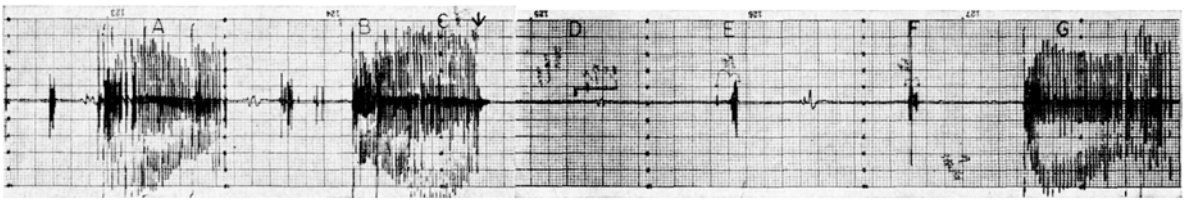

Fig. 5. The effect of halothane vapor on spontaneous potentials from an explant of chick embryo telencephalic tissue in culture. The lower trace is a direct continuation of the upper trace. A and B are normal series of potentials. At the arrow at C, a dose of halothane vapor (1 cm^3 air saturated with halothane vapor at 37°C) is administered and then washed out repeatedly with warm air at D, E, and F. G is the point of return of the spontaneous potentials. X axis — each division equals 1 sec. Y axis — eight large divisions equals 30 μV.

serum stopper containing an air filter (to prevent any change in the pressure inside the Kahn tube) and a length of Teflon tubing to allow the introduction of the anesthetic gases. The stopper was inserted so as to allow the egress of the two platinum electrodes. The Kahn tube was placed in a shielded incubator at 37°C and connected by shielded cables to amplifiers and a paper strip recorder.

An injection of a volume of warm air similar to that already in the Kahn tube caused only a transient incidental electrical disturbance as can be seen at the time of injection in any of the illustrations. The anesthetic gas to be used was firstly carefully warmed to 37°C before injection by syringe into the outer end of a piece of Teflon tubing. The anesthetics used were: Chloroform (Figure 2), di-ethyl ether (Figure 3), divinyl ether (Figure 4), and Halothane (Figure 5). Each of the anesthetics had the same general effect. They caused an initial short period of increased frequency of potential production—a stage of 'excitement' which is best seen in Figure 1 where low concentrations of anesthetics were used. It is also visible in the traces seen after the introduction of some of the anesthetics (Figure 3 and 4). After this excitement phase, the potentials were diminished or suppressed by the presence of anesthetic.

This effect can be reversed if the anesthetic is washed out with warm air or when a low concentration of anesthetic is used the explant will slowly recover activity with the passage of time even if the anesthetic is not physically washed out. High concentrations of anesthetic will cause permanent suppression of the potentials if the anesthetic is not removed rapidly by the immediate and repeated replacement by warm air. After a dose of anesthetic had been washed out with warm air, the explant was then susceptible to further doses of the same or other anesthetics.

The increase in frequency of potential production or 'excitement phase' is of interest. It is notable that in spite of the second dose of anesthetic being five times that of the first (Figure 1) the interval between the end of injection and onset of excitation is 80 sec in each case. Possibly this is because the vapor had to penetrate through the same thickness of explant to reach the active focus in each occasion. Unless an inhibitory effect by some of the cells in the explant is present and removed by anesthetic action, this 'excitement phase' must be due to direct stimulation by the anesthetic. This suggests that the excitement stage encountered in humans and animals during induction with anesthetics may also be due to direct stimulation of cortical cells and not to paralysis of inhibitory action.

Zusammenfassung. Die spontanen Potentiale von Telencephalon-Explantaten *in vivo* werden durch anästhetische Gase nach anfänglichem Potentialanstieg vermindert bzw. gehemmt. Die Potentiale kommen in ihrer ursprünglichen Form nach Entfernung des Betäubungsmittels mit warmer Luft wieder zurück, und die Explantate sind dann wieder durch dasselbe oder andere Betäubungsmittel beeinflussbar.

