Table 1. Measurements of DNA-Feulgen complex on nuclei of embryos at the 32-cell stage from the large and small forms of *Pseudocalanus*. Amount of chromatophore is determined from average extinctions and from photometric areas of 532 μ^2 for the small form and 2084 μ^2 for the large form. Average extinctions are from averages of duplicate readings of ten nuclei from each embryo.

Average extraction (mean ± S.D.)	Total relative amount of chromatophore
Individual small embryos	
0.122 ± 0.025	63.8
.138 ± .021	72.2
.141 ± .036	73.7
.115 ± .012	60.2
.146 ± .016	76.4
Small embryos combined	
.132 ± .022	69.0
Individual large embryos	
.241 ± .030	502.4
.233 ± .028	485.7
.279 ± .030	581.6
.202 ± .026	421.1
.238 ± .012	496.1
Large embryos combined	
.239 ± .035	498.2

gen reaction (*3*) was employed as a measure of relative amounts of nuclear DNA. Embryos of both forms were treated concurrently to avoid variability due to the staining procedure. Several nonhydrolyzed embryos served as controls. Measurements were made on five embryos of each form, all at the 32-cell stage. Individual embryos were mounted on separate slides in appropriate oil of refractive index. The cytophotometric technique used was the aperture method, and all measurements were made with the two-wavelength method at λ 570 mμ and λ 500 mμ (*4*). Ten nuclei were measured from each embryo. Table 1 shows the results.

It is evident that nuclei of the large form have a greater DNA content, which has occurred by an increase of size rather than number of chromosomes. Considering that the ratio of volumes of somatic nuclei among older stages of the large and small forms was shown to be about 3 : 1, like the ratios of egg and body volumes (*1*), the ratio of DNA amounts (about 7 : 1) is somewhat curious. However, the point to be made here is that the two forms are similar except in size and development rates, these being related to their DNA contents. It is known that nuclear size (chromosome number, size, or DNA content) influences cell size and inversely affects both cellular metabolic and division rates, without necessarily affecting qualitative characters of the organism. Several theoretical discussions of these relationships have recently been offered (*5*). Copepods show determinate growth and probably determinate cell number (*1*), so that an increase in DNA content may be expected to increase size and to reduce development rate of the entire organism, as found in the large form of *Pseudocalanus*.

The possible adaptiveness of the large form will not be considered here in detail. It is noteworthy that *P. minutus*, which is especially large in ordinary arctic waters, is greatly reduced in size by the unusually warm waters of the aforementioned semilandlocked fiords (*1*). The large form may represent an evolutionary attempt to restore "normal" size and development rates for these high latitudes.

The increase of chromosome size presumably results from polyteny, that is, from multiplication of DNA-containing strands, rather than from differential degrees of tightness of coiling in the chromosomes. Although chromosome size was of sporadic concern to earlier workers, Hughes-Schrader and Schrader (*6*) were apparently the first to implicate polyteny as a factor in evolution within a closely related group of animals. In the literature there seems to be no explicit demonstration that polyteny is responsible for size forms of the sort demonstrated here, but there are suggestions that others occur among copepods. Three cryptic species of the well-known genus *Calanus* are now recognized from the North Atlantic (*7*). *Calanus finmarchicus* is a mid-latitude form which is smaller than the northern *C. glacialis* where they occur together in subarctic waters. Similarly, *C. finmarchicus* is smaller than *C. helgolandicus* where their ranges overlap in the eastern North Atlantic. Harding (*8*) reported that both *C. finmarchicus* and *C. helgolandicus* have haploid chromosome sets of 17. Noting that the eggs of *C. helgolandicus* are larger (about 172 μ) than those of *C. finmarchicus* (about 145 μ), he suggested that the chromosomes of the former were larger, although his evidence was inconclusive. A form producing large eggs (about 170 μ) with distinctive membranes in northern Norway (*9*) probably belonged to *C. glacialis*, which was not then described. Similar large (178.6 ± 2.5 μ), membraned eggs were produced by *C. glacialis* in Frobisher Bay (June 1965), and aceto-orcein squashes revealed haploid chromosome sets of 17. It may be noted that the eggs of the two large forms of *Calanus* are about twice the volume of those of *C. finmarchicus*. Analogies with the polytenic form of *Pseudocalanus* are suggested.

Examples of "dualism" among freshwater copepods (*10*) and seasonal variations in egg size, especially where larger eggs occur in the warm season (*11*), should be examined more closely, along with bimodal size distributions (*7*), to see if polytenic and cryptic species are involved.

