the supralittoral tidal zone and subjecting them to various levels of hydrostatic pressure to determine a survival curve. At pressures from 1 to 500 atm and short exposure intervals 100-percent survival was observed. In view of the relative

Table 1. Effect of hydrostatic pressures on sex ratio in *Tigriopus*.

Pressure (atm)	Surviving/ treated	Percent survival	Males		Females	
			Total	Percent	Total	Percent
1	168/175	96	142	84.5	26	15.5
450	295/300	98	236	80.0	58	20.0
500	175/200	88	131	75.0	44	25.0
550	242/253	95	167	69.0	75	31.0
600	151/225	67	55	36.5	96	63.5
650	36/300	12	1	3.0	35	97.0
700	3/300	1	0	0.0	3	100.0

insensitivity of the adults to increased pressure, larval forms were used in the succeeding experiments in the hope they would prove more sensitive.

Female copepods with ripe egg sacs were collected and washed with distilled water to cleanse them of unicellular contaminants. The egg sacs were then removed from the females and placed in a growth medium, where they were allowed to hatch. The larvae hatch almost immediately after dissection of the egg sac. These larvae were pooled and stirred with a pipette to obtain a random distribution. Seven samples were selected from the nauplii pool, each sample comprising 300 larvae. Six of the samples were subjected to hydrostatic pressure from 450 to 700 atm, at intervals of 50 atm. The seventh sample was held at 1 atm as a control. The samples were raised to pressure and held at that level for 2 hours. The time of pressure rise was approximately 60 seconds. The time of reduction from each pressure level to 1 atm was approximately 30 to 60 seconds. The pressure bombs were immersed in a water bath at 22°C. Following depressurization, the samples were cultured in the standard laboratory growth medium. Upon maturing, the copepods were counted and the percent survival and sex ratio determined. No morphological mutants were observed among the progeny of the survivors in any of these experiments. The results are shown in Table 1.

It can be seen from Table 1 that in the control population, 84.5 percent of the copepods were males. No males were found in the surviving fraction of the 700-atm sample. It is evident that the fraction of females of the survivors increases with the increase of pressure. It is apparent that a definite shift in sex ratio exists, since the ratio in the control was approximately 8 males to 1 female and at the 50-percent survival level a sex ratio of 1 to 1 was observed. These data may represent either selection for females in this population by increased pressure or an actual sex conversion in the early stages of development. Examination of the data from the 600-atm sample shows that: if all the individuals dying were males the sum of surviving males plus all dead animals equals 57.6 percent males in the population. This is lower than observed at any pressure below 600 atm and suggests that conversion may be the mechanism.

Takeda (*1*) has presented evidence of sex conversion in *Tigriopus* induced by temperature or chemical agents or both. He concludes that *Tigriopus* is to a degree sexually neutral during its six naupliar stages and does not differentiate until the onset of the five copepodid stages. With this precedent it seemed worth while to attempt to determine whether sex conversion could be induced by hydrostatic pressure by the following experiment. A population of newly hatched nauplii was obtained with the procedure outlined above. Twelve hundred nauplii were counted out and 100 were placed in each of twelve 3-ml test tubes. Six of these tubes were subjected to 600 atm of pressure for 2 hours. The six remaining tubes were used as controls. The times of pressure rise and pressure reduction to 1 atm were approximately 1 minute. The temperature during the 2 hours of exposure to pressure was 23°C. The samples were then cultured separately in standard laboratory growth medium. Upon maturing, the cultures were examined and percent survival and sex ratio determined. The survival in the pressure-treated group was much lower than in the previously described results from Table 1; that is, 5.2 percent as compared to 67.0 percent. These data are insufficient for interpretation regarding sex conversion, since the survival at 600 atm was too low. However, they were consistent with the preceding results in respect to the stringent selection for females.

At this stage of the work it is impossible to distinguish between selective effects of the pressure and sex conversion. Regardless of which mechanism is responsible for the preponderance of females in the survivors of exposure to pressure, it seems reasonable to assume that there might be a biochemical basis. If some essential component is altered by pressure in the young larvae, two possibilities exist: (i) If the pressure effect is selective, the larvae which would become males upon maturation fail to develop as a result of the alteration, as this substance may be required by the organism for advancing through one or more of its 11 metamorphic stages; (ii) If the mechanism is actually sex conversion, the destruction of a male-determining substance would lead to the development of a larger number of females and a concomitant loss in males. While other alternatives exist, those enumerated seem to be the most immediately accessible to experimentation.

In order to document the presumptive evidence regarding sex conversion, experiments are now being carried out to obtain more quantitative data (*2*).

VICTOR VACQUIER, JR.
Department of Marine Genetics, University of California, La Jolla

References and Notes

1. N. Takeda, *Physiol. Zool.* **23**, 288 (1950).
2. I am greatly indebted to Dr. William L. Belser for his interest and guidance concerning this project and Mr. James R. Lance for his assistance and advice; also, to Dr. Claude E. Zobell for the use of his apparatus. This report represents the results of research conducted under the Marine Life Resources program, Scripps Institution component of the California Cooperative Oceanic Fisheries Investigations, a project sponsored by the Marine Research Committee.

23 October 1961

Induction of Changes in Surface Activity of Strain L Cells at Gas-Membrane Interfaces

Abstract. The injection of smoke and non-smoke gases into a perfusion chamber containing monolayers of strain L or HeLa cells in a fluid medium produces cellular changes. The most striking change is surface "blebbing," produced when gas bubbles pass over the surface of the cells. This reaction can be promptly reversed by the injection of fresh medium into the chamber.

During an investigation of the effects of various gaseous agents on the growth of strain L and HeLa cells in cell culture, the immediate direct effect of the passage of particulate and nonparticu-