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The Effect of Puromycin on Protein Metabolism and Cell Division in Fertilized Sea Urchin Eggs[1]

It has recently been shown that puromycin is a specific inhibitor of protein metabolism at the S-RNA-ribosome level[2,3]. Preliminary experiments on the effects of this drug on early sea urchin development indicated that it inhibits the mitotic activity of fertilized eggs in a very characteristic way. When puromycin was added to the eggs some minutes before fertilization at concentrations above $10^{-4}M$, activation and early development proceeded in a seemingly normal way until the 'clear streak' stage, but then development came to a standstill. The 'clear streak' attained a rigid appearance with abnormally sharp borders. The nucleus gradually swelled, but no spindle was observed and no cell divisions occurred. The present report deals with an attempt to correlate this antimitotic effect of puromycin with its inhibitory action on the incorporation of labeled amino acids into protein by the same eggs, *in vivo* and *in vitro*.

Experimental. Puromycin (Lederle Laboratories Division) at varied concentrations was added to unfertilized eggs of the sea urchin *Paracentrotus lividus* (LM), 10 min before fertilization. At various intervals after fertilization, equal egg samples (approximately 10 mg protein) were withdrawn from the egg suspensions. After sedimentation of the eggs, the volume of the medium was reduced to 4 ml, and 75 mμ*M* of ^{14}C-*L*-valine (6.53 mc/m*M*) was

[1] Supported by a grant from the Swedish Natural Science Research Council. Experiments carried out at the Zoological Station, Naples (Italy).

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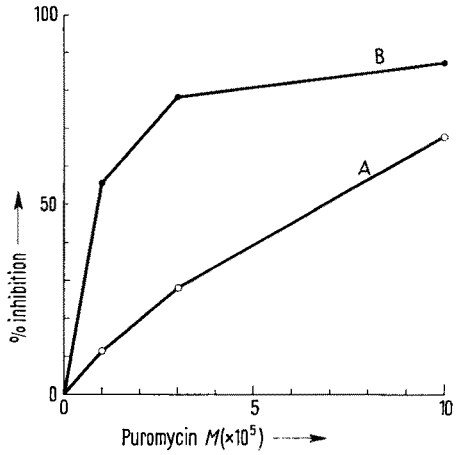

Fig. 1. Inhibition by puromycin of the incorporation of ^{14}C-*L*-valine into protein by fertilized *Paracentrotus* eggs. A: Intact eggs 35–45 min after fertilization ('clear streak' stage). The eggs (approximately 10 mg protein) were incubated for 10 min (20°C) with 75 mμM ^{14}C-*L*-valine. Puromycin as indicated was added 10 min before fertilization. B: Mitochondria-free egg homogenate, 40 min after fertilization. Incubation system: 0.75 ml homogenate, 10 μM phosphoenol pyruvate, 1 μM ATP, 0.075 μM ^{14}C-*L*-valine, and puromycin as indicated. Incubation period 45 min (25°C).

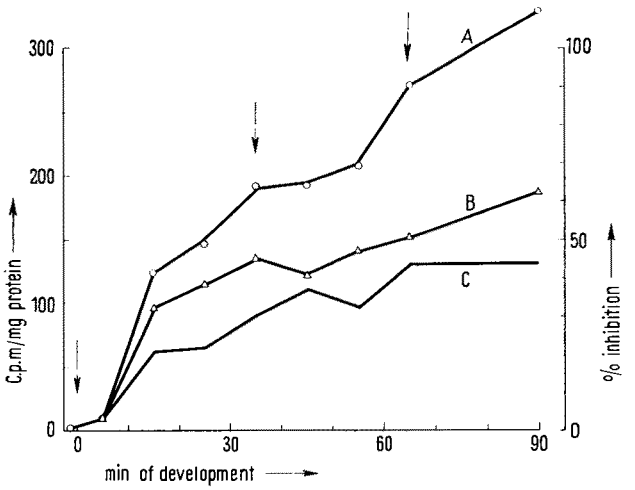

Fig. 2. Effect of puromycin on the velocity of ^{14}C-*L*-valine incorporation by *Paracentrotus* eggs at different periods of development. A: Eggs in plain sea water. B: Eggs in sea water with 10^{-5} *M* puromycin. C : % inhibition. Incubation system as in Figure 1 A. Arrows symbolize (from left) Fertilization, 'clear streak', first cleavage in control suspension. Measurements are plotted in the middle of the incubation periods (10 min).

