action, and that aminoacetonitrile inhibits the activity of these enzymes.

Further experiments will be made in order to see if this assumption can be supported. We intend also to establish whether AAN inhibits the demethylation of DMNA in vitro.

Institute of General Pathology,

S. Roffia

L. FIUME

G. Ciamician Institute of Chemistry, University of Bologna, Italy.

¹ Fiume, L., and Favilli, G., *Nature*, **189**, 71 (1961). ² Fiume, L., J. Path. Bact., **83**, 291 (1962).

- ³ Fiume, L., and Laschi, R., Lo Sperimentale, 113, 193 (1963).
- ⁴ Fiume, L., J. Path. Bact., 84, 256 (1962).
- ⁵ Fiume, L., Nature, 197, 394 (1963).
- ⁵ Flume, L., Nature, 197, 394 (1963).
 ⁶ Flume, L., Lo Sperimentale, 112, 865 (1962).
 ⁷ Magee, P. N., in Cancer Progress Volume, 56, edit. by Raven, R. W. (Butterworths, London, 1963).
 ⁸ Magee, P. N., and Lee, R. Y., Ann. N.Y. Acad. Sci., 104, 916 (1963).
 ⁹ Mizrahi, I. J., and Emmelot, P., Cancer Research, 22, 339 (1962).
 ¹⁹ Craddock, V. M., and Magee, P. N., Biochem. J., 89, 32 (1963).

- ¹¹ Magee, P. N., Biochem. J., 70, 606 (1958).
- ¹² Fiume, L., Nature, 201, 615 (1964).
- ²³ Heath, D. F., and Jarvis, J. A. E., Analyst, 80, 613 (1955).
- 14 Magee, P. N., Biochem. J., 64, 676 (1956).
- ¹⁵ Magee, P. N., and Vanderkar, M., Biochem. J., 70, 600 (1958).

Transport of Sodium and Water by Rabbit lleum, in vitro and in vivo

PREVIOUS studies from this laboratory^{1,2} have indicated a deficiency of Na⁺ ion absorption from the gastro-intestinal tract in cholera¹, and an inhibition of Na⁺ transport in the short-circuited frog skin in the presence of cholera stool. We are studying animal models to eluci-date intestinal mucosal cell function in cholera. A difference in function has been noted when Na⁺ ion transfer in the everted intestinal sac in vitro was compared with data from loops of the adult rabbit in vivo.

Table 1 shows typical data obtained with the everted rabbit ileal sac. It is evident that glutamine markedly stimulates water transport and increases the unidirectional and net transport of Na⁺ ion. Although these data are from experiments specifically designed to compare the in vitro and in vivo preparations, many closely related experiments have consistently shown this stimulation in the presence of glutamine. The data in Table 1 suggest a modest stimulation of net Na⁺ ion and water transport when glucose is added; this stimulation by glucose has been observed only during the late spring and summer months. Repeated efforts during the colder winter months failed to demonstrate any stimulation.

The data in Table 2 clearly show that in the rabbit ileal loop in vivo, glucose markedly stimulates water and sodium transport, while glutamine does not. Since the failure of glutamine to stimulate net water and Na⁺ ion transport was so unequivocal, no studies of unidirectional fluxes were made in the presence of glutamine.

It should be noted that the ionic composition of the in vitro and in vivo media is not identical. The reason for this is that these two experimental models were designed in a totally separate manner and the optimal conditions for each were evolved with no original intention of comparison. Also, the fact that calcium ion was not added to the *in vitro* system and was presumably supplied by the blood to the in vivo system leaves unanswered in these experiments the possible role of calcium in sodium and water transport.

In comparing the data in vitro and in vivo it appears that water transport is greater in the loop than in the sac. Glutamine in vitro and glucose in vivo appear to exert their action on sodium transport by augmentation of the 'mucosa to serosa' unidirectional flux. It is of some interest that the sodium flux rates from 'mucosa to serosa'

Table 1. SODIUM AND WATER TRANSPORT BY EVERTED ILEAL SACS in vitro H.O transport Sodium transport (umoles/cm²/h)

babbA	(mmoles/cm ² /h)	pourant oranoport (pintoroo) one (m)		
substrate	M→S	$M \rightarrow S$	$S \rightarrow M$	Net $M \rightarrow S$
None (6)	0.6 ± 0.07	$9{\cdot}2\pm0{\cdot}78$	$8 {\cdot}7 \pm 0 {\cdot}65$	$0{\cdot}5\pm0{\cdot}13$
Glucose (6) Glutamine (6)	$\begin{array}{c} 0.7 \pm 0.21 \\ 1.2 \pm 0.15 \end{array}$	$\begin{array}{c} 9 \cdot 2 \pm 0 \cdot 34 \\ 12 \cdot 1 \pm 1 \cdot 03 \end{array}$	$\begin{array}{c} 8{\cdot}0\pm 0{\cdot}55\\ 8{\cdot}9\pm 0{\cdot}90 \end{array}$	${}^{1\cdot2}_{3\cdot2}{}^{\pm}_{\pm}{}^{0\cdot09}_{0\cdot34}$

