micrographs (*9*). If we attribute the core resistance entirely to the junctional membranes and take the area of electrical junction of a cell pair as 5 $\mu^2$ (*10*), we obtain 0.02 ohm-cm$^2$ for the specific resistance of junctional membrane. The equivalent specific core resistance is 180 ohm-cm.

## IV

*General aspects.* The specific core resistances along the chains of interconnected cells in the renal, salivary gland, and bladder epithelia are not substantially greater than those of cytoplasm alone. This underlines the absence of significant barriers to diffusion, of at least some ionic species, in the cell-to-cell direction along the chains. Diffusion in the interior-to-exterior direction, on the other hand, is limited by a strong barrier all along the surface of the cell chain, as is shown by the high surface resistances in these epithelia (Table 1).

The four epithelia described here, and the gland cell epithelium described earlier (*1*), all seem to conform to a common scheme: a cell system with a fairly continuous interior, at least as far as much of its ion content is concerned, bounded by a diffusion barrier which extends and is continuous along the entire external surface of the system. The system rather than its individual cell components constitutes the unit of ion environment. There are differences in spatial arrangement of the systems in the various epithelia. In the salivary gland and renal epithelia, communication between a cell and any of its nearest neighbors is equally good, and this one system thus comprises virtually the entire epithelium. In the bladder and sensory epithelia, on the other hand, connections are more restricted, resulting in tortuous channels in the former, with, perhaps, several such systems arranged in parallel.

At the structural level these epithelia have one feature in common: their cell membranes are closely joined at their surfaces of contact. In salivary gland (*11*) and Malpighian tubular cells (*12*), the membranes are joined periodically, and in bladder cells (*10*) they are joined at a certain region of their surfaces of contact (*13*). These junctional regions are likely to be the coupling elements which permit diffusion from cell to cell.

The general functional adaptation of intercellular communication is probably one that allows the cell system to operate in concert. Ions, metabolites, and, perhaps, hormonal and genetic information may conceivably flow from one cell to another, with economy of external nervous and humoral controls (*1*). A special variant shows itself in the sensory epithelium. Here intercellular communication appears to have adapted to a properly electrical role, namely, to transferring and synchronizing excitation over various sensory units. The connected system acts as a signal amplifier in this case.

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### References and Notes

1. W. R. Lowenstein and Y. Kanno, *J. Cell Biol.* **22**, 565 (1964).
2. We are indebted to Dr. G. Palade, Rockefeller Institute, who suggested to us the toad urinary bladder as a convenient material.
3. For details of technique, calculations, and definitions, see W. R. Loewenstein and Y. Kanno (*1*).
4. A. Barets and T. Szabo, *J. Microscop.* **1**, 47 (1962); V. B. Wigglesworth and M. M. Salpeter, *J. Insect Physiol.* **8**, 229 (1961).
5. W. R. Loewenstein, *Nature* **188**, 1034 (1960).
6. Part of this work was done at the Estación de Biología Marina, Universidad de Chile, Montemar. The University of Chile and the director of the Marine Biological Station provided most generous hospitality and facilities. We are indebted to them.
7. A major difficulty was the fragility of the cell membranes in the urinary bladder epithelia. The membranes often ruptured upon penetration. In such a connected system this, of course, ended the experiment. Hence the number of cell chains which could be identified, as well as the number of experiments which could be completed, was severely limited. Acceptable results were obtained only with electrodes of especially fine tips, and then only at the expense of a large number of trials. The bladder was kept at about 8°C to minimize smooth muscle movement. For reviews of physiological studies on the bladder, see A. Leaf, *Gen. Comp. Endocrinol.* **2**, 148 (1962); H. Frazier, *J. Gen. Physiol.* **45**, 515 (1962); and Peachey and Rasmussen (*8*).
8. L. D. Peachey and H. Rasmussen, *J. Biophys. Biochem. Cytol.* **10**, 529 (1961).
9. L. D. Peachey provided the electron micrographs used for surface estimates of bladder epithelial cells. We are indebted to him.
10. The "electrical" junction is taken here as a band of 0.5 × 10 $\mu$ which appears as tight intercellular junction (*zona occludente*) in electron micrographs of G. Palade (personal communication), of L. D. Peachey (personal communication) (see also *8*), and of J. K. Choi [*J. Cell Biol.* **16**, 53 (1963)].
11. J. Wiener, D. Spiro, W. R. Loewenstein, *J. Cell Biol.* **22**, 587 (1964).
12. I. Tsubo and P. W. Brandt, *J. Ultrastruct. Res.* **6**, 28 (1962).
13. Structural details of the junctions in the sensory epithelium of Lorenzinian ampullae are not known to us.
14. Compare, for example, P. Fatt and B. Katz, *J. Physiol.* **120**, 171, 177 (1953).

