dissection and sexual maturity revealed that in the spring and late winter, the conduction rate was 0.48 m/sec in all animals, while in the summer and fall, and early winter, diminished conduction rate was only evident in a fraction of the mature specimens examined (Figure 2).

The present report points out larger individual differences in conduction rates than had previously been reported in a study by GOLDMAN[5]. He observed a mean conduction velocity of 0.50 m/sec, with a range of 0.43 to 0.55 m/sec in data obtained from 12 animals; and his measurements were in agreement with earlier work on another species by TAUC[6]. In the work described here, velocities were seen to range from 0.24 to 0.69 m/sec. Since the very slow conduction rates were observed only in adult animals dissected in fall and winter, when a part of the *Aplysia* population appears to be dying of old age (see Methods), the speculation that the velocity diminution is geriatic may be warranted. However, only a mature-immature classification of specimens is permitted in the present paper, and a stronger statement about the age-relatedness of this phenomenon is impossible.

It is interesting to note that only the 3 slowest fibres had substantial, positive X-intercepts (2.3, 2.5 and 2.5 mm respectively). Since the spread of electrotonic currents is greater beyond a conduction block than beyond the active region of a normally propagating spike[7], it is possible that these large intercepts indicate the point of arrest of active propagation. Overdevelopment of membrane invagination 'trophospongium'[2] might cause such a failure.

One final observation on the very slow R2 neurons concerns the arrival of synaptic potentials evoked by the same stimulus used to start the antidromic spike. KANDEL and TAUC[8] noted that, in *A. depilans*, an evoked excitatory postsynaptic potential (EPSP) occasionally arrived at the intracellular recording electrode before the antidromic spike elicited by the same, right connective stimulus, and they used this observation as an argument for the monosynaptic nature of the EPSP. I have found that an EPSP from the right connective can arrive at the intracellular electrode sooner than the antidromic spike (Figure 1, inset), but also, that this result reflects only an abnormally slow conduction rate in the R2 axon. That is, in the few preparations where this happened, the conduction velocity of the fibres bringing the EPSP was greater than that of the R2 axon. The velocity of these EPSP fibres was normal, as viewed in the context of measurements on more than 20 preparations.

Summary. In late winter and in spring, the conduction velocity of the R2 axon of *Aplysia californica* is 0.48 m/sec or more in all specimens. However, in the summer, fall and early winter, some sexually mature animals exhibit markedly diminished R2 conduction rates (as low as 0.24 m/sec). It is possible that this reduced velocity is a reflection of the age of the specimen from which the axon is taken.

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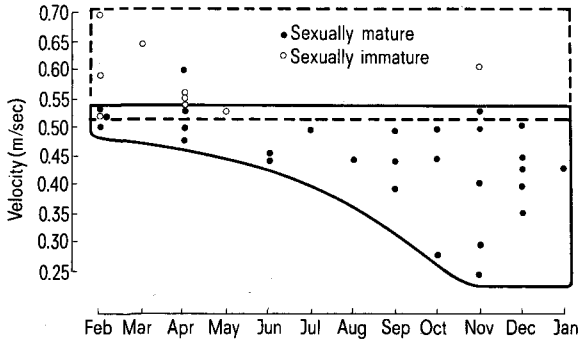

Fig. 2. R2 conduction rates in relation to the season of dissection and the sexual maturity of the specimen. The solid line indicates the hypothesized domain of conduction rates for mature animals, while the dashed line indicates the hypothesized domain for immature animals.

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[10] M.S.R.L. Contribution Number 176.

Protection of Sea Urchin Embryos Against the Action of Some Neuropharmacological Agents and Some Detergents by Endogenous Gangliosides

It is known that a number of neuropharmacological drugs block the cleavage divisions of early sea urchin embryos and inhibit macromolecular syntheses; these effects result from the suppressing of endogenous acetylcholine and monoamine functions[1-4]. It is also known[5,6] that: 1. increase of the concentration of the embryos strongly diminishes their sensitivity to neuropharmaca as well as to some detergents; 2. this phenomenon is based on the release of highly active endogenous substances which lower the sensitivity of the embryos either to neuropharmaca (An_1-factor) or to detergents (An_2-factor), but do not affect the sensitivity to other development blocking agents; 3. after removal of Ca^{++} from the incubation media, the protective action of these factors persists (against neuropharmaca – partly, against detergents – completely), whereas the protective action described earlier of neurotransmitters[1,2] is not observed.

