cells might be described as "naturally resistant" to 5-fluorouracil. Response to 5-fluorouracil therapy in human leukemia has not been impressive. The extent of the conversion of 5-fluorouracil to nucleotides may offer a suitable predictive index of drug response in man, and may be an aid in selecting patients for 5-fluorouracil therapy.

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References and Notes

1. To obtain survival data, intraperitoneal injections (30 mg/kg) were given, from day 1 to day 10, to animals after inoculation with 10^6 tumor cells. Survival increase = 100 $(T-C)/C$, where T is the mean survival time (in days) of tumor-bearing animals receiving the drug and C is the mean survival time of the untreated (control) animals. Each point on the figure represents the data obtained from at least ten mice.
2. Rapid diffusion of 5-fluorouracil into Ehrlich ascites cells was reported by J. A. Jacquez, *Proc. Soc. Exp. Biol. Med.* **109**, 133 (1962).
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4. This buffer contained 62 m*M* tris, *p*H 7.2; 65 m*M* NaCl, 15 m*M* KCl, and 8m*M* $CaCl_2$.
5. 5-Fluorouracil-2-C^{14} (20 mc/mmole) was obtained from Calbiochem Corp.
6. Nuclear-Chicago Solubilizer (NCS) was furnished by the Nuclear-Chicago Corporation for solubilizing proteins for scintillation counting. Substitution of similar products, such as hyamine or ethanolamine, was satisfactory.
7. This was prepared by mixing 600 ml of toluene, 400 ml of pure methyl cellosolve, 4 g of 2,5-bis-[2(5-*tert*- butylbenzoxazolyl)] thiophene (BBOT) (Packard Instrument Co.), and 60 g of naphthalene.
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10. The most useful systems for separating nucleotides of 5-fluorouracil were those often used for the corresponding uracil analogs: (i) a mixture of 20 ml of 5*M* ammonium acetate, *p*H 9, 80 ml of saturated sodium tetraborate, 180 ml of ethanol, and 0.5 ml of 0.5*M* ethylenediaminetetraacetate suggested by P. Reichard and O. Sköld, *Biochim. Biophys. Acta* **28**, 376 (1958); (ii) a mixture of 100 ml of isobutyric acid and 60 ml of 1*M* NH_4OH (adjusted to *p*H 4.6), H. A. Krebs and R. Hems, *Biochim. Biophys. Acta* **12**, 172 (1952).
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14. We thank L. Nigrosh, N. Kuribayashi, and S. Blakely for technical assistance. Supported by Cancer Chemotherapy National Service Center contracts PH 43-62-169, PH 43-66-541, and PH 43-65-61 and by grant C-6516 of NCI.

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Cilia Regeneration in the Sea Urchin Embryo: Evidence for a Pool of Ciliary Proteins

Abstract. *Late gastrulae of* Paracentrotus lividus *regenerated cilia after being deciliated in hypertonic sea water. Regeneration was not affected by actinomycin D or puromycin. Actinomycin D also did not affect ciliary protein synthesis during regeneration, although overall embryonic synthesis was depressed. Puromycin inhibited both total embryonic and ciliary protein synthesis. The data indicate that ciliary protein synthesis is controlled by a stable template and that the regenerating cilia are formed from a pool of ciliary proteins. It is suggested that the proteins of the mitotic apparatus and of the cilia may be related.*

There is fairly general agreement that the template- or messenger-RNA requisite for protein synthesis during early development is present in an inactive state in the unfertilized egg of the sea urchin and that fertilization or parthenogenetic treatment of the egg is required for it to become active (*1, 2*). Although RNA, some or all of which may be informational, is synthesized early in development, it appears to remain in a masked form until later (*2, 3*).

The overall pattern of early protein synthesis has been examined by Spiegel *et al.* (*4*) and by Terman and Gross (*5*) who used gel-electrophoretic procedures on soluble proteins isolated from embryos in various stages of development. In agreement with the work of Pfohl and Monroy (*6*), the former group found no marked qualitative difference during early development, either in the presence or absence of actinomycin D, a compound which has been shown by Gross *et al.* (*2*) to inhibit RNA synthesis during this period. Terman and Gross (*5*) found systematic changes in the pattern of protein synthesis, either in the kinds of proteins made or in the changing rate of synthesis of one particular protein, but these changes were not affected by actinomycin D.

Some of the specific proteins made during early development appear to be associated with the mitotic figure (*7*) and, more specifically, with the microtubular elements of the spindle region (*8*).

