tion against the two neuropharmaca but not against the detergent. At the same time, monosialo-ganglioside and monosialo-ganglioside-like fractions from the blastulae were not active against neuropharmaca, but showed protective action against the detergent OP-10. The effective protective concentrations of the gangliosides are given in the Table. These concentrations are from 20 to 1000 times lower than the toxic concentrations of the neuropharmaca and 10–20 times lower than the concentrations of the detergent. At the same time, the di- and trisialoganglioside-like fractions isolated from sea urchin embryos were not effective either against neuropharmaca or against the detergent OP-10.

The factor decreasing the sensitivity of embryos to neuropharmaca was shown also to be present in the chloroform-methanol extract of conditioned sea water (CSW), i.e. sea water, in which dense suspensions (about 30,000 embryos/ml) of *S. intermedius* embryos were incubated.

TLC of the CSW chloroform-methanol extract revealed the presence of resorcine-positive substances coinciding, by their Rf-values, with the hematoside-like fraction of sea urchin embryos and with rat liver hematoside (Figure). We found that the sialic acid concentration in CSW was about 1×10^{-5} *M*. Neither the gangliosides from the sea urchin embryos or from rat liver nor the chloroform-methanol extract of the CSW or CSW itself affected the sensitivity of the embryos to puromycin. In previous experiments[5], it was shown that the sensitivity of the embryos to this antibiotic did not depend on their concentration (in the concentration interval from 10^2 to 10^4 embryos/ml); consequently the An_1- and A_2-factors were not effective in this case.

As can be seen from these results, the An-factors may comprise either the gangliosides themselves (i.e. An_1-hematoside, An_2-monosialoganglioside) or mixtures containing these gangliosides. To solve this problem it will be necessary to estimate the concentration of the gangliosides in CSW. Such experiments may be of interest in studies of the biological role of gangliosides. They also could be helpful for understanding the intracellular regulatory functions of acetylcholine and monoamines[13].

ВЫВОДЫ. Чувствительность ранних эмбрионов морских ежей к эмбриотоксическим нейрофармакологическим препаратам и детергентам резко снижается при повышении концентрации эмбрионов. Этот эффект, по-видиому, обусловлен выделением в инкубационную среду ганглиозидов из эмбриональных клеток. При испытании защитного действия фракций ганглиозидов, выделенных из эмбрионов морских ежей, наиболее эффективными антидотами против нейрофармакологических препаратов была гематозидоподобная фракция и против детергентов – фракция моносиалоганглиозидов.

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Intracellular Recording of Secretory Potentials in a 'Mixed' Salivary Gland

Secretory potentials evoked by nerve stimulation have been recorded in mammalian[1,2] and insect[3] salivary glands. These responses, which can also be elicited by applications of neurotransmitters[4,5], are apparently hyperpolarizations of the basal cell membrane of acinar cells. Recently biphasic responses (depolarization followed by hyperpolarization) have been noted in cat and rabbit submaxillary glands[6].

It has been assumed with some justification[7] that such secretory potentials are genuine membrane responses and are recorded with the microelectrode tip inside a gland cell. Direct evidence is lacking, however, that under these circumstances the microelectrode is intracellular. Moreover, in most glands so far examined electrophysiologically there are at least two cell types in each acinus. For example, in the cockroach salivary gland, which shows secretory potentials presumably homologous with those in mammals, the acinus consists of peripheral cells and central cells. The main features of these cells have been described[8]. The peripheral cell is notable for its large intracellular ductule (contiguous with the excretory duct) and its numerous mitochondria whereas the central cell has large granules probably containing enzymes secreted by this gland.

The aims of the present experiments were to establish, first, whether the tip of the electrode had an intracellular location when a secretory potential was observed and, secondly, whether such responses could be recorded from both peripheral and central cells.

Methods. Salivary glands were dissected from cockroaches, *Nauphoeta cinerea*, kept under conditions described previously[3]. The preparation was mounted in a chamber[9] and perfused with a solution containing 160 m*M* NaCl, 1 m*M* KCl, 5 m*M* $CaCl_2$, 1 m*M* NaH_2PO_4 and 1 m*M* $NaHCO_3$. The salivary duct nerves[10] were drawn into a suction electrode and stimulated with pulses (0.5 msec, 10–60 V) from a square pulse stimulator.

