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*).

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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.

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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-

late gases over the surface of these cells was observed. The L cells were grown in monolayer cultures on Leighton cover slips in medium NCTC-109 with 10-percent horse serum, and HeLa cells in Eagle's balanced salt solution with added L-glutamine and 10-percent horse serum. No antibiotics were added. About 48 to 72 hours after preparation, the cover slips were put into Rose perfusion chambers, filled with fresh medium, and mounted on a special carrier on the stage of a microscope. A camera attached to the microscope permitted continual observation of any changes.

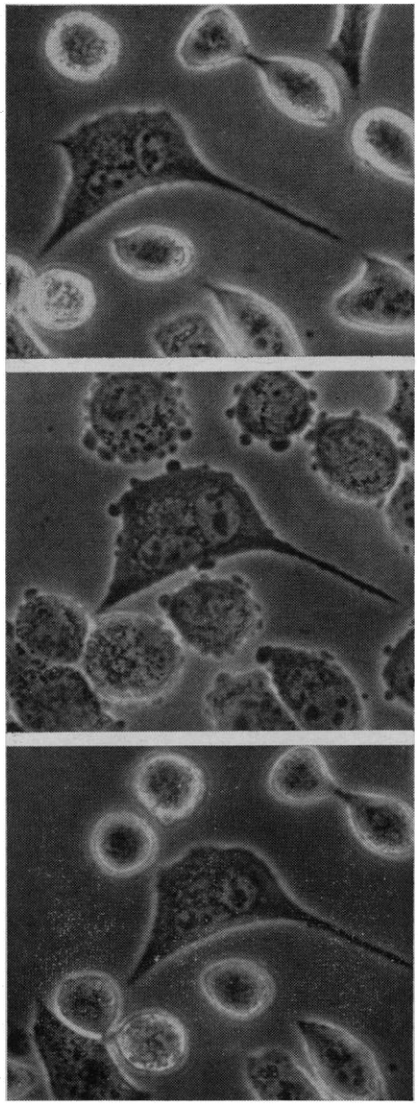

Fig. 1. Changes in L cells caused by exposure to illuminating gas. (Top) Before exposure to gas. (Middle) Immediately after injection of gas, cells show swelling, increased granularity, and surface "blebbing." (Bottom) Cells appear to have returned to their original state, 2 minutes after removal of gas bubbles by change of media. (about × 1000)

Smoke from the combustion of cigarettes, of cigarette tobacco only, of cigarette paper, and of onion skin paper and non-smoke gases such as carbon monoxide, hydrogen sulfide, illuminating gas, oxygen, and helium were injected in volumes of 0.2 to 0.3 cm^3. The gases were obtained and injected under controlled conditions.

Immediately after the injection of all gases tested there is a very rapid increase of the surface area of the cell as well as increased granularity. Most evident, however, is "blebbing" of the surface of the cells. Lewis (*1*) described the formation of "blebs" along the edge of tissue cultures exposed to alkali and Buchsbaum and Kuntz (*2*) noted "blebs" after the injection of certain drugs into a perfusion chamber. Dornfeld and Owczarzak (*3*) demonstrated that ethylenediaminetetraacetic acid produces surface bubbling. Landau (*4*) described surface "blebs" in cells following release of high hydrostatic pressure.

We found that "blebbing" is most pronounced immediately after bubbles of gas move over the surface of the cells. It is most marked near the center of the bubble. The reaction occurs on cell surfaces that are free and exposed and is not seen on the surfaces of neighboring cells. The "blebbing" persists as long as bubbles of gas remain in the chamber. If the contaminated medium is replaced by fresh medium within 10 to 15 minutes after contamination, the cells are promptly restored to their original appearance. If "blebbing" is allowed to continue for more extended periods, cellular activity gradually decreases and the cells die.

Further observations showed that "blebbing" does not occur when cells in a chamber without medium are exposed to air or medium alone, under varying degrees of increased pressure. The pressure in the chamber was increased by occluding the outlet needle while the gas or medium was being injected. Cells grown on a Leighton cover slip were observed microscopically while the cover slip, moistened with medium, was exposed to air and the medium was allowed to evaporate. "Blebbing" did not develop.

Cellular changes in control, experimental, and recovery phases, after injection of illuminating gas, are shown in Fig. 1. The effects of the other gases are similar.

It appears from our observations that the "blebbing" results from a disturbance of the sol-gel relationship of the cell surfaces and apparently can occur at a gas-membrane interface. Further studies now in progress may reveal some of the factors involved in this phenomenon.

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18 August 1961

Spontaneous Discharge of Single Neurons during Sleep and Waking

Abstract. Populations of single neurons in visual cortex, middle suprasylvian association cortex, and brain stem of cat show greater variance of spontaneous discharge rates during waking than during sleep; this change in variance occurs in the absence of significant changes in mean discharge rates. Neurons which discharge rapidly during sleep tend to discharge even more rapidly during waking, whereas neurons with relatively low rates of discharge during sleep tend to have reduced spontaneous activity during waking.

Recent studies of the effects of sleep and waking on spontaneous discharge of single neurons have indicated that neurons may show either increases or decreases of spontaneous activity as a result of arousal (*1–5*). The proportion of units showing one or the other of these responses to arousal varies both as a function of the cerebral area from which recordings are obtained and as a function of the behavior of the experimental animal during the waking state (*2*). Our observations provide information about a characteristic of unit discharge, during sleep, which is associated with the direction of change of discharge rate which occurs with waking.

Experiments were carried out in unrestrained cats (*3*, *4*). Microelectrodes of a variety of types were employed. Units in visual cortex were recorded exclusively with tungsten microelectrodes (*6*). Brain stem units were recorded with tungsten or steel (*7*) microelectrodes. Initially, steel electrodes were used to facilitate identifica-