gentle agitation. This was followed by washing with a large excess of C^{12}-L-valine and three washings with ice-cold sea water. The eggs were processed by a modification (*4*) of a filter-paper technique (*5*) for determination of the radioactivity of the proteins insoluble in trichloroacetic acid. A liquid scintillation spectrometer with a counting efficiency of 50 percent was used. A cell suspension consisting of a mixture of micromeres, mesomeres, and macromeres, in the normal ratio of 4:8:4, was prepared, incubated, and processed in similar fashion to the micromere suspension to serve as control. For cell counts, three 0.05-ml portions were taken from each suspension, appropriately diluted, and all the cells enumerated under the microscope at × 100 (Table 1).

On a "per-cell" basis, micromeres incorporate C^{14}-L-valine into protein at a rate about 17 to 32 percent of that exhibited by the mixed cell suspension. The diameters of micromeres, mesomeres, and macromeres are approximately 22 μ, 34 μ, and 44 μ, respectively, in *Lytechinus pictus*. Calculated as a sphere, the average volume of a cell in the mixed suspension (with the normal 4:8:4 ratio of numbers of micromeres to mesomeres to macromeres) is approximately 4.1 times that of a micromere. When the radioactivity of the micromeres is converted to radioactivity per unit volume (multiplication of column 2, Table 1, by 4.1) the results show no significant difference between micromeres and mixed-cell suspensions in ability to incorporate C^{14}-L-valine into protein.

It appears, then, that there are no marked differences in the rates of protein synthesis among these cell types. This conclusion is subject to the general or implied assumption in experiments in incorporation of labeled precursors into macromolecular substances in vivo, and sometimes in vitro, that there are no appreciable differences in the precursor pool. In the present instance there are no a priori reasons for expecting appreciable differences. Also, the relatively small changes in amino acid pool that have been noted (*6*) during these early stages of development in sea urchins tend to argue against there being appreciable differences in the different cell types.

At present, then, the results are consistent with the view (*4*) that the bulk of the protein synthesis during early development employs messenger RNA that was present (in inactive form) in the unfertilized egg. This maternal messenger RNA does not appear to be distributed differentially to any great extent among the three cell types of the 16-cell stage. While there is evidence (*7*) that new messenger is also being formed during this period, it, in turn, appears to remain inactive, or relatively so, until later stages of development.

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8. Supported by NSF grants G 21810 and GB-28 and NIH grant GM-12777.

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Rotifer Ecology and Embryological Induction

Abstract. *The rotifer* Asplanchna *releases into its environment a. water-soluble, nondialyzable, pronase-sensitive factor which causes uncleaved eggs of another rotifer,* Brachionus calyciflorus, *to develop into individuals with a pair of long, movable spines which neither their mothers nor the unaffected controls have. These appendages protect the* Brachionus *from* Asplanchna *predation.*

Beauchamp (*1*) briefly described a remarkable predator-prey relationship between the rotifers *Asplanchna* and *Brachionus calyciflorus* (*2*). When *B. calyciflorus* were introduced and maintained as food organisms in cultures of the considerably larger and predominantly carnivorous *A. brightwelli*, the female, parthenogenetic offspring of these prey animals had, in addition to the normal complement of three relatively short pairs of spines, a pair of long posterolateral spines—structures that were completely lacking in the previous generation. The production of these extra spines was mediated by a factor released into the medium by *Asplanchna* and represents a phenotypic response of undoubted adaptive significance for the *B. calyciflorus*, long-spined forms being more difficult for *Asplanchna* to eat.

This interaction between *Asplanchna* and *B. calyciflorus* is of such unusual biological interest with respect to exogenous substances, population dynamics, evolutionary biology, and embryological induction that a more detailed analysis of the phenomenon was undertaken (*3*).

Clones of *Asplanchna sieboldi* Leydig (*4*), collected by N. D. Meadow in the Philadelphia area, and a Lake Washington strain of *A. girodi* de Guerne were reared in an inorganic medium and fed *Paramecium bursaria* that had been harvested by centrifugation from dilute, xenic, wheat-grain media kept under constant illumination. A clone of *B. calyciflorus* from Lake Washington was cultured in inorganic medium and fed *Euglena gracilis* Klebs, strain Z, obtained from the Culture Collection of Algae at Indiana University (*5*). Stock cultures of all three rotifers were maintained in Syracuse watch glasses and incubated at 25°C in the dark. The *B. calyciflorus* clone never produced individuals with posterolateral spines unless the medium was affected by *Asplanchna*. Hereinafter, all references to spines are to the posterolateral spines, unless otherwise stated.

