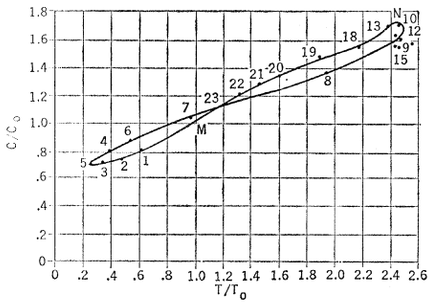

Fig. 2. Normalized concentration—traffic curve at East 45 Street.

The data obtained from the carbon monoxide samplers operated during portions of 1967 indicate that: (i) Business-day traffic determines the basic shape of the curve for diurnal carbon monoxide concentration in Manhattan. (ii) Local traffic conditions are the primary influence upon concentrations of carbon monoxide measured close to street level. The problem of automobile exhaust for a large city of metropolitan area may be the sum of local problems related to local traffic conditions.

An effort is being made to predict the hourly average concentrations of carbon monoxide for each day. When the average concentration of carbon monoxide is plotted against the time of day, the resulting curves are similar in shape from station to station, although their amplitudes and time phases differ. Therefore, we attempted to predict hourly concentrations of carbon monoxide from readings taken in the very early morning. Accordingly, the curves were smoothed through the use of a 2-hour moving average. Each of the smoothed hourly values was then divided by the midnight average, C_0. To predict the hourly concentrations of carbon monoxide, we first determined C_0 by direct measurement. We then forecast the concentration at each hour of the following day by multiplying C_0 by the ratio indicated on the average curve for that station. When this method was used to predict daily peaks, the predicted values fell within 5 parts per million of the measured values about 70 percent of the time for stations at Park Avenue South, Times Square, Herald Square, and East 45 Street.

The limitations of this predictive method are dictated by the dispersion in the hourly averaged data. The mean daily standard deviation is between 4 and 5 parts per million. This is consistent with the error in the predictions. There is a cause-and-effect relationship between traffic and carbon monoxide concentration. Hourly averages of traffic volume for the five sites were obtained from the Traffic Department. These data were smoothed and normalized in exactly the same fashion as the carbon monoxide data were. In order to eliminate time between the two sets of data, the relative carbon monoxide concentration, C/C_0, was plotted against T/T_0. In Fig. 2, a curve has been drawn through the points in the chronological order in which they appear.

Carbon monoxide concentration and traffic volume are periodic functions of time, and each can be represented by a Fourier series. When time is eliminated between the two functions and they are plotted as orthogonal coordinates (Fig. 2), closed curves somewhat akin to Lissajous figures should result.

The carbon monoxide concentration and the traffic volume are not in phase, and accordingly the increases and decreases in the curves do not coincide. The carbon monoxide concentration lags the traffic volume during the increase and much of the decrease in the curve. Carbon monoxide concentration does not build up as rapidly as the traffic perhaps because the traffic exhausts into a relatively clean atmosphere in which dilution is relatively good. A relatively dirty atmosphere prevents the carbon monoxide concentration of the traffic from decreasing as rapidly as the traffic does. This phase shift is consistent with a diffusion process.

For all sites the carbon monoxide concentration and the traffic volume are nearly in phase from midnight until some hour between 3 and 5 a.m. Thus, the decay rate of the carbon monoxide increases after the concentration and traffic volumes fall below a certain value characteristic of each station. This phenomenon may explain the crossing of traffic volume and carbon monoxide curves as each begins to increase and again when they fall off.

KENNETH L. JOHNSON *
L. H. DWORETZKY †
AUSTIN N. HELLER
New York City Department of Air Pollution Control,
51 Astor Place, New York 10003

Notes

* Public Health Service. Temporary assignment to New York City Department of Air Pollution Control.
† General Electric Company, Re-entry Systems Department. Temporary assignment to New York City Department of Air Pollution Control.