Microelectrodes were filled with 5% Procion Yellow (M-4R) by 2 methods. In one, the electrodes were pulled conventionally, their tips were broken by gentle pressure against tissue paper and they were then back-filled from a syringe with a Touhy-Borst adapter (Becton, Dickinson & Co.). These electrodes had resistances in the range 10–30 $M\Omega$. Alternatively normal unbroken electrodes were filled by the method described by THOMAS[11]; such

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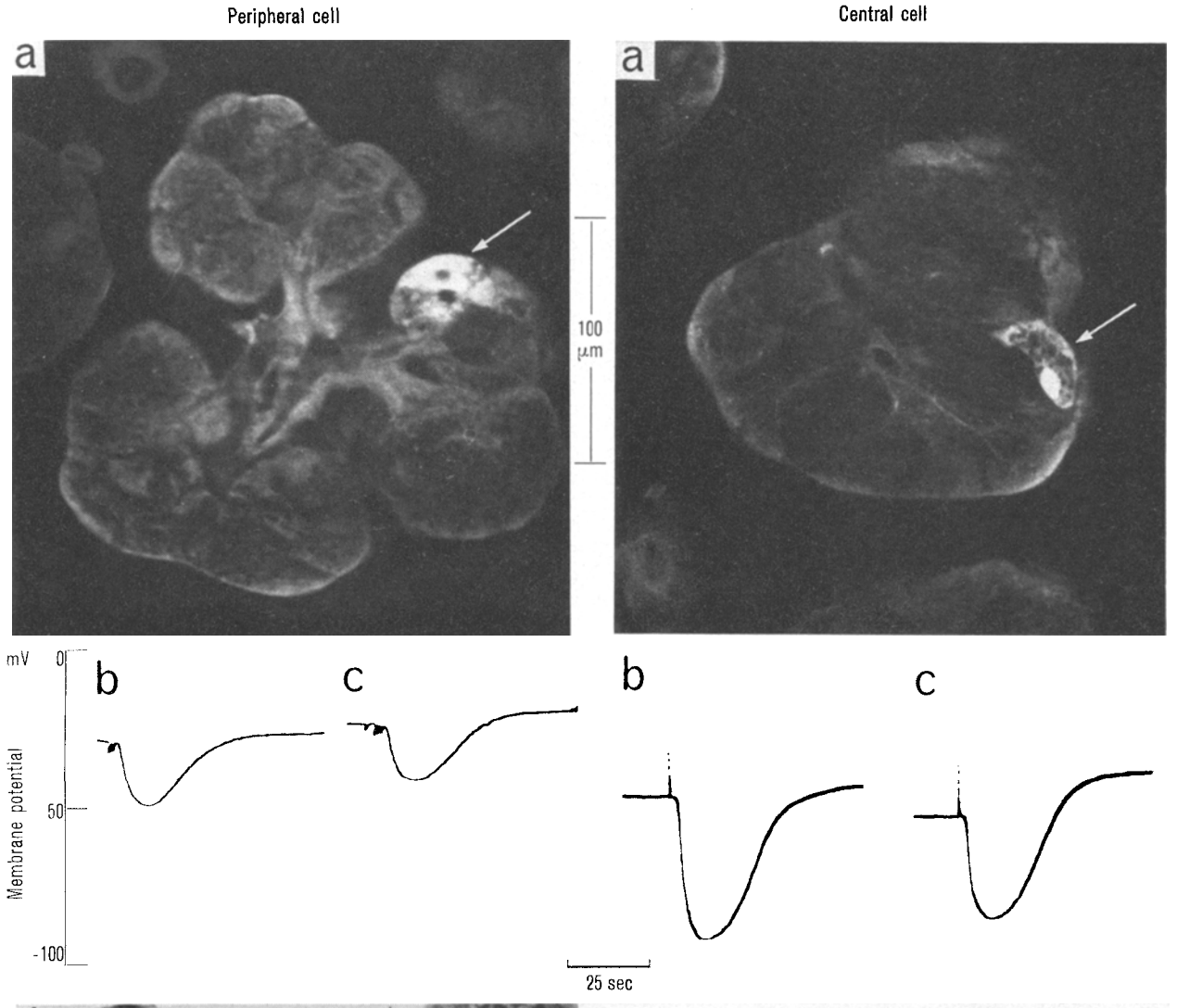

Secretory potentials recorded from peripheral and central cells. a) and d) are respectively fluorescent and light microscope pictures of identical sections showing that each cell type has been stained with Procion. In d) the borders of the cells have been accentuated by the interrupted white lines. b) and c) show the secretory potentials recorded from these cells before and 10 min after dye injection. To elicit the peripheral cell responses the nerve was stimulated with 10 pulses at 100 Hz; the central cell responses were evoked by a single stimulus. It has been demonstrated[12] that Procion Yellow ejected from high resistance (30–100 $M\Omega$) electrodes usually forms a precipitate at their tips whereas that from low resistance electrodes does not. These features of dye injection are evident in a), as the peripheral cell was impaled with a 20 $M\Omega$ electrode and has filled more evenly than the central cell impaled with a 80 $M\Omega$ electrode. However, the electrical records indicate that smaller resting and secretory potentials were observed with the broken tip electrodes (peripheral cell; b, c) than those obtained with the higher resistance electrode (central cell; b, c). It is considered that the difference in membrane potentials is due to the different type of electrodes rather than to some property of the cell types.

electrodes had resistances in the range of 50–100 $M\Omega$ and recorded larger resting and secretory potentials than the other. The methods of recording membrane potential have been described previously[3,9].