Newly hatched, spineless *B. calyciflorus* cultured in media extensively conditioned by *A. sieboldi* remain visibly unaffected throughout their lifetime, but their first offspring invariably have long posterolateral spines. Rotifers with these *Asplanchna*-induced evaginations of the body wall also have significantly longer anterior and posteromedian spines (Figs. 1 and 2, Table 1), the amount of elongation being roughly directly proportional to the length of the posterolateral spines. There are no associated changes in body length.

The altered morphology of these individuals is determined before the eggs from which they hatch are extruded from the maternal body cavity and begin to cleave. Lacto-orcein squash preparations and direct observations with a Zeiss-Nomarski differential interference-contrast microscope (*6*) showed that

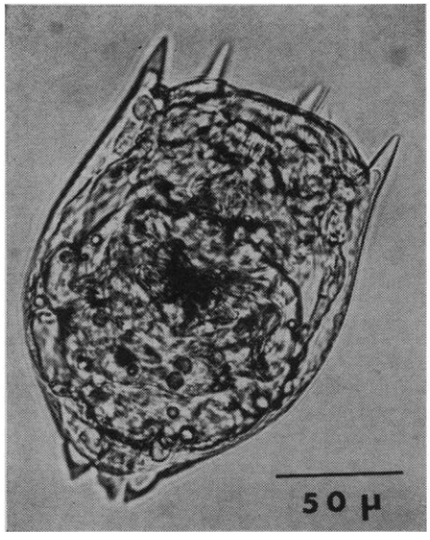

Fig. 1. Young, living *Brachionus calyciflorus* with no posterolateral spines. Note short posteromedian, anterolateral, and anteromedian spines.

eggs which have been extruded for up to 20 minutes have no more than a few nuclei, and usually have only one. Eggs formed in mothers grown in *Asplanchna*-conditioned medium and transferred to fresh medium immediately after extrusion develop into long-spined animals; those formed in mothers grown in fresh medium and transferred to *Asplanchna*-conditioned medium upon extrusion hatch into spineless individuals. The refractoriness of the uncleaved, extruded egg to the *Asplanchna*-factor could be due to very early embryonic determination of spine morphology or to inability of the factor to penetrate the membrane of the sensitive egg.

Body and posterolateral spine measurements were made in a microcompression chamber on live, long-spined *B. calyciflorus* twice during their lifetime—once when newly-hatched and again when nearly fully grown. Growth of these spines is highly negatively allometric whether the neonates are grown in *Asplanchna*-conditioned or fresh media, average k values (*7*) being 0.45 and 0.42, respectively ($t = 0.33$, $p < .8 > .7$). The *Asplanchna*-factor therefore has no effect on the postnatal growth of posterolateral spines.

The degree of spine production in the offspring of single, spineless mothers grown since birth in different samples of the same conditioned medium is affected by a number of variables. Especially in weakly conditioned media, there is a direct correlation between the volume of medium per mother and the extent of spine production in the offspring. Even when identical volumes of a given conditioned medium are used, however, there is great variability in spine production. Two factors are largely responsible for this. First, successive offspring from the same mother exhibit decreasing amounts of spine production. This is a consistent pattern which occurs too rapidly to be explained by the gradual deactivation of conditioned medium that occurs under similar experimental conditions but in the absence of *B. calyciflorus* (Table 2). Second, spine production in offspring of the same parity varies directly with maternal growth rates when a volume of 1 ml or more of conditioned medium is used for each mother. These effects must be controlled before spine production in *B. calyciflorus* can validly assay the activity of *Asplanchna*-conditioned media.

The minimum of *Asplanchna*-conditioning that will induce the first offspring of single, spineless mothers grown in 1 ml of medium to produce posterolateral spines of near maximum size is about .070 mm^3 of living *A. sieboldi* per milliliter for 30 minutes (*8*). When diluted 1 : 15 and 1 : 63, the conditioned medium has slight and no activity, respectively. Media conditioned by *A. girodi* and *A. sieboldi* are similarly active when comparable volumes of each species are used.