5 February 1968

Salt Transport in Valonia: Inhibition of Potassium Uptake by Small Hydrostatic Pressures

Abstract. *Internally perfused cells of the marine alga* Valonia *actively transport potassium from external seawater into the cell vacuole. This active uptake of potassium is reduced by restoring the normal turgor pressure of 1 atmosphere by means of a mercury manometer attached to the internal perfusion system. This inhibition of salt uptake by a small hydrostatic pressure suggests that* Valonia *and other walled cells may regulate their turgor pressures by adjusting their rates of salt uptake.*

Cells of most plants and microorganisms are protected from osmotic swelling by a rigid cell wall which surrounds the protoplast (*1*). The presence of this cell wall permits the development of a turgor pressure which is approximately equal to the difference between the osmotic pressures of the intracellular and extracellular fluids. Turgor pressures vary widely among walled cells, ranging from about 1 atm in the giant-celled algae *Valonia* and *Halicystis* to about 20 atm in certain microorganisms (*2, 3*).

In some algae the turgor pressure is nearly constant over a wide range of environmental salinities (*3, 4*). In *Chaetomorpha linum,* for example, the turgor pressure is 13 to 14 atm, although the osmotic pressure of the environment ranges from about 0 to 23 atm (freshwater to seawater) (*4*). This leads one to ask whether walled cells regulate their turgor pressures and, if so, by what mechanism.

A possible answer to this question is suggested by the early work of Blinks and Jacques (*5, 6*), who impaled cells of *Valonia* and *Halicystis* on glass capillaries and then observed the rate at which fluid (sap) moved up the capillary. Jacques (*6*) observed that the rate of fluid uptake was 10 to 15 times higher in impaled than in control cells which grew at a rate of 0.8 to 1.0 percent per day. The only large variable in Jacques's experiments was the turgor pressure, which was 0.5 to 2 atm in normal cells but virtually zero in impaled cells. In other experiments, Jacques abolished the turgor pressure by immersing *Valonia* cells in hypertonic seawater, and again the cells responded by rapidly absorbing salts and water until the normal turgor pressure was restored.

Jacques's results are surprising be-

cause pressures of less than 2 atm should not detectably affect either the passive or the metabolic driving forces for fluid transport. First, the electrochemical potential of an ion is virtually insensitive to physiological pressures, that is, about 0.2 mv/atm (*7*). Second, to markedly affect the rate of a chemical or metabolic reaction involving solid or liquid phases, pressures of more than 100 atm are required (*8*). Finally, although removing the turgor pressure creates an inward chemical potential gradient for water, this gradient is nearly abolished by the inward osmotic flow which occurs during the first few hours after impalement (*6*). Therefore, the sharp increase in the steady-state rate of fluid uptake by these impaled cells cannot be attributed to large changes in any of the obvious driving forces for salt or water movement.

I have tested directly the effects of small hydrostatic pressures on salt transport in *Valonia ventricosa*. In most experiments I studied potassium transport because, in *Valonia*, potassium is the predominant intracellular cation and is actively absorbed from seawater into the vacuole against an electrochemical gradient of about 110 mv or 2500 cal/mole (*9, 10*). Spherical cells about 1 cm in diameter were shipped by air from Miami and maintained in laboratory cultures as described previously (*9*). The methods of perfusing the vacuole of *Valonia* and of measuring solute and water fluxes across the protoplast are also described elsewhere (*10, 11*). Briefly, a cell was perfused by means of two micropipettes (tip diameter, about 0.2 mm) inserted through the outer cell wall (7 to 13 μ thick) and underlying protoplasm (7 to 12 μ thick) into the vacuole or sap cavity. After a 1-hour recovery period, the vacuole was perfused with artificial sap (*12*) at a rate of 50 to 100 μl/min. Solute fluxes were measured by adding a suitable tracer either to the artificial sap or to the external seawater (*12*) and then measuring the rate of appearance of tracer on the unlabeled side after sufficient time had elapsed for the specific activity of the protoplasm to become constant.