Previous study of ciliary protein synthesis associated with the initial formation of cilia during the blastula stage of *Arbacia punctulata* has indicated that ciliary proteins are made during early development and that they form a reserve necessary for ciliary development (*9*). Actinomycin D partially reduced the incorporation of precursors into ciliary proteins, but cilia still developed.

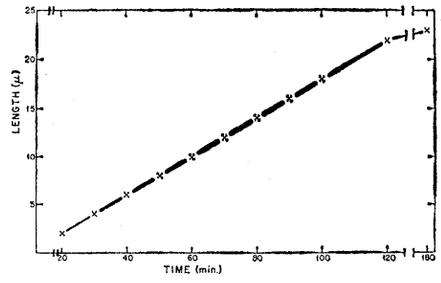

Fig. 1. Growth rate of regenerating cilia of 24-hour-old gastrulae of *Paracentrotus lividus* at 20°C.

To determine whether these conditions also hold later in development, 18- to 24-hour-old embryos of the sea urchin, *Paracentrotus lividus*, in the late gastrula stage were used for the regeneration experiments. Adults collected from the Bay of Naples region shed their gametes when 0.5*M* KCl was injected into their peritoneal cavities. The eggs were fertilized and placed overnight in continuously stirred, Millipore-filtered sea water at 20°C. Deciliation was induced by short exposure of the embryos to sea water whose osmolarity was doubled by the addition of 29.2 g of NaCl per liter of filtered sea water. The embryos were rapidly returned to continuously agitated, normal sea water at 20°C.

The regenerating cilia at the embryo surface were measured with oil immersion-phase contrast optics. Measurable cilia were first seen 20 minutes after deciliation as stiff rods 2 μ long. The rate of growth was 1 μ/5 min for nearly 2 hours; the rate then decreased over the next 2 to 3 hours until the cilia reached a final length, 24 to 25 μ, equal to that of the original cilia (Fig. 1). The cilia of the apical tuft do not regenerate to their original length of 70 to 75 μ. Extrapolation of the curve in Fig. 1 to the abcissa indicates that there is a 10-minute lag period between deciliation and the beginning of ciliary regeneration if one assumes a constant rate of growth. This may be caused by disturbing influences

Table 1. Effect of actinomycin D (25 μg/ml) and puromycin (50 μg/ml) on incorporation of C^{14}-L-leucine (specific activity, 150 mc/mmole; concentration, 0.1 μc/ml) and C^{14}-L-glutamic acid (specific activity, 208.5 mc/mmole; concentration, 0.1 μc/ml) into TCA-precipitable protein of regenerated cilia and of the total embryo after an 80-minute pulse. Figures in parentheses refer to the percentage of inhibition by each antibiotic.

Antibiotic	Incorporation (count/min per microgram)	
	Ciliary protein	Embryo protein
	L-Leucine	
Control	137 (0)	17.8 (0)
Actinomycin D	140 (0)	11.8 (34)
Puromycin	9 (93)	2.0 (89)
	L-Glumatic acid	
Control	7.1 (0)	1.0 (0)
Actinomycin D	6.9 (0)	0.3 (67)
Puromycin	1.7 (76)	0.3 (67)

of the treatment with hypertonic sea water.

To ascertain whether either DNA-mediated RNA synthesis or ciliary protein synthesis on a stable template is a prerequisite for ciliary regeneration, we treated embryos with actinomycin D (25 and 50 μg/ml) or puromycin (50 and 200 μg/ml) 2 hours before deciliation. The embryos were then deciliated in the presence of the antibiotics and were returned to normal sea water containing the antibiotics at the same concentrations. The rate of ciliary regeneration was equal to that in controls.

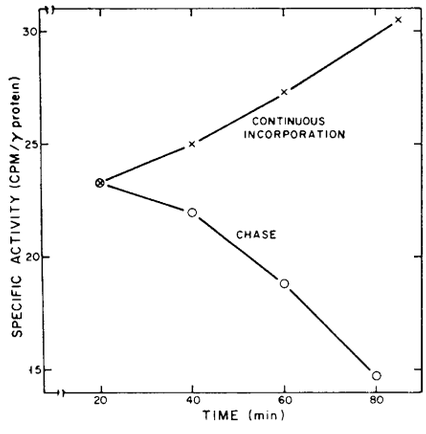

Fig. 2. Determination of the effectiveness of the dilution of the labeled amino acid pool by the chase medium. Embryos (24 hours old) are deciliated and placed in sea water containing streptomycin sulfate (0.1 mg/ml) and C^{14}-L-leucine (150 mc/mmole, 0.1 μc/ml). After 20 minutes half of the embryos are washed and placed into a solution containing unlabeled L-leucine at a concentration 10^5 times greater than that of labeled leucine. A number of embryos are also removed at this time and placed in a final concentration of 5 percent TCA containing unlabeled leucine. At each succeeding time interval a number are removed from both cultures, protein is extracted as described, and the specific activity is determined. There is a steady decrease in the specific activity of protein from embryos maintained in the chase medium, indicating effective dilution of the labeled amino acid.

