quently falling off. This type of curve is similar to that observed when treatment is carried out with protein synthesis inhibitors under the same experimental conditions[5].

2. Evaluation of the inhibitory capacity on protein synthesis. In order to test the effect of RNase on protein synthesis, 3 concentrations were tested: 100, 500 and 1000 µg/ml. The criterion used to quantify protein synthesis inhibition was to consider the radioactivity observed in the control samples as 0% inhibition. The results obtained are set out in figure 2 from which we may deduce that 500 µg/ml of RNase is a suitable concentration, as it gives 90% inhibition of total protein synthesis.

3. Cytological study of RNase as a protein synthesis inhibitor in mitotic prophase. The inhibitory effect of RNase on protein synthesis in mitotic prophase was observed by using a synchronous binucleate population produced by caffeine and RNase 500 µg/ml from the 24th h after the end of caffeine treatment. Together with the enzyme, we administered colchicine 0.1% in continuous treatment in order to observe the metaphase accumulation.

Figure 3 shows the behaviour of the binucleate population on the presence of RNase-colchicine, and a comparison is made with its behaviour in the presence of colchicine alone. The same figure demonstrated how a similar curve is obtained from the evolution of the relative number of biprophases, both in control samples with colchicine and in the samples treated with enzyme-colchicine. The bimetaphase curve, however, is markedly different. These results strongly suggest that prophase in the presence of RNase neither go through to metaphase nor remain blocked in prophase but rather regress to interphase.

Using experiments combining protein synthesis inhibitors and RNA synthesis inhibitors, region of return, was situated earlier than the region where prophasic RNA is synthesized (blocking region). The experiments described in this paper themselves reafirm the earlier demonstration, since RNase acts simultaneous as a RNA digestive enzyme and as a protein synthesis 'inhibitor'. The response of the synchronous population to RNase treatment is the same as its response to protein synthesis inhibitors.

On the other hand, we observed no inhibition of metaphase, anaphase and telophase processes nor of cytokinesis, which would suggest that these events are dependent neither on RNA nor, in the short term, on protein synthesis.

Free choline in sea urchin embryos (Paracentrotus lividus)

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Summary. A sensitive radio-enzymatic assay for choline, applied to extracts of developing sea urchin embryos, revealed that free choline in eggs declines during cleavage and rises abruptly previous to gastrulation, indicating an important shift in choline metabolism between these two stages.

Choline has three major roles in cellular physiology and biochemistry. First choline occurs in animal cell membranes as phosphatidyl choline, a principal component of both plasma and mitochondrial membranes. A second function of choline depends on its oxidation by choline oxidase to betaine, an important source of methyl groups in biological methylations. A third role for choline is as precursor for acetylcholine formed in the presence of choline acetylase.

While mammalian cells receive their required choline from their medium, embryos developing independently of exogenous nutrients contain phospholipid reserves in yolk and other organelles and these reserves are metabolized progressively during early development. In sea urchin embryos although the net amount of phospholipids is constant during early development the synthesis of phospholipids increases suggesting that new phospholipids are made from the degradation products of those in reserve[1].

Since knowledge of the quantities of free choline available to embryos during development could tell us something about their regulation of choline metabolism we have measured the freely extractable choline from whole embryos by means of a highly sensitive and specific assay and found important changes in the amounts of free choline available from embryos at different stages.

Materials and methods. Eggs and embryos of the sea urchin *Paracentrotus lividus* were extracted in lots of 5000 embryos at various developmental stages in perchloric acid and neutralized. Dried extracts were assayed by a radioenzymatic assay dependent on choline kinase[2]. Choline kinase was prepared as described by ACARA and RENNICK[3].

Fifty µl of the incubation medium consisting of 10 m*M* *Tris* buffer at pH 9.5, 5 m*M* $MgSO_4$, 0.1 m*M* ATP, 2 µg of purified choline kinase and 1 µCi of ^{32}P-ATP were mixed with 1 to 10 µl of dissolved extract from the embryos. The samples were incubated 3 h, chilled and 10 µl of 0.045 *M* phosphoryl choline were added to each tube and the entire mixture placed on 2 ml columns of BioRad AG 1-X8 (200–400 mesh) which had been equilibrated with 10 m*M* *Tris*, pH 11 in 10 m*M* $MgSO_4$, 10 m*M* NaF and 30 m*M* NaCl. The labelled phosphoryl choline formed by the reaction of choline with ^{32}P-ATP in the presence of choline kinase was eluted from the column with 7 ml of *Tris* buffer at pH 11, collected in scintillation vials, and the radioactivity determined by Cerenkov counting. Triplicate samples of each sea urchin extract, with and without added choline, were analyzed to test for any inhibition of the assay system caused by the extract.

Results and discussion. The developmental pattern of the free choline in sea urchin embryos is shown in the Figure. The unfertilized eggs contain 0.2 picomoles choline per egg, which constitutes a relativly large reser-

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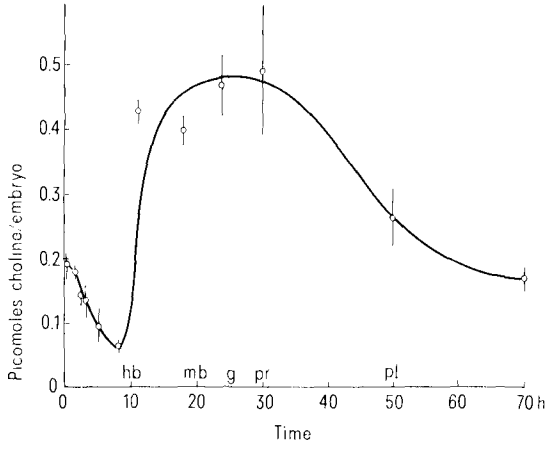
voir of unbound choline. In an egg of about 3×10^5 μm^3 the intracellular concentration of choline is 0.7×10^{-3} M choline.

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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