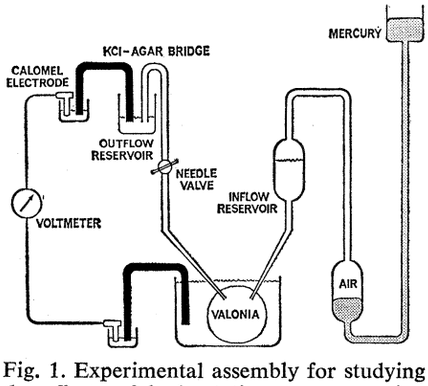

Fig. 1. Experimental assembly for studying the effects of hydrostatic pressure on ion transport and electrical potentials in *Valonia ventricosa*. Not shown are the micromanipulators holding the inflow and outflow pipettes, and a dissecting microscope which supports the cell chamber.

Figure 1 shows schematically the experimental arrangement for measuring solute fluxes in the presence and absence of an applied hydrostatic pressure. Pressure was applied to the vacuole by a mercury manometer attached to the inflow reservoir, and the rate of perfusion was controlled by a needle valve on the outflow tube. The potential difference across the protoplast was monitored continuously and provided a sensitive indicator of the condition of the cell. Serious leaks which developed occasionally caused a rapid drop in the potential difference to zero. The normal vacuole potential in illuminated cells of *V. ventricosa* ranges from +10 to +30 mv, the vacuole being positive to external seawater (*9*). Cells were illuminated (3000 to 4000 lux) throughout these experiments, and the temperature was 25° ± 1°C.

One atmosphere of hydrostatic pressure always caused a marked decrease in potassium influx, and the effect was always reversible (Fig. 2 and Table 1) (*13*). The efflux of potassium, on the other hand, was apparently increased by 1 atm of hydrostatic pressure, but the effect was not so large as the effect on potassium influx (Table 1). The precise time course of the change in potassium flux is uncertain (indicated by the broken line in Fig. 2) because of the large dead space in the cell vacuole (about 1 cm^3) and the low rate of perfusion. For comparison, the influx of a nonelectrolyte, urea (*12*), was measured in two cells. Urea influx apparently was not affected by hydrostatic pressure, however (Table 1).

Collectively these results indicate that the net uptake of potassium by *Valonia* is stimulated by abolishing the turgor pressure, which is essentially what Jacques (*6*) reported. Before concluding that pressure directly affects the mechanism of potassium transport in *Valonia*, however, we must consider two alternative explanations. First, applied pressure might mechanically damage the cell surface, either by stretching the membranes or by creating leak pathways around the micropipettes. Second, a change in hydrostatic pressure alters the chemical potential of water and, consequently, the rate of water flow across the protoplast. This might affect ion movements if there were frictional interactions between ions and water crossing the protoplast. With regard to possible mechanical effects, Villegas (*14*) showed that the wall of *V. ventricosa* stretches slightly under a 1-atm pressure, but the surface area increases less than 1 percent. Furthermore, any serious mechanical damage to the cell surface would have been indicated electrically and by a change in the urea flux. With regard to possible interactions between ions and water crossing the protoplast, in several experiments I reversed the direction of water flow by adding 200 m*M* mannitol (about 5 atm of osmotic pressure) to the external solution. This reversal of water flow had no obvious effect on potassium fluxes in either the presence or absence of applied hydrostatic pressure; this finding agrees with my previous results indicating an absence of solute-solvent interactions in the membranes of *Valonia* (*10, 15*).

Table 1. Effect of hydrostatic pressure on potassium and urea fluxes in *Valonia ventricosa*. Values are the means ± standard errors; in parentheses are the numbers of cells. The flux units are 10^{-12} mole cm^{-2} sec^{-1}. ΔP is the difference in hydrostatic pressure between the inside and outside of the cell.