Glutamine (6) $1\cdot 2\pm 0\cdot 15$ $12\cdot 1\pm 1\cdot 03$ $8\cdot 9\pm 0\cdot 90$ $3\cdot 2\pm 0\cdot 34$ The serosal and mucosal solutions for the everted sacs of rabbit ileum contained the following electrolytes, in m.equiv./l.: Na⁺, 163; K⁺, 4; Mg⁺⁺, 0.7; Cl-, 70; HCO₃⁻, 17; HPO₃⁻⁻, 20. Mannitol 30 mM was added to adjust the osmolality to 290 milliosmols. The concentration of added glucose and glutamine was 20 mM; the control solutions contained an additional 20 mM mannitol. The pH was 8.0 and the gas phase was oxygen. The volume of mucosal solution was 25 ml; the serosal solution was about 3 ml. Tracer quantities of ²⁴Na and ²⁴Na were added to the serosal and mucosal solutions respectively. Water movement was determined gravi-metrically. The sacs were placed in 125-ml. flasks and shaken for 1 h in a 37° water bath. Flgures in parentheses indicate the number of different animals on which the studies were performed. Studies were done in duplicate on each animal. Variations are expressed as standard errors. Note that water movement is about 1,000 times greater than that of sodium.

Table 2. SODIUM AND WATER TRANSPORT BY ILEAL LOOPS in situ H₂O transport Addad Sodium transport (umole/cm²/b)

nauca	(mmores) on /)	a) bouran	manapore (pm	0.0701011111111111111111111111111111111
substrate	M-→S	· M→S	S→M ¨	Net $M \rightarrow S$
None (6)	12.3 ± 0.51	8.8 ± 0.13	4.6 ± 0.15	4.2 ± 0.14
Glucose (6)	19.5 ± 0.39	12.1 ± 0.17	4.8 ± 0.06	7.1 ± 0.10
Glutamine (4)	11.1 ± 3.9			4.3 ± 0.16
		1 1	- · · · · · · · · · · · · · · · · · · ·	#1

The solution introduced into the lumen of loops of rabbit ilcum in vivo contained the following electrolytes, in m.equiv./l.: Na⁺, 140; K⁺, 10; Cl⁻, 105; HCO₇, 35. The concentration of added glucose and glutamine was 25 mM. The *p*H of this solution was 8-0. 5-ml. volumes, at 37° C, were placed in the loops for 15 min. The animal was labelled with ²²Na and tracer quantities of ²⁴Na were added to the mucosal fluid.

are nearly identical for the two preparations. The 'serosa to mucosa' rate is much lower in the in vivo preparation and the net flux is therefore greater, even in the absence of added glucose or glutamine.

The mechanism for this disparity of stimulation by added substrates in vivo and in vitro remains unexplained. Rabbit ileum vigorously oxidizes carbon-14 labelled glutamine to CO₂ in vitro, but it also converts labelled glucose to CO₂, although at a lesser rate (unpublished data). Therefore, there is no question of limited permeability to glucose in vitro. Presumably the permeability of ileum to glutamine should be at least as adequate in vivo as in vitro.

Although no mechanism has been elucidated, these data indicate the necessity for the exercise of considerable caution in extrapolating data obtained from the everted sac in vitro to the preparation in vivo.

This study was supported in part by funding under Public Law 480, Section 104(c). The opinions and assertions contained herein are ours and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

А.	Н.	G.	LOVE

- T. G. MITCHELL
- E. M. NEPTUNE, JUN.
- U.S. Naval Medical Research Unit No. 2,

Box 14, APO San Francisco.

¹ Phillips, R. A., Blackwell, R. Q., Wallace, C. K., and Huber, G. S., Fed. Proc., 22 (1963).

² Huber, G. S., and Phillips, R. A., SEATO Conference on Cholera, Dacca, East Pakistan, 37 (National Institutes of Health, 1962).

PHYSIOLOGY

High-precision Repetitive Firing in the Insect Optic Lobe and a Hypothesis for its Function in Object Location

WE have encountered, in the optic lobe of the insect Calliphora erythrocephala, a layer of elements firing spontaneously at a very constant rate of about 50 spikes per sec. The observed waveform depends on the position of the pick-up electrode and on the temperature, but in a given spike-train the rise-time is constant within 20 µsec. A photograph of a typical spike is shown in Fig. 1. In these experiments (more than a hundred), the animals were intact except for a small window cut in the back of the head, and were able to survive for many hours.