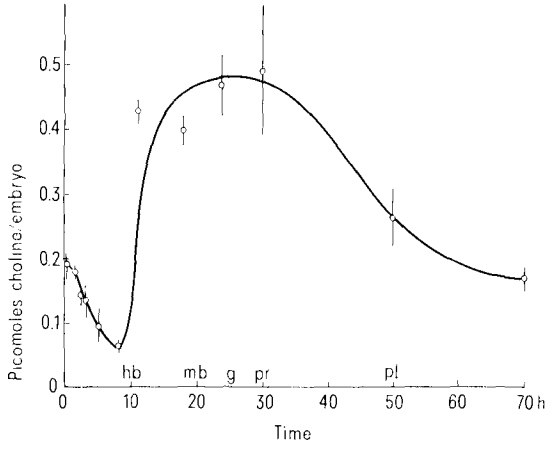
voir of unbound choline. In an egg of about 3×10^5 μm^3 the intracellular concentration of choline is 0.7×10^{-3} M choline.

During cleavage the free choline of the egg gradually dwindles to about one quarter in hatching blastulae. At the end of cleavage there is an abrupt change in the level of free choline which rises rapidly before gastrulation. The maximum free choline in the early embryos is double the amount in eggs and represents a 7-fold increase since the end of cleavage. The amount of free choline per embryo declines again in later prism and pluteus stages.

Free choline appears to be available to eggs and embryos of all stages of sea urchin development through the pluteus larva. The developmental pattern of decline, rise and decline of free choline may depend on changes in choline metabolism, or on variations in extractability, or both. We assume that the level of free choline in embryos depends on rates of utilization or production of choline, since there is no reason to suspect variations in extractability, and no way of measuring them in any case.



Free choline in eggs and embryos of *Paracentrotus lividus* of various stages. Whole organisms were extracted with perchloric acid as unfertilized eggs, 2-, 4-, 8-, 32-cell embryos, 8-hour blastulae, hatched blastulae (hb), mesenchyme blastulae (mb), gastrulae (g), prisms (pr) and plutei (pl). Choline was assayed by means of choline kinase and ^{32}P-ATP. Each point represents the average of 5–12 assays, and the vertical bars show the standard error.

Not a great deal is known concerning the acetylation of choline, or its oxidation to betaine, during cleavage in sea urchins[4–7] however considerable interest has focused on phospholipid metabolism in these organisms, in view of the importance of membrane formation in the development of embryos. The total phospholipid content of *Paracentrotus* embryos remains at 1 mg per 10^5 embryos, from fertilization through blastula[1]. Active phospholipid synthesis occurs in these and other sea urchin embryos during cleavage[1,8,9]. Efforts to determine the timing of the activation of phospholipid synthesis in development by measuring the incorporation of labelled choline into lipids of eggs and embryos have not been conclusive because of uncertainties regarding the amounts of free choline within the embryos at different stages[8–10]. Our data provide a basis for estimates of the dilution of labelled choline taken up into embryos at various stages. Our data also suggest the occurrence of a major shift in choline metabolism beginning at the end of cleavage.

The developmental pattern for free choline which we observed from fertilization through pluteus formation (Figure) in *Paracentrotus* parallels the developmental pattern of activity of the enzyme choline phosphotransferase in *Arbacia punctulata*[11]. Ewing has proposed that since the reaction catalyzed by the phophotransferase proceeds rapidly in the direction of degradation of phosphatidyl choline and is associated with yolk-containing fractions, the function of the enzyme might be to transfer phosphoryl choline from yolk, for embryonic use in phospholipid synthesis. The similarities in pattern of choline phosphotransferase activity and the pattern of free choline levels during development support the hypothesis that the two are metabolically linked and that at least some of the free choline is derived from the breakdown of phospholipid reserves packaged in the egg during oogenesis.

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Thrombosis of sympathectomized rats after strong excitation provoked by sound stimuli

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Summary. The data obtained are evidence of the decreased resistance of sympathectomized animals to excitement caused by afferent stimuli.

It is known that sympathectomized animals are far less resistant to critical conditions than normal animals[1–3]. The objective of our work was to investigate the response of sympathectomized animals to strong excitation in response to afferent stimuli.

Rats of KM strain were chosen for these experiments, these animals being susceptible to audiogenic seizures. A loud sound provokes in them violent locomotion (running, jumping) culminating in an epileptiform convulsive fit[4].

Chemical sympathectomy was performed on 146 rats, using guanethidine which is known to destroy sympa-