Asplanchna-conditioned media and aqueous extracts of *Asplanchna* were prepared and then assayed for activity by using them as culture media for newly hatched, spineless *B. calyciflorus*. Assays were made both by serially diluting the test media and by measuring the extent of spine production in the offspring. When heated at 100°C, no appreciable loss of activity occurred un-

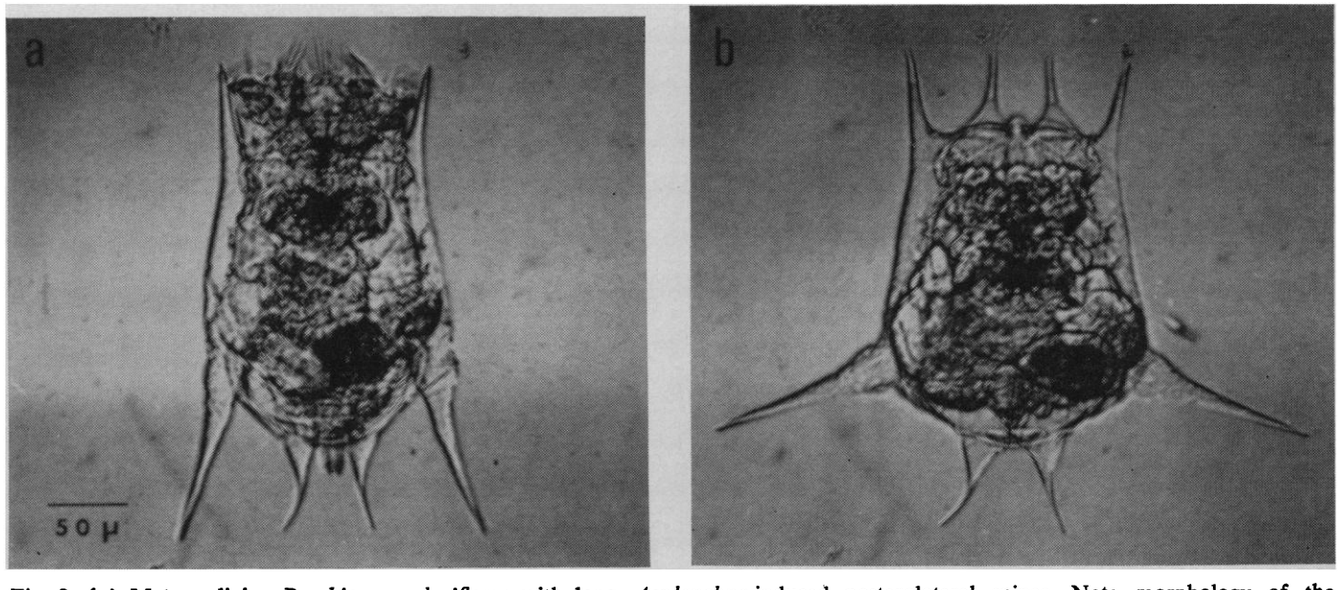

Fig. 2. (*a*) Mature, living *Brachionus calyciflorus* with long, *Asplanchna*-induced posterolateral spines. Note morphology of the articulation of these spines with the body and long posteromedian spines. (*b*) Same living specimen as in (*a*) but with retracted corona, posteriorly displaced internal structures, and laterally extended posterolateral spines. The long anterolateral and anteromedian spines can be seen because corona has been withdrawn.

Table 1. Average spine and body lengths, with 95 percent confidence limits, of adult *Brachionus calyciflorus* after culture for an equal number of generations with and without *Asplanchna*-factor.

Spine length (μ)				Body length (μ)
Postero-lateral	Postero-median	Antero-lateral	Antero-median	
		Without Asplanchna-*factor*		
0	26.3±3.1	19.2±1.2	33.9±2.1	211±5.8
		With Asplanchna-*factor*		
70.7±7.8	38.6±2.8	39.2±2.7	46.9±2.1	210±6.9

Table 2. Effect of age on the activity of media conditioned by .070 mm³ *A. sieboldi* per milliliter for 30 minutes and stored at 25°C for periods of up to 9 days. Activity is expressed as mean ratios of spine length to body length of first offspring from replicate assays, with 95 percent confidence limits.