Flux	$\Delta P = 0$ atm	$\Delta P = 1$ atm
Potassium influx	68 ± 29 (5)	22 ± 9 (7)
Potassium efflux	16 ± 4 (3)	29 ± 11 (3)
Urea influx	9.7 (1)	9.8 (1)
Urea efflux	13 (1)	15 (1)

The simplest interpretation of my results, therefore, is that hydrostatic pressure directly affects the mechanism

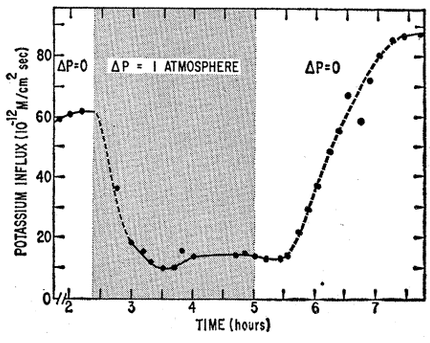

Fig. 2. Effect of hydrostatic pressure on potassium influx in a single cell of *Valonia ventricosa*.

responsible for active uptake of salt in *Valonia*. A possible function of this effect of pressure would be to enable a growing cell to maintain a rather constant turgor pressure and, if other environmental factors were favorable, a constant rate of expansion. Cells subjected to changing salinities would especially benefit by having a feedback mechanism for controlling their turgor pressures. The mechanism by which pressure might affect salt transport is not clear, however, and offers an intriguing area for further study.

JOHN GUTKNECHT*
School of Biological Sciences,
University of East Anglia,
Norwich, England

References and Notes

1. J. Dainty, *Annu. Rev. Plant Physiol.* **13**, 379 (1962).
2. A. Rothstein, in *The Cellular Functions of Membrane Transport*, J. F. Hoffman, Ed. (Prentice-Hall, Englewood Cliffs, N.J., 1964), p. 23.
3. J. Gutknecht and J. Dainty, in *Oceanography and Marine Biology: Annual Review*, H, Barnes, Ed. (Allen and Unwin, London, in press).
4. H. Kesseler, *Kiel. Meeresforsch.* **14**, 23 (1958); *ibid.* **15**, 51 (1959); *Proc. Marine Biol. Symp. Botanica 5th Gothoburgensia* **3**, 103 (1965).
5. L. R. Blinks, *J. Gen. Physiol.* **18**, 409 (1935).
6. A. G. Jacques, *ibid.* **22**, 147 (1938); *ibid.*, pp. 743, 757 (1939); *ibid.* **23**, 41 (1939).
7. D. C. Spanner, *Introduction to Thermodynamics* (Academic Press, New York, 1964).
8. F. H. Johnson, J. Eyring, M. J. Polissar, *The Kinetic Basis of Molecular Biology* (Wiley, New York, 1954).
9. J. Gutknecht, *Biol. Bull.* **130**, 331 (1966).
10. ———, *J. Gen. Physiol.* **50**, 1821 (1967).
11. ———, *Science* **158**, 787 (1967).
12. The approximate concentrations (millimoles per liter) of major ions in the seawater were Na, 505; K, 11; and Cl, 600. The concentrations of major ions in the artificial sap were Na, 50; K, 615; and Cl, 650. The artificial sap contained 1 part seawater and 9 parts 0.67*M* KCl. Potassium fluxes were measured with K^{42}. Urea labeled with C^{14} was used in two experiments at an external concentration of 10 mmole/liter.
13. Pressures were applied and released gradually over a 10-minute period; sudden changes in pressure sometimes caused aggregation of the chloroplasts. The hydrostatic pressure of the perfusion system alone was about 15 cm of water, which was negligible compared to the experimental pressure of 1 atm.
14. L. Villegas, *Biochim. Biophys. Acta* **136**, 590 (1967).
15. J. Gutknecht, *Biophys. J.* **8**, A-126 (1968).
16. I thank Drs. J. Dainty, J. F. Thain, and N. A. Walker for helpful suggestions. I thank Miss Anitra Thorhaug for supplying the *Valonia* and Mrs. Lyn Barber for technical assistance. Supported in part by PHS postdoctoral fellowship GM-13, 804.
* Present address: Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27706.