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Circadian Rhythm for Tryptophan Pyrrolase Activity and Its Circulating Substrate

Abstract. *Hepatic tryptophan pyrrolase and its circulating substrate, whole blood tryptophan, have a circadian rhythmicity in mice. Intact adrenocortical function is required for the normal rhythmicity of both enzyme and substrate although an altered but less apparent rhythm persists in the adrenalectomized state.*

Circadian rhythm for the enzyme, kidney transamidinase, has been well described as occurring in mice (*1*). The lighting regimen was the dominant synchronizer of this enzyme; reversal of day-night relationships could reverse enzyme rhythmicity. A dependent relation to plasma corticosterone rhythmicity was excluded. We now report

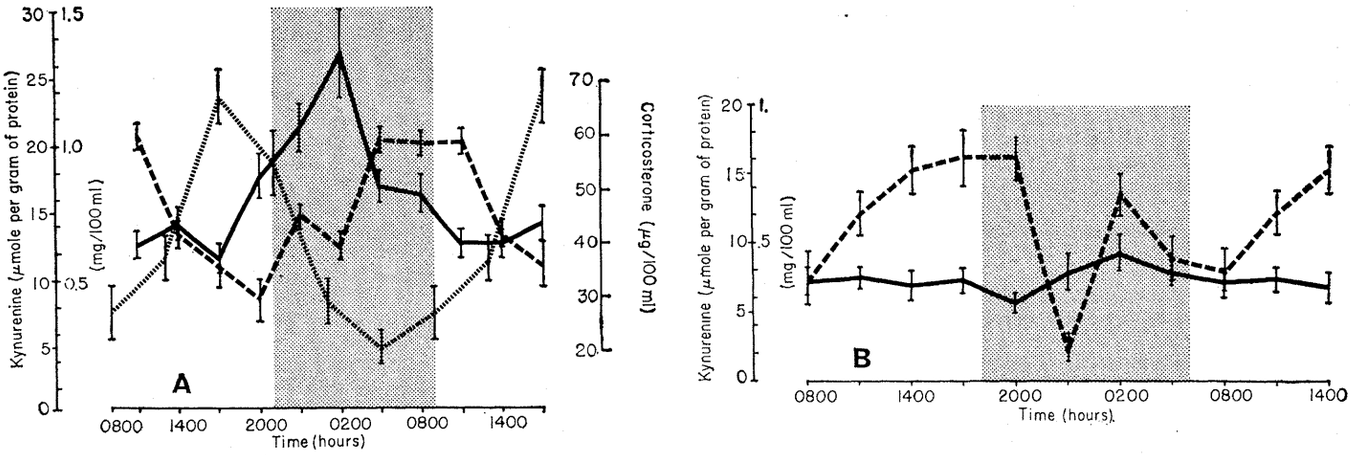

Fig. 1. The relation in normal CD-1 male mice (A) and in adrenalectomized, mice (B) of tryptophan pyrrolase activity (solid lines), whole blood tryptophan concentrations (dashed lines), and plasma corticosterone concentrations (dotted lines) over a 24-hour period. Shaded portion indicates dark.

that there is a circadian pattern for the hepatic enzyme, tryptophan pyrrolase (TP), as well as its circulating substrate, whole blood tryptophan. In contrast to kidney transamidinase, TP seems dependent on intact adrenocortical function for its rhythmicity.

Male CD-1 mice (Charles River) weighing 12±2 g were used. Mice of the same strain and of similar weight, adrenalectomized 5 to 7 days earlier, were used for comparison. Mice were housed in an environmental chamber at 21°±2°C with artificial lighting maintained from 0600 to 1800 hours. They were fed Rockland mouse chow as desired; the drinking water for adrenalectomized mice contained 0.9 g percent NaCl. At various periods, groups of four mice were killed and the livers were removed, pooled, and homogenized in seven volumes of cold 0.14*M* KCl containing 0.0025*N* NaOH. The homogenate was centrifuged at 11,000*g* (Spinco centrifuge) for 30 minutes. The enzyme-containing supernatant was incubated for 60 minutes at two concentrations (*2*) with added hematin (*3*) for determination of TP activity. Results were expressed as micromoles per hour of kynurenine formed per gram of biuret protein.