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# Medial Neurosecretory Cells as Regulators of Glycogen and Triglyceride Synthesis

Abstract. *In the female mosquito, medial neurosecretory cells restrict synthesis of glycogen from sugar and stimulate triglyceride synthesis. Removal of these cells greatly increases the storage capacity for glycogen at the expense of triglyceride storage.*

When emerging female mosquitoes are starved, glycogen and triglycerides virtually disappear and subsequent feeding produces new pools of these products (*1*). In the experiments described here, we used this technique to study the role of the neurosecretory system in glycogen and triglyceride metabolism.

Three species, *Aedes taeniorhynchus*, *A. sollicitans*, and *A. aegypti*, were reared as described previously (*2*). In mosquitoes, two groups (12 to 15 cells each) of medial neurosecretory cells lie in a cluster on either side of the midline of the protocerebrum, easily distinguishable by their bluish appearance. Within 1 hour after emergence of the mosquitoes, these cells were surgically removed along with a small amount of surrounding tissue. The technique was the same as that described by Thomsen for the blowfly *Calliphora erythrocephala* (*3*), except that the head capsule was sealed by drying with air.

The medial neurosecretory cells do not affect the utilization of glycogen or fat, because, when experimental and control animals were starved, glycogen and triglycerides disappeared in the same time. Starved mosquitoes were fed, in a single dose, 2 $\mu$l of a solution of equal parts of glucose and fructose from a micropipette; the insects were

maintained at 27°C on a light-dark cycle of 12 : 12 (hours) and were given access to water only. At intervals of 0, 4, 8, 16, 24, 48, and 72 hours, at least six mosquitoes were killed and analyzed individually for glycogen (*4*) and triglycerides (*5*). These techniques allow quantitative estimation of as little as 0.01 mg of each of the components (*6*). Because carbohydrate and fats are not comparable on a weight basis, all values are expressed as calories per mosquito: 1 mg of carbohydrate equals 3.7 cal and 1 mg of triglyceride equals 9 cal. Immediately before the mosquitoes were fed, neither of these components exceeded 0.1 cal. All data are expressed as net changes.

In the control *A. taeniorhynchus* fed 4 cal of sugar, 0.25 to 0.30 cal of glycogen was deposited in 6 to 8 hours (Fig. 1*A*) and the excess sugar was used for triglyceride synthesis (Fig. 1*B*) rather than for additional glycogen accumulation. When the controls were fed 1 cal of sugar, the same amount of glycogen was deposited in the same time, so that limitation of the glycogen pool after the controls were fed 4 cal of sugar was not due to lack of sugar for synthesis. When the medial neurosecretory cells were removed, glycogen synthesis did not slow down within 6 to 8 hours in insects fed 4 cal of sugar, but continued at the same rate for 24 hours. This proves that in the normal mosquito the size of the glycogen pool is limited by the medial neurosecretory cells; concurrently, fat synthesis is greatly depressed. Glycogen synthesis in the experimental mosquitoes paralleled triglyceride synthesis in the controls, so that within 48 hours both had stored the same amount of caloric reserves (Fig. 1).

Most experiments were performed with *Aedes taeniorhynchus*, but similar effects were observed in *A. sollicitans* and *A. aegypti*. For the last two species, only data obtained 48 hours after feeding are presented (Table 1, experiments f and g) because in all three species this time interval appeared to be a maximum for triglyceride storage in controls and for glycogen storage in experimental mosquitoes.

In experimental *A. taeniorhynchus* fed 4 cal of sugar, the maximum amount of glycogen appeared in 48 hours (Fig. 1*A*). If at this point a second dose of sugar was fed, fat synthesis was not resumed, but glycogen accumulation was greatly increased (Table 1, experiment d).

Table 1. Effect of removal of medial neurosecretory cells (MNC) on net increases of glycogen and triglycerides in three mosquito species 48 hours after they were fed 4 cal (2 $\mu$l, 55 percent) of sugar.

| Experiment | Treatment | *N* | Net increase ± S.E. (cal/mosquito) | |
|---|---|---|---|---|
| | | | Glycogen* | Triglyceride† |
| | *Aedes taeniorhynchus* | | | |
| a | Both groups of MNC removed before feeding (from Fig. 1) | 14 | 1.1 ± .09 | 0.34 ± .03 |
| b | One group of MNC removed before feeding | 12 | 0.23 ± .04 | 1.2 ± .12 |
| c | Controls | 17 | 0.20 ± .03 | 1.3 ± .09 |
| d | Both groups of MNC removed before feeding, fed 4 cal of sugar 48 hours after first feeding, and examined 44 hours later. | 6 | 1.9 ± .10 | 0.30 ± .04 |
| e | Both groups of MNC removed 21 hours after feeding | 9 | 0.55 ± .05 | 0.66 ± .12 |
| | *Aedes sollicitans* | | | |
| f | Controls | 5 | 0.50 ± .10 | 1.20 ± .20 |
| | Experimental | 5 | 1.40 ± .20 | 0.62 ± .07 |
| | *Aedes aegypti* | | | |
| g | Controls | 15 | 0.24 ± .02 | 1.25 ± .09 |
| | Experimental | 12 | 0.48 ± .04 | 0.61 ± .09 |

* Milligrams × 2.7. † Milligrams × 9.