Activity of *Asplanchna*-conditioned media of various ages (mean ratio of spine length to body length)				
0–1 day	2–3 days	4–5 days	6–7 days	8–9 days
0.264±.030	0.127±.019	0.104±.069	0	0

til after 5 to 10 minutes. Thereafter, activity gradually decreased [sometimes remaining] for 60 to 80 minutes. Conditioned media, but not extracts, were deactivated by passage through a 0.45-μ Millipore filter. After conditioned medium had been centrifuged at 105,400*g* for 3 hours, the upper layer had lost no activity. The active factor, which thus appears to be water-soluble, is nondialyzable and resistant to treatment with both .01*N* $NaIO_4$ for 8 hours at 25°C in the dark and .01 percent ribonuclease (Calbiochem, 5X crystallized, bovine pancreas) for 1 hour at 37°C. Incubation of conditioned media with .01 percent pronase (Calbiochem, type B) for 2 hours at 37°C destroyed all activity; the result suggests that the *Asplanchna*-factor is a protein. That conditioned medium is somehow deactivated by mixing with bentonite and by extraction with chloroform provides corroborating evidence for this interpretation.

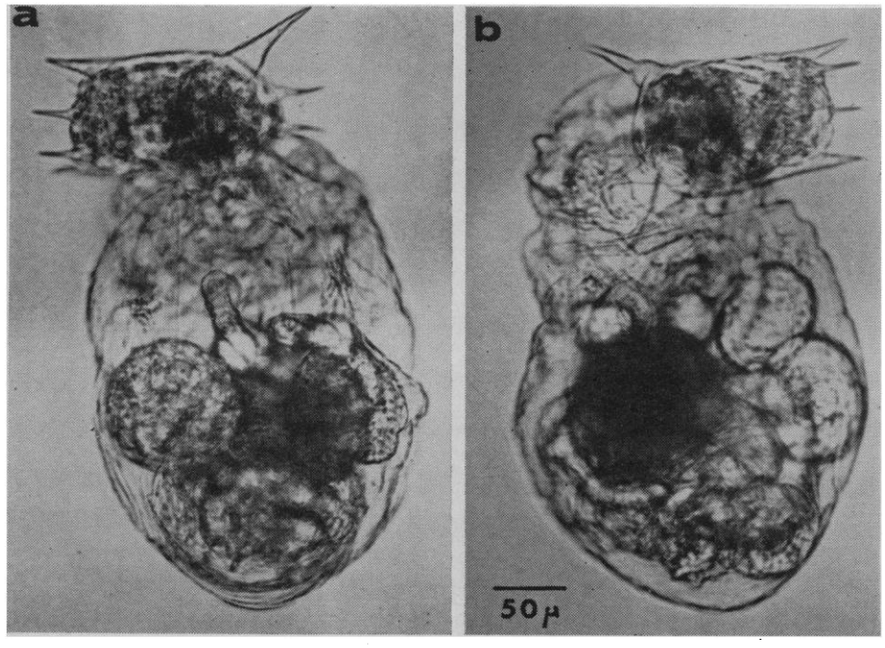

Fig. 3. *Asplanchna sieboldi* in process of trying to capture a *Brachionus calyciflorus* with long, *Asplanchna*-induced posterolateral spines. Note coronal retraction and posterolateral spine extension of the *B. calyciflorus*. The inedibility of these long-spined organisms is clearly illustrated. (*a*) Predator attempting to entrap prey in its corona. (*b*) Predator and prey soon after initial contact.

The long posterolateral spines of *B. calyciflorus* differ from those of other species in the genus in that they have an articulation with the body. They usually project backward, parallel to the long axis of the body, but extend laterally whenever the animal's corona retracts and causes positive pressure inside the body cavity (Fig. 2). The length and coordinated movements of these spines provide mechanical defense against predation by *Asplanchna*. If a *B. calyciflorus* touches or is touched by a sizeable object, its corona by reflex withdraws for as long as the stimulus persists. Thus when a long-spined form is contacted by an *Asplanchna*, this same reflex response will cause an extension of the spines and thereby possibly prevent the predator from trapping or swallowing it (Fig. 3).

The degree to which a spined *B. calyciflorus* evades *Asplanchna* predation depends on the size of its body and posterolateral spines and on the size, species, and degree of satiation of the *Asplanchna*. Hungry, adult *A. girodi* (750 μ in length) capture 25 percent of the young, spineless *B. calyciflorus* (150 μ in length) contacting their corona and manage to ingest 33 percent of these, but they are completely unable to capture young animals with spines in the 60- to 80-μ range. Hungry, young *A. sieboldi* (560 μ in length) capture 89 percent and 14 percent, respectively, of the young spineless and spined *B. calyciflorus* contacted; all of the captured animals are ingested. Hungry, adult *A. sieboldi* (870 μ in length) capture 100 percent of the adult. spineless *B. calyciflorus* (200 μ in length) that they contact but only 78 percent of those with spines in the 65- to 100-μ range; practically all (> 95 percent) of the captured animals are subsequently ingested, although sometimes only with great difficulty. Individuals of both species of *Asplanchna* become satiated when they ingest several *B. calyciflorus* in rapid succession, and they fail to respond with reflex searching and grasping movements when additional prey contact their corona.