We next tested the effect of these antibiotics on ciliary protein synthesis by treating embryos with the antibiotic, removing their cilia, and then placing the embryos in filtered sea water containing streptomycin sulfate (0.1 mg/ml), C^{14}-L-leucine (150 mc/mmole) at a concentration of 0.1 μc/ml, and either actinomycin D (25 μg/ml) or puromycin (50 μg/ml). After 20 minutes, a portion of embryos was taken from the control culture, washed in sea water containing excess unlabeled leucine at a concentration 10^5 times greater than that of the labeled amino acid, and kept in this medium until 80 minutes after the initial deciliation. This solution effectively diluted, or chased, the labeled amino acid (Fig. 2). All embryos were again stripped of their cilia by being placed in a solution of 10 percent ethanol and 0.1 percent ethylenediaminetetraacetic acid (EDTA) in 0.5*M* sodium acetate having a *p*H of 6; addition of one part of 1*M* $CaCl_2$ to 99 parts of this solution induced deciliation (*9, 10*). The embryos were separated from the cilia by centrifugation (1000*g* for 20 minutes) over a gradient of 0 to 60 percent sucrose in 0.5*M* sodium acetate. The cilia were then collected by centrifugation (5000*g*, 30 minutes), the supernatant was removed, and the pellet was resuspended in 0.5*M* acetate. This procedure was repeated. We examined a sample of the isolated cilia preparation with oil immersion–phase contrast microscopy to be certain it was not contaminated (Fig. 3).

Protein was extracted from both isolated cilia and the deciliated embryos with 5 percent trichloroacetic acid (TCA). Nucleic acid and lipid were extracted with hot TCA and a mixture of chloroform and ether, and the concentration of protein was determined by the method of Lowry *et al.* (*11*). The protein was reprecipitated with TCA, the precipitate was collected on a Millipore filter, and the radioactivity was measured with a gas-flow counter.

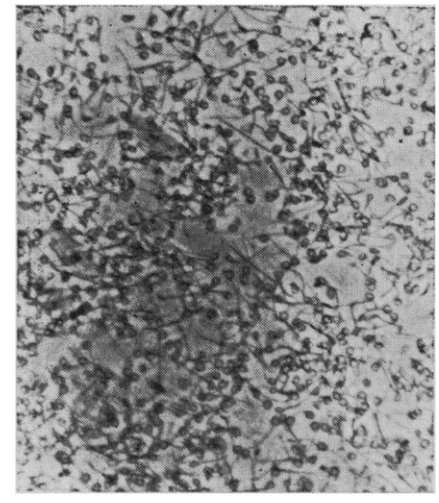

Fig. 3. Oil immersion–phase contrast micrograph showing part of a suspension of isolated cilia.

There is a steady increase in the incorporation of labeled amino acid into both ciliary and total embryo TCA-precipitable protein, but the specific activity of ciliary protein is seven to

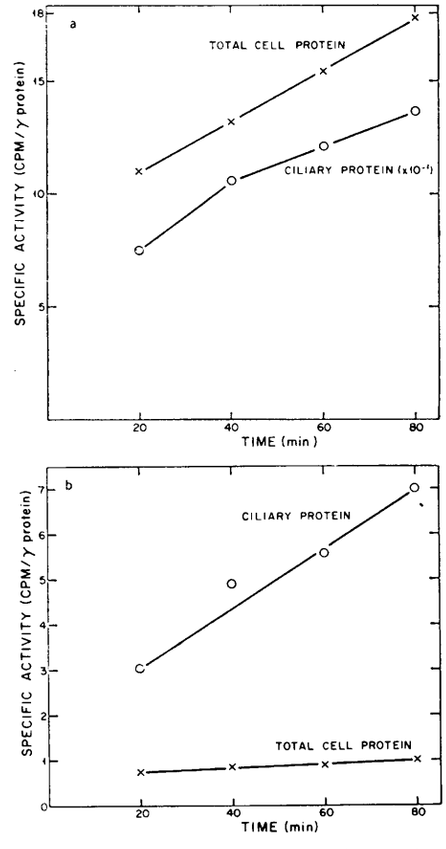

Fig. 4. Incorporation of (a) C^{14}-L-leucine and (b) C^{14}-L-glutamic acid (each amino acid at a concentration of 0.1 μc/ml) into TCA-precipitable protein of regenerated cilia and deciliated 24-hour-old gastrulae.