In control *A. taeniorhynchus* fed 4 cal of sugar, the amount of glycogen decreased after 21 hours (Fig. 1*A*). If at this point a second dose of sugar was fed, glycogen synthesis was not resumed, but fat accumulation was greatly increased; if, instead of feeding a second dose of sugar, the medial neurosecretory cells were removed after 21 hours, glycogen synthesis was resumed at the expense of further growth of the fat pool (Table 1, experiment e). This shows once more that, in normal mosquitoes fed with sugar, synthesis of glycogen is limited and synthesis of triglycerides stimulated by the neurosecretory cells.

Three experiments show that the effects on metabolism were not due to unspecific brain damage. (i) In mosquitoes from which either the right or the left group of medial neurosecretory cells was extirpated along with adjacent brain tissue, metabolism was not different from that of controls. (ii) In mosquitoes with both groups of cells intact, but with bilaterally ablated brain tissue (ablated as close to the neurosecretory cells as was technically feasible), metabolism was no different from that of controls (*7*). (iii) In mosquitoes with the combined nervus corporis allati and oesophagi sectioned bilaterally at the point where they enter the neck, metabolism was the same as in mosquitoes from which both groups of medial neurosecretory cells had been extirpated. Cutting the nerves severs the medial

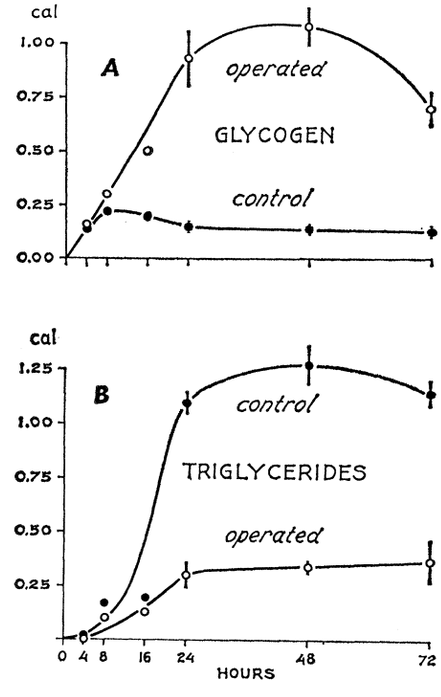


Fig. 1. Effect of removal of medial neurosecretory cells on net changes of glycogen and triglycerides in female *Aedes taeniorhynchus* fed 4 cal (2 $\mu$l, 55 percent) of sugar. Points represent averages of 6 to 17 individuals. Vertical bar represents 2 standard errors (1 cal glycogen = 0.27 mg; 1 cal triglycerides = 0.11 mg). The controls consisted of insects not operated on or insects from which either the left or right group of medial neurosecretory cells had been removed. The curve labeled "operated" is for those insects from which both right and left groups of MNC were removed.

neurosecretory cells from their axons, but the brain and the perikaryon of the cells are left untouched.

The results of experiment iii may account for our failure to restore the metabolism to normal by implantation of medial neurosecretory cells and should caution against the premature postulation of the involvement of a hormone.

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#### References and Notes

1. E. Van Handel and P. T. M. Lum, *Science* **134**, 1979 (1961); E. Van Handel, *J. Physiol.*, in press.
2. A. O. Lea, *J. Insect Physiol.* **9**, 793 (1963).
3. E. Thomsen, *J. Exptl. Biol.* **29**, 137 (1952).
4. A. E. Osterberg, *J. Biol. Chem.* **85**, 97 (1929).
5. E. Van Handel, *J. Clin. Chem.* **7**, 249 (1961).
6. ———, *Anal. Biochem.* **11**, 266 (1965).
7. A remote possibility remains that the results are due to ablation of cells so close to the medial neurosecretory cells that our present surgical techniques cannot differentiate between them.
8. Supported by NIH grants (AI-05054, AI-03112). The assistance of J. E. Guira and D. G. Evans is gratefully acknowledged.