2 February 1968

Ciguatoxin: More Than an Anticholinesterase

Abstract. *Anticholinesterase action of ciguatoxin has been reported. A study of the pharmacology of the action of the toxin on the respiratory system suggests that additional properties may be involved in the respiratory failure—the usual cause of death in ciguatera poisoning.*

Although anticholinesterase activity (*1, 2*) and the general parasympathomimetic properties of ciguatoxin (CT) have been described (*3*), there is some doubt that the entire action of this toxin can be explained so simply. Banner (*4*) has pointed out that, in some of the earlier reported cases, symptoms of ciguatera poisoning were alleviated when atropine or neostigmine was given (although the latter might be expected to potentiate the effects of an anticholinesterase). We now report on the effects of CT, with particular reference to the respiratory actions of the toxin which apparently cause death in ciguatera by asphyxia (*1*).

Female albino rats (180 to 250 g) were anesthetized with pentobarbital sodium (25 μg per gram of body weight). Respiratory movements were recorded on a Grass polygraph with a thin wire hook in the diaphragm connected to a Grass force-displacement transducer (FTO3C); one jugular vein was cannulated for intravenous injection. The animal was prepared for artificial respiration by cannulation of the trachea; arterial blood pressure was monitored from one common carotoid with a Statham P23 pressure transducer (*5*). Absolute quantities of toxin injected cannot be compared because the activity of the crude methanol extracts of CT varies considerably, depending on the toxicity of the fish from which it was extracted. Intravenous dosages were normally between one-tenth and one-half of the intraperitoneal LD_{50} (lethal dose for 50 percent of animals tested) determined for each batch of extract used, and are thus in the low lethal range for intravenous injection. The toxic oil, prepared by the method of Scheuer *et al.* (*2*), was injected as an emulsion in human serum; equivalent quantities of human serum had no effect on respiration.

Within 5 to 10 seconds after CT was injected, a brief (2 to 5 seconds) increase in respiratory frequency occurred, and was followed by apnea lasting about 8 to 20 seconds (Fig. 1A), after which the amplitude of diaphragm movements sometimes increased again. With smaller doses, a transitory increase in frequency sometimes occurred, whereas with larger doses this phase was short or absent, and slowing of the respiratory rhythm was observed. As the rhythm slowed, the movements took on a gasping appearance, and the whole respiratory musculature became involved. The time from initial apnea to final respiratory failure depended on the dosage. With very large intravenous doses of CT there was no recovery from the initial apnea.

When the vagi were sectioned before injection of CT, the apneic phase did not occur. However, the initial increase in frequency, the subsequent slowing of the respiratory rhythm, and the time of final respiratory failure were not affected. The effects of prior vagotomy on tidal volume after CT injection were complex. At low doses, tidal volume decreased (Fig. 1B) rather than increased; when doses were increased this effect was reversed. Very large doses (twice the intraperitoneal LD_{50}) caused a marked increase in tidal volume within 5 seconds of injection, and respiration ceased 20 to 30 seconds later.

The pattern of respiratory failure caused by the anticholinesterases physostigmine (0.5 to 1.5 μg/g) and paraoxon (1.5 to 7.5 μg/g) showed only the most superficial similarities to the effects of CT. The initial progressive reduction in tidal volume became, at the larger doses, so pronounced as to constitute an apneic period followed by gradually increasing, irregular gasping movements. However this apnea had a longer latency (25 to 35 seconds as opposed to 5 to 15 seconds with CT) and lacked the sudden onset characteristic of the initial apnea produced by CT. Bilateral vagotomy, rather than suppressing the apnea, enhanced the reduction in tidal volume, thereby producing an apneic phase at dosage (0.5 to 1.0 μg of physostigmine per gram of body weight) at which this is not normally seen. Increasing the dosage did not reverse the apnea.

The final respiratory failure occurring after both CT and physostigmine is not a peripheral neuromuscular effect, as has been shown by stimulating the phrenic nerve with single maximum shocks at intervals during the experiment. There was some increase in twitch height, followed by reduction of the