Six to eight separate groups of mice were sacrificed at 3-hour intervals. Immediately before the animals were killed and the livers removed, whole blood was obtained by cardiac puncture, and each sample was placed on a separate piece of filter paper (Schleicher and Scheull 903) and analyzed by the method of Efron, *et al.* (*4*) with a Spinco analytrol densitometer. Large sheets of Whatman No. 3MM paper were used so that numerous samples with standards could be placed together on the same sheet of paper. Approximately 80 of the aforementioned blood samples were analyzed at the same time and in the same solvent system to minimize variations in technique. The chromatograms were sprayed with ninhydrin and developed in an oven at 110°C for 8 minutes. In this system, it is difficult to separate tryptophan from valine and methionine. Therefore identical samples of whole blood were again chromatographed with a tryptophan standard. These chromatograms were not stained. The areas corresponding to the tryptophan position were eluted and analyzed quantitatively by the modified Fischl procedure (*5*). Results were expressed in milligrams per 100 milliliters.

Plasma corticosterone determinations were made by a fluorometric technique (*6*). Each determination represented pooled plasma of four mice. Six pools were obtained every 3 hours.

Determinations of enzyme activity in intact mice at various times of the day and night are shown in Fig. 1A with each point representing the mean ± one standard error (S.E.). In agreement with Squibb's observations (*7*) of a circadian periodicity to serum amino acid concentrations in chickens, individual amino acids separated by our technique in the mouse plasma also exhibit cyclic peak activities. Peak tryptophan concentrations reach a plateau between 0200 and 0800 hours.

A marked reduction in TP activity is apparent with values less than 30 to 50 percent of those in intact mice (Fig. 1B). Furthermore, the previously observed circadian patterns in the intact mice are flattened and reversed.

The pattern of circulating tryptophan is also affected by adrenalectomy. Overall concentrations are lower with flattening and reversal of the amino acid rhythm so that peak values are obtained between 1400 and 2000 hours. The lowest concentration occurs at 0800.

The presence of a normal circadian rhythm in mice for tryptophan pyrrolase activity and its circulating substrate is apparent from these studies. The requirement for intact adrenocorticoid function to maintain the normal rhythmicity is equally apparent. Data presented are insufficient to define the exact relation between enzyme activity and substrate values and the relation of these to plasma corticosteroids.

Large doses of infected tryptophan increase enzyme activity in rats within 4 hours of administration (*8*). However, we observed peak activity of TP 15 to 21 hours after peak tryptophan values. Thus, the circadian rhythm of circulating tryptophan is not a likely basis for rhythmic changes in enzyme activity. From our data it is impossible to determine whether the change in circulating tryptophan is a result of rhythmicity of hepatic TP.

Adrenalectomized mice had a lower, less sharpened, and reversed rhythmicity of amino acid and enzyme. Whereas an intact adrenal gland is required for normal periodicity, the periodicity is altered in the absence of intact adrenocortical function. The mechanism of enzyme and amino acid rhythmicity in the adrenalectomized state has not been elucidated by these studies.

Exogenous cortisol administration increases hepatic protein synthesis. Furthermore, there are circadian patterns in DNA synthesis and glycogen metabolism in the liver (*9*). Possibly

TP synthesis occurs in a circadian fashion, and this enzyme may represent only part of an overall rhythmic pattern in hepatic protein synthesis. Also, intact adrenocortical function may play at least a permissive role in the maintenance of this rhythmicity.

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Rate of Protein Synthesis: Regulation during First Division Cycle of Sea Urchin Eggs

Abstract. *Protein synthesis in fertilized sea urchin eggs, or in 12,000g supernatants derived from them, increased linearly during the period preceding prophase of the first mitotic cycle, dropped during metaphase and anaphase, and increased again after telophase. Similar results were observed for whole cells incubated in the presence of colchicine. These changes in the rate of protein synthesis during the mitotic cycle may be regulated at the translational level.*

The overall rate of protein synthesis in the fertilized sea urchin egg increases throughout the early cleavage stages until blastulation (*1*). In addition to this general increase, fluctuations exist in the rate of protein synthesis during the first few division cycles (*2*). Similar variations have been observed in dividing mammalian cells in tissue culture (*3*). We report here that these variations in the rate of incorporation of labeled amino acids into protein occur in vivo, in vitro, and in the presence of colchicine, and they are detectable at the polysome level during the first division cycle of the sea urchin egg.

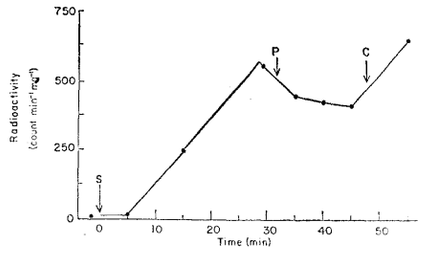

Fig. 1. Incorporation of C^{14}-leucine (231 mc/m*M*) into 5 percent trichloroacetic acid–precipitable protein during the first division cycle. At the indicated time after fertilization the eggs (at 26°C) were "pulse" labeled for 2.5 minutes with C^{14}-leucine (0.03 μc/ml). Eggs were fertilized at zero time. Radioactivity is expressed as counts per minute per milligram of protein; time, as minutes after fertilization. *S* indicates when sperm was added; *P* is prophase; and *C*, cleavage.