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## Growth Rate of Giant Clam *Tridacna gigas* at Bikini Atoll as Revealed by Radioautography

Abstract. *At Bikini Atoll, radioactivity from strontium-90 deposited in the growing shell of a giant clam, presumably during the testing of nuclear weapons in 1956 and 1958, produced unmistakable lines on radioautographs made from transverse sections of the shell. The regular banding seen in the sections is interpreted as annular in nature. One annulus precedes the 1956 layer of radioactivity, two intervene in 1958, and six follow to the time of collection, so that this clam (length, 52 centimeters) was in its 9th year of life.*

Written records of the giant clam *Tridacna gigas* Linné have existed for centuries. Considered remarkable at first simply because of its large size (2 m in greatest length and several hundred kilograms in weight), the clam was later (*1*) found to contain symbiotic algae within its tissues. Yonge proposed that the clam attained such size by "farming" the zooxanthellae within the greatly expanded tissues of the siphons, and by utilizing the photosynthetic products in nutrition (*2–4*). However, in spite of an almost universal curiosity about the age of these giants of the coral reefs, no studies on their rate of growth have been reported. The rate of growth of *T. gigas* over a period of several years can now be estimated by reading the annulations in the shell and relating them to marks introduced by radioactivity from nuclear detonations.

Although the ability of *T. gigas* to concentrate $Co^{60}$ in its soft parts has been emphasized (*5*), little is known concerning the uptake of radionuclides by the shell, which is shown here to contain $Sr^{90}$. To elucidate the pattern of deposition of nuclides in the shell after nuclear detonations, one valve of a specimen 52 cm in length (*6*) from Bikini Atoll was transversely sectioned with a 51-cm circular diamond saw. Figure 1 shows a section, 6 mm in thickness, from the region immediately anterior to the umbo. Figure 2 shows a radioautograph resulting from exposing the section to "No Screen" x-ray film for a period of 3 months. Two lines each about 2 mm wide, representing layers of radioactive material, appeared on the film. Other sections farther from the umbo also showed these marks. Records (*7*) reveal that tests of nuclear devices were conducted at Bikini Atoll only in 1946, 1954, 1956, and 1958. It is reasonable to attribute the layers of radioactivity to the two most recent test series. The 1956 Redwing series at Bikini extended from 20 May through 20 July, and the 1958 Hardtack series from 11 May through 22 July.

The positions of the layers containing radioactivity were determined by superimposing the radioautograph on the shell section, and are shown as stippled lines in Fig. 1, top. This view by transmitted light accentuates the conspicuous alternating dark, relatively opaque, layers, as contrasted with the lighter, more translucent bands, clearly indicating apparent years of age. Up to the 1956 line the clam was in its first year of life. Two years intervene between the two stippled lines, to 1958, and then six more years to the inner surface of the shell representing 1964, so that the clam was in its 9th year. The 1956 line corresponds to a shell length of about 10 cm, and the 1958 line, to about 24 cm.

It is of special interest that a tropical organism living in water with a mean monthly temperature varying less than 3°C (*8*) throughout the year should display distinct annulations. Seasonally varying environmental factors other than temperature, such as winds, currents, weather, light, and the abundance of planktonic food, could influence growth. At Bikini Atoll the relatively constant winter trade winds from the east are frequently interrupted in summer by other winds, particularly from the south (*9*), and surface currents would be similarly influenced.

Spawning is probably of a seasonal nature and thus may influence shell growth. Yonge (*2*) cites the spawning of the closely related genus *Hippopus* in January of the Australian summer and gives 30°C as the minimum temperature for spawning of the giant clam (*3*). Wada (*10*) reported that *Tridacna* collected in the Palau Islands in April, May, and June of 1938, 1940, and 1941 frequently discharged sperm and eggs when brought into the laboratory, although he said nothing of those collected in other seasons.

During growth, new shell material is added exclusively on the inside. Although the mantle is attached only at the pallial sinus, it contacts and deposits new material (aragonite) upon the entire inner surface of the shell. The extrapallial portion of the shell, distal to the pallial sinus and comprising about half of the total inner surface, is prismatic, while the central basal part of the shell is nacreous. In macroscopic views of sections (Fig. 1, bottom) the distal, prismatic part is relatively opaque and shows only faint layering; the central, nacreous part is more translucent and distinctly layered. The two areas are clearly demarcated by a boundary layer leading from the basal edge of the existing pallial sinus obliquely through the shell toward the umbo at the base (Fig. 1, *P*).

Figure 3 shows a low-power photomicrograph obtained by using crossed polaroid discs of a thin (15 to 20 $\mu$) shell section at the position indicated by the dashed lines of Fig. 1 (top). Although Fig. 3 shows the outer border of the shell at the upper left, it does not extend to the inner border. The prismatic outer layer occupies the first and most of the second column of photographs down to the sloping light area which is the pallial layer marked *P*, while the rest of column 2 and all of columns 3 and 4 consist of nacre. The prismatic layer is composed of vertical columns about 45 $\mu$ in thickness disposed normally to the outer surface of the shell. The nacreous layers below are more irregular, with only slight or localized indications of vertical striae, but with both primary, coarse layering and fine striations oriented approximately parallel to the inner shell