The experiments were performed in the following manner. An acinus was impaled with a Procion electrode and, provided the resting potential did not decline, the salivary duct nerves were stimulated to elicit a secretory potential. Procion was injected into the acinus by passing hyperpolarizing current pulses (10–20 nA, 100 msec) at 5 Hz through the microelectrode. Procion injection was continued for periods of 5 min, interrupted for about 1 min solely to monitor resting and secretory potentials. The microelectrode remained inside the cell for up to 30 min as judged by these criteria. The salivary gland was removed from the chamber about 30 min after the end of the experiment and fixed in 10% formalin. After wax embedding, 10 µm sections were prepared and mounted in Gurr's Uvinert mountant for examination by fluorescence microscopy and then photographed. The cover slips were floated off the slides by prolonged immersion in xylene and the tissue sections stained with Ehrlich's Haematoxylin and Eosin and finally mounted in Gurr's XAM for examination by light microscopy.

Results and discussion. In 11 experiments it was demonstrated that the site of microelectrode recording of secretory potentials was the interior of gland cells. The Figure shows representative results from 2 experiments where responses were obtained from each of the two cell types present in the acinus. Examination with light microscopy (d) established that the cells stained with Procion under fluorescence microscopy (a) were peripheral and central cells. In sections stained with Haematoxylin and Eosin the peripheral cells appear pink, are generally pyramidal in shape and show a prominent brush border lining the lumen of an intracellular ductule. In Figure a) (peripheral cell) the lumen of this ductule can be seen to be distended and free of Procion Yellow. Some of the dye appears to have spread from the peripheral cell across its apical margins to neighbouring central cells. The central cells stain purple to varying degrees, show the presence of large granules and do not contain an intracellular ductule since they discharge their secretion into the central lumen of the acinus[8]. It can be seen in Figure a) (central cell) that Procion Yellow has been deposited preferentially in the nucleus, although this may be fortuitous.

In 9 other experiments it was found that 3 of the cells were peripheral and 6 were central. Thus it seems that responses can be recorded from either kind of cell and therefore, in the light of the previous evidence[3], from any cell in an acinus. It seems unlikely that each cell has its own innervation in view of the sparse number of nerve fibres running to acini[10]. Although this possibility cannot be excluded by the present experiments, it seems more probable that acinar cells, even of different kinds, are coupled electrically. It might be that a secretory potential is evoked in a single cell, or perhaps a few cells, and spreads electrotonically. Electrophysiological evidence for electrical coupling in this gland has already been reported[9] and is consistent with the presence of septate desmosomes[8] between acinar cells. Also consistent with this suggestion is the observation that in the experiments described above (peripheral cell, a), Procion Yellow appears able to spread from one cell to another; however, further experiments are required to investigate this point.

Summary. Secretory potentials evoked by nerve stimulation have been recorded from both types of cell (peripheral and central) present in the acini of cockroach salivary glands.

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A Preliminary Report on the Fine Structure of *Tripneustes esculentus* Eggs[1]

The past decade has given us much information about the structural organization of unfertilized, fertilized and centrifuged sea urchin eggs[2–4], but the methods of fixation employed in those studies resulted mostly in general coarse preservation of cytoplasmic organelles.

It is essentially because of the importance of the sea urchin egg in the study of embryology that work involving modern fixation methods of this material for electron microscopy observation has been in progress in our laboratory for some time. Our aim was to arrive at a suitable fixation procedure that enable us to make more meaningful interpretations of the ultrastructure of the eggs in normal or experimental development.

The purpose of this communication is to report the best preservation for eggs of the sea urchin *Tripneustes esculentus* so far obtained through extensive screening and modification of various methods.

Methodology. Sea urchins of the species *Tripneustes esculentus* and sea water were obtained from the Carribean Research Centre, Martinique, and kept at 22 °C for a few hours. The eggs, obtained by injection of 0.53 *M* KCl in the vicinity of the oral region, were gently filtered through cheese-cloth and repeatedly washed with millipore-filtered sea water. The eggs were then fixed in the following pH 7.4 mixture: Glutaraldehyde-paraformaldehyde (50%), prepared in Martinique sea water. The chemicals were mixed previous to use, and the washed cells were suspended in this solution for only 5 min at room temperature approximately (23 °C).

Since longer fixation periods can result in leaching out of the pigment granules, the fixation time was kept at a minimum, and 5 min was found to be most appropriate. This was followed by treatment of the eggs with a 1% solution of osmium tetroxide (OsO_4) in sea water at 4 °C. The eggs were then dehydrated in Epon using standard techniques. To improve contrast, the sections were double-stained with uranyl nitrate and citrate. The preparations were examined with a JEM 7-A electron microscope.

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