eight times greater than that of embryonic protein. This specific activity indicates selective synthesis of ciliary protein (Fig. 4a and Table 1). Actinomycin D does not affect ciliary protein synthesis. The fact that total protein uptake is decreased by about one-third demonstrates that actinomycin D did enter the embryo. Puromycin inhibits uptake into both total embryonic and ciliary protein by 89 and 93 percent, respectively.

Similar results are obtained if C^{14}-L-glutamic acid (208.5 mc/mmole) is used as the precursor instead of leucine (Fig. 4b and Table 1). Since glutamic acid is also utilized by the embryo as a metabolite in the Krebs cycle, the specific activity of the proteins is much less when this amino acid is used than when leucine is the precursor. But the specific activity of ciliary protein is still seven to eight times greater than that of the total embryonic protein. It is thus unlikely that the greater specific activities of ciliary proteins, being nearly the same in both experimental conditions, reflect greater amounts of leucine and glutamic acid in ciliary protein than in total cellular protein.

The fact that actinomycin D does not affect the initial appearance of cilia (*9*) nor their synthesis in the regenerating system supports evidence that ciliary protein synthesis in the sea urchin embryo is controlled by a stable maternal template RNA. When exposed to puromycin, a substance which inhibits overall protein synthesis, embryos can regenerate cilia four times over a 10-hour period. This finding suggests the presence of an extensive intracellular pool of ciliary proteins.

The need for a large reserve may stem from the frequent cell divisions of the ciliated ectodermal cells, this reserve assuring the daughter cells an adequate supply of proteins for immediate cilia formation. To account for such an apparently extensive pool, one must postulate that the proteins synthesized during regeneration mix readily with the preformed pool, thus labeling the cilia. In two species of bacteria newly synthesized flagellar proteins do mix freely with the preexisting pool, and, although flagellar protein synthesis is inhibited by chloramphenicol, the regeneration of new flagella is not affected (*12*).

The process of aggregation of proteins to form cilia is not understood, but it probably is the key to the initial appearance of cilia in the blastula, since ciliary proteins are probably already available (*9*). It is possible that cilia and mitotic apparatuses have proteins in common, or have ones that are structurally similar, and they may have similar mechanisms for organizing the protein. Colchicine, at a concentration ($10^{-4}M$) that inhibits organization of the mitotic figure, immediately stops ciliary growth. Both structures may be under the organizational influence of homologous organelles (the centriole and basal body); both contain submicroscopic microtubular elements (*8, 13*), have adenosine triphosphatase activity (*14*), and incorporate amino acids during early development (*7–9*). Kane (*15*) and Stephens (*16*) have described an extensive pool of a mitotic apparatus protein making up about 10 percent of the total cell protein in unfertilized sea urchin eggs.

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Comparative Mutagenicity of Two Chemosterilants, Tepa and Hempa, in Sperm of Bracon hebetor

Abstract. *In an assessment of the mutagenic efficiency of the alkylating agent, tepa, and its nonalkylating analog, hempa, both chemicals induced a high frequency of recessive lethal mutations in the sperm of the parasitic wasp,* Bracon hebetor *Say* (Habrobracon), *although tepa was the more efficient mutagen.*

Because certain recently discovered insect chemosterilants with low mammalian toxicity were classified chemically as nonalkylating agents (*1*), some investigators may have assumed that they were also nonmutagenic. Since any chemical that produces sterility in insects without affecting the hereditary material would act quite differently from most male chemosterilants currently available, we were interested in determining whether the nonalkylating analog of tepa, known as hempa (*2*) (Fig. 1), is indeed nonmutagenic. Both tepa and hempa produce sterility in several economically important insects, although tepa is more efficient (*1*). If one of these chemicals were mutagenic and the other not, we would then have some basis for assuming that the type of sterility they produced was different. If both compounds were mutagenic, even though one were more effective than the other, the type of sterility produced by each would probably be similar. The two chemicals might differ only in their availability at critical sites.

The insect utilized for the tests was the parasitic wasp, *Bracon hebetor* Say (also known in the literature as *Habro-*