Gametes of the sea urchin (*Lytechinus variegatus*) were obtained by the KCl method described by Harvey (*4*). Eggs were washed three times in filtered sea water, washed three more times in Millipore-filtered sea water, and then incubated in Millipore-filtered sea water containing 75 μg of streptomycin sulfate and 300 units of penicillin per milliliter.

The eggs in suspension were fertilized, and portions of the suspension were transferred to separate incubation vessels. After fertilization, eggs were exposed to C^{14}-amino acids for the times indicated in each experiment. Unfertilized control eggs were treated similarly. After incubation an equal volume of 10 percent trichloroacetic acid (TCA) was added and the resultant precipitates were homogenized. These homogenates were then centrifuged and washed three times with 5 percent TCA, heated to 90°C in 5 percent TCA for 30 minutes, again washed three times with 5 percent TCA, and extracted twice with a mixture of ethanol and ether (3 : 1) and once with acetone. Dried precipitates were dissolved in 88 percent formic acid, plated on either preweighed aluminum planchettes or Whatman GF/C glass filter pads, and dried. Radioactivity was measured with a Nuclear-Chicago gas-flow counter or a Packard scintillation counter (efficiencies, 33 percent and 63 percent, respectively). No corrections for self-absorption were required. The amount of protein was determined by weight.

In the experiments on in vitro synthesis of protein, eggs were first incubated at 30°C to the appropriate stage, rapidly cooled to 5°C, washed three times with a mixture of cold isotonic NaCl and KCl (19 : 1) and once with cold homogenizing medium (0.01*M* $MgCl_2$, 0.24*M* KCl, and 0.01*M* tris-HCl, *p*H 7.6), resuspended in four

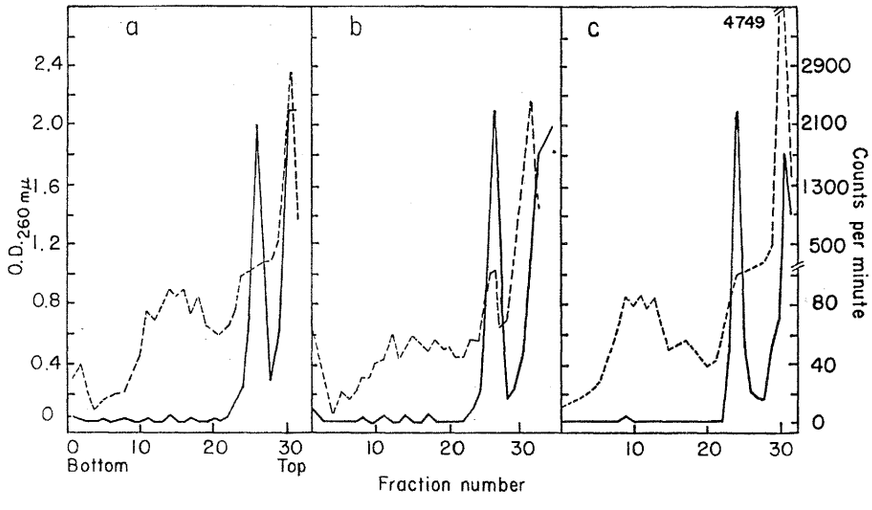

Fig. 2. Sucrose-gradient analysis of the 12,000*g* supernatant from eggs homogenized after exposure to C^{14}-leucine (231 mc/m*M*) at different stages during the first division cycle. Eggs were incubated in the presence of C^{14}-leucine from (a) 17 to 22 minutes, (b) 28 to 33 minutes, and (c) 43 to 48 minutes after fertilization. After fertilization eggs (at 30°C) were "pulse" labeled at the indicated times with C^{14}-leucine (0.025 μc/ml), then homogenized and centrifuged at 12,000*g* for 30 minutes; 0.25 ml of the supernatants of equal $O.D._{260}$ were layered on 29 ml of 15 to 50 percent sucrose gradients. The gradients were centrifuged for 3 hours at 24,000 rev/min in a SW-25 rotor. The polysome region lies between fractions 0 and 20. Solid line is $O.D._{260}$; dotted line is counts per minute.