We have tested the antiserotonins 1-benzyl-2-methyl-3-(2′-aminoethyl)-5-methoxyindole (BAS), 3-(2′-methyl-2′-aminopropyl) indole, 3-indolylacetaldehyde and β-

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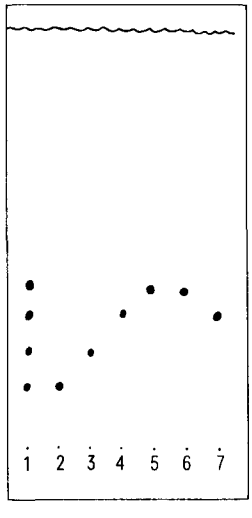
The influence of gangliosides on the sensitivity of early sea urchin embryos to neuropharmaca (BAS and aprophen) and detergent OP-10

Drugs	Concentrations of gangliosides showing a protective action				
	Hematosides from		Monosialogangliosides from		Di- and trisialogangliosides of sea urchin embryos
	Rat liver	Sea Urchin embryos	Rat liver	Sea urchin embryos	
BAS ($1.5–3 \times 10^{-5}$ *M*)	1×10^{-6} *M*	1×10^{-7} *M*	no protection	no protection	no protection
Aprophen ($7–11 \times 10^{-5}$ *M*)	5×10^{-8}–1×10^{-7} *M*	1×10^{-7}–1×10^{-6} *M*	no protection	no protection	no protection
OP-10 (2.5–10 μg/ml)	no protection	no protection	1×10^{-7}–5×10^{-7} *M*	1×10^{-6} *M*	no protection

diethylaminoethyl-1,1-diphenyl propionate (Aprophen), and the detergents – Triton X-100, and OP-10 a non-ionic detergent (mixture of polyethylene glycole ethers of mono- and dialkyl phenols).

This paper presents results of our attempt to identify the An-factors. On the basis of earlier data[5–7], we have considered An-factors most probably to be lipid substances. Therefore, we first tested whether the total lipids of early sea urchin embryos or any of the isolated lipid fractions affect the sensitivity of the embryos to neuropharmaca and detergents. The lipids were extracted[8] from fertilized eggs and blastulae of *Arbacia lixula*, *Strongylocentrotus intermedius* and *S. nudus*; for activity tests, fertilized eggs of these species as well as of *Paracentrotus lividus* were used. Gangliosides were obtained from blastulae of *S. intermedius* and tested on fertilized eggs of *A. lixula* and *P. lividus*. Incubation of the embryos and sensitivity determinations were performed under standard conditions[4].

Individual lipid and ganglioside fractions were separated by column chromatography on silica-gel[9] followed by preparative thin-layer chromatography (TLC)[10,11]. In some experiments, gangliosides were separated by DEAE-cellulose column chromatography[12].



Thin-layer chromatogram of gangliosides. 1. Rat liver total gangliosides (I, hematoside; II, monosialoganglioside; III, disialoganglioside; IV, trisialoganglioside). 2.–5. Ganglioside-like fractions isolated from the blastulae of *S. intermedius*. 6. Ganglioside-like fraction from CSW. 7. Rat liver monosialoganglioside.

As can be seen from the Figure, chromatography of the total gangliosides of the *S. intermedius* blastulae yielded 4 fractions coinciding by their Rf-values with rat liver hematoside, mono-, di- and trisialogangliosides.

Prior to testing, aliquotes of chloroform-methanol solutions of the fractions were evaporated to dryness on a water bath; the residues were eluted by artificial sea water. The total neutral lipids, as well as 3 main phospholipid fractions (phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine) isolated from the eggs and embryos, did not lower the sensitivity of early sea urchin embryos to antiserotonins, BAS and Aprophen even when the lipids were used in high concentrations (up to 100 μg/ml). Phosphatidylcholine protected the embryos against equal and lower concentrations of cytotoxic detergents (OP-10, triton x-100, digitonin). Lysophosphatidylcholine isolated from the embryos, as well as from other natural sources, was highly cytotoxic at low concentrations ($3–4 \times 10^{-5}$ *M*) and did not decrease the sensitivity of the embryos to neuropharmaca and detergents.

The total gangliosides of the sea urchin embryos showed a marked protective action against the neuropharmaca mentioned. In most cases, full protection was observed, i.e. the embryos developed normally to the blastula stage. In certain cases, the protection was less complete and development was arrested after several cleavage divisions. In control experiments (neuropharmaca without gangliosides) the embryos did not cleave at all.

Data on the protective action of the individual ganglioside fractions are given in the Table; for comparison rat liver gangliosides were also tested. It can be seen that rat liver hematoside and the hematoside-like fraction of the blastula gangliosides gave complete or partial protec-

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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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