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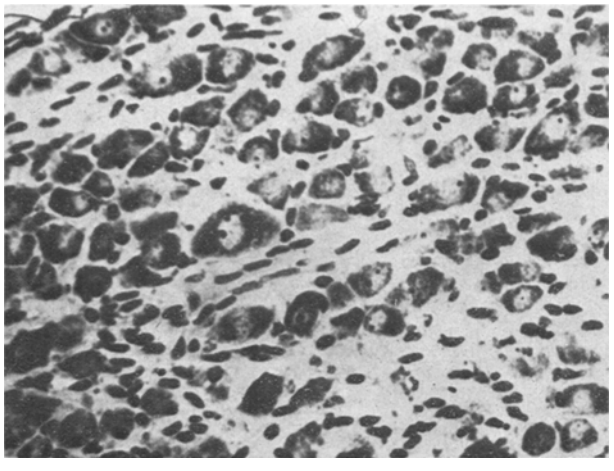
thetic cells when administered to newborn animals[5,6]. Guanethidine ('pliva', Yugoslavia) was injected subcutaneously to newborn rats daily, for 4 weeks, beginning with the first day of life, in doses of 25 mg/kg. The effectivity of sympathectomy was evaluated by the decrease in number of Stellate ganglia neurons in histological sections. Fixed in Carnua's fluid, the ganglia were then embedded in paraffin and cut into series of 10 μm sections. Every 10th section of a series was stained with toluidin blue and the number of nervous cells in it were determined. The number of neurons in all the stained sections of a given ganglion served as index of the total amount of cells in it. These calculations were performed in 4 ganglia of sympathectomized animals and 4 ganglia of controls.

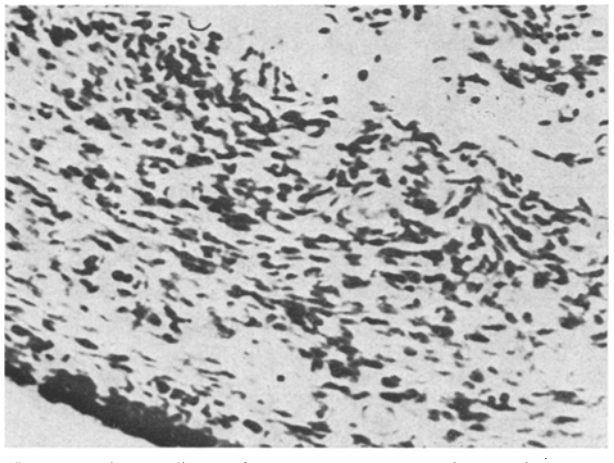
Experiments on audiogenic susceptibility were performed on chemically sympathectomized rats at the age of 1.5 months. Audiogenic sensitivity in rats was determined in animals placed in a plexiglass 42 × 26 × 50 cm chamber and exposed for 1.5 min to the loud sound (a bell), about 110–115 db above the human auditory threshold.

In the figure sections of stellate ganglia of intact sympathectomized rats are shown. On emay notice that after prolonged postnatal guanethidine treatment practically no neurons are present in the ganglion; it was established that these ganglia contain less than 0.5% of the normal amount of neurons. Thus it may be stated that under the schedule of guanethidine treatment of our experiment nearly complete sympathectomy was achieved.

A



B



[a] Sympathetic ganglion of intact rat; [b] sympathetic ganglion of rat chemically desympathized with guanethidine (see text).

The sound stimulus provokes vigorous locomotion both in sympathectomized and intact animals of KM strain, the difference being in almost instantaneous death of the former while the control animals stayed alive after suffering the usual audiogenic fit. 116 rats from the total of 146 animals (80%) died within 10–15 sec after the stimulus onset. It was shown previously that exposure of KM rats to prolonged (15–20 min) sound stimulation may be lethal to a certain percent (up to 20%) of animals provided the sound was introduced in special schedule. In such cases cerebral hemorrhages were shown to be the cause of death[4].

Contrary to this finding, the autopsy of sympathectomized rats revealed not a single cerebral hemorrhage in any case. In all animals which perished in this experiment thrombi in the auricular cavity were found. The thrombi were so large that the entire cavity was filled with them. In a few animals, some smaller thrombi were discovered also in the ventricular cavity and the aorta.

Thrombosis was produced by exposure to intensive sound not only in chemically sympathectomized rats but also in rats with pharmacologically blocked sympathetic system. In this case, mortality induced by sound exposure was total (10 deaths in 10 animals). As in the case of chemically sympathectomized rats, in these animals death also occurred within 10–15 sec after the stimulus onset.

In this experiment, the adult intact KM rats at the age of 2–3 months were injected with 25 mg/kg guanethidine 10–15 min before the sound exposure. This interval was considered sufficient for this drug to disturb the transmission of impulses through the sympathetic system[7,8].

As the cause of death in sympathectomized animals was shown to be thrombosis, it was assumed that death may be prevented by the injections of heparin which is known to inhibit the formation of thrombi. Heparin (100 units per 200 g of body weight) was administered intravenously, just before the sound exposure to 16 rats with a pharmacologically blocked sympathetic system. In this case the sound stimulation provoked the death of one-half of animals. Post-mortem examination revealed no thrombi in them but extensive pulmonary hemorrhages were visible with the naked eye as large scarlet patches on the surface of the lungs.

No lethal effect was observed when heparin and guanethidine were administered without sound stimulation, or when rats were exposed to sound with heparin but no guanethidine injected.

Thus, the lethal effect of pathological excitement, evoked in KM rats by a sound stimulus, is caused by the formation of thrombi in the cases of destruction, or pharmacological blockade of the sympathetic nervous system. If thrombus formation is prevented, the death is caused by pulmonary hemorrhage. Evidently there are many vulnerable points in the organism of animals deprived of sympathetic regulation and the protection of one of them brings into play another one.

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