added. The suspension was gently shaken for 10 min (20°C), after which the incorporation was stopped by the addition of trichloroacetic acid. Protein purification and radioactivity determinations were made as described previously[4]. The visible effect of the puromycin treatments on cleavage and development was checked under the microscope on egg samples taken at intervals from the same suspensions.

The effect of puromycin on ^{14}C-*L*-valine incorporation by cell-free systems was studied in parallel on eggs from the same batch. After fertilization the eggs were freed from jelly by acid treatment[5], and homogenates were prepared as described previously[4]. The homogenates were centrifuged at $12000 \times g$ for 8 min (0°C), and 0.75 ml of the supernatant was incubated for 45 min at 25°C with 10 μM of phosphoenolpyruvate, 1 μM of ATP, and 0.075 μM of ^{14}C-*L*-valine in the presence of puromycin at varied concentrations. After incubation, the proteins were isolated and the radioactivity determined as in the *in vivo* experiments.

Results and Discussion. The inhibitory effect of puromycin on amino acid incorporation by fertilized *Paracentrotus* eggs *in vivo* and *in vitro* is illustrated by Figure 1. In the *in vivo* part of this experiment the egg samples, treated with puromycin at the indicated concentrations, were incubated with ^{14}C-*L*-valine in the interval between 35 and 45 min after fertilization. In $10^{-4}M$ puromycin 50% of the eggs stopped developing after the 'clear-streak'-stage of the first division, while the rest of the eggs reached the two-cell stage. Development was definitely arrested, however, at the corresponding phase of the second cleavage, when the same typical picture of inhibition was displayed. In spite of the perfectly normal elevation of the fertilization membrane, the two cells became tightly adherent. At the lower puromycin concentrations, a larger portion of the eggs divided, and at a concentration of $10^{-5}M$, 40% of the eggs reached the 4-cell stage. The inhibitory effect of puromycin on the incorporation of ^{14}C-*L*-valine *in vitro* was studied on a second part of the same egg suspension. The homogenate was prepared 40 min after fertilization.

As is shown by Figure 1, the cell-free incorporation system was considerably more sensitive to puromycin than were the intact eggs. Also with the whole eggs, however, the inhibition became very marked at the higher puromycin concentrations.

The experiment shown in Figure 2 illustrates the gradual development of the puromycin inhibition. In this experiment, the puromycin concentration was $10^{-5}M$, and some of the eggs finally reached the 8-cell stage. In the cases, however, when the third cleavage occurred, it was usually unequal and gave rise to 1–4 micromeres.

The mitotic block induced by puromycin is probably a direct effect of the impaired protein metabolism. Special kinds of proteins of importance for the initiation of mitosis may not become produced in sufficient amounts under these conditions. Which functions these proteins have can only be a matter of conjecture. The present data are consistent, however, with the idea that some of them may be related to the formation of the mitotic spindle.

Résumé. La puromycine inhibe fortement l'incorporation de la ^{14}C-*L*-valine dans les protéines, aussi bien dans les œufs fécondés d'oursins intacts que dans les préparations subcellulaires faites à partir de ceux-ci. Cependant ces préparations se sont montrées nettement plus sensibles à l'action de la puromycine que les œufs entiers. Parallèlement le blocage des divisions cellulaires a été proportionnel à cette action inhibitrice sur le métabolisme des protéines. Le développement s'est arrêté au stade «clear streak».

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The Wenner-Gren Institute, University of Stockholm (Sweden), May 15, 1961.

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