While *Asplanchna*-induced spine pro-

duction is probably highly adaptive to *B. calyciflorus* in nature, the effect of the phenomenon on *Asplanchna* is more difficult to assess. If alternative food species in great abundance were available to *Asplanchna*, whose diet includes ciliates, rotifers, cladocerans, and colonial algae, a complete or partial restriction in the intake of one food organism—*B. calyciflorus*—might have little or no effect on its total input. However, if *B. calyciflorus* and *Asplanchna* comprised a very high proportion of the total net plankton, as they often do, the effect could be either detrimental or beneficial. For example, a species like *A. girodi*, which is unable to capture long-spined *B. calyciflorus*, might soon starve. On the other hand, a species like *A. sieboldi*, which is only partially prevented from capturing and ingesting long-spined forms, might avoid the danger of rapidly depleting its own food supply and persist in a more or less stable association with its prey.

The morphological response of *B. calyciflorus* to the *Asplanchna*-factor is probably a frequent occurrence in nature and not an artifact. An extensive survey of the literature and numerous personal observations indicate that there is usually a very close correlation between the appearance in natural populations of long-spined *B. calyciflorus* and the presence of *Asplanchna* (*9*). More conclusive are our findings that the amount of *Asplanchna*-factor in supernatants of net-filtered pond water, as determined by spine production assays in the laboratory, is correlated both with the abundance of *Asplanchna* and with the spine length of *B. calyciflorus* in the sampled environment (*10*). The existence of threshold quantities of *Asplanchna*-factor in nature is consistent with its stability at 25°C (Table 2) and the small amount of *Asplanchna*-conditioning required to activate fresh media in the laboratory.

The importance of exogenous substances in the regulation or dynamics of populations is becoming increasingly evident. The direct influence of vitamins and inhibitors on the reproduction of natural populations is considerable, and there is a growing body of data showing or suggesting that more or less specific substances released into the environment have direct and vital effects on a wide variety of life history phenomena, such as the control of asexual and sexual stages. The *Asplanchna*-factor seems to be unique, however, in that it is a specific embryological inducer having a dramatic and direct effect on another organism's morphology. As a result, it also indirectly controls the ecological interactions of predator and prey between the two species populations.

The *Asplanchna*-factor differs from classical embryological inducers in several important respects. First, it is a substance produced by one species which affects the developmental pattern of another species. Second, it exists in effective concentrations and in a free state in the organisms' external environment. Typical inducer substances both form and operate within a single organism and are closely associated with cells or cell layers. Finally, the *Asplanchna*-factor acts prior to cleavage, probably during oogenesis, whereas other inducers appear and exert their influence during or after gastrulation.

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References and Notes

1. P. de Beauchamp, *Compt. Rend.* **234**, 573; *ibid.* **235**, 1355 (1952).
2. *Brachionus calyciflorus* is preferable to, but synonymous with, *B. pala*, which is the name used by Beauchamp.
3. The present article is a brief summary of an extensive report now in preparation.
4. Beauchamp's *A. brightwelli* sensu latissimo includes *A. sieboldi* and the poorly defined *A. intermedia* [P. de Beauchamp, *Bull. Biol. France Belg.* **85**, 137 (1951)].
5. J. J. Gilbert, *J. Exp. Zool.* **153**, 113 (1963).
6. I thank R. D. Allen, Princeton University, for the privilege of using this instrument.
7. $k = dy \cdot x/dx \cdot y$, where x and y are the first body and posterolateral spine-length measurements and dx and dy are the length increments in these structures after the second measurements.
8. Conditioned media were prepared by allowing a number of live, starved *A. sieboldi* adults (675 to 750 μ in length) to remain in as many milliliters of fresh, food-free medium for 30 minutes. *Asplanchna* volumes were calculated by measuring living animals in a microcompression chamber and assuming their shape to be cylindrical.
9. Temperature and probably food density [H. Buchner, F. Mulzer, F. Rauh, *Biol. Zentralbl.* **76**, 289 (1957); L. A. Erman, *Zool. Zh.* **41**, 998 (1962); F. Rauh, *Z. Morphol. Oekol. Tiere* **53**, 61 (1963)] also affect posterolateral spine production, often making field interpretations of this sort very difficult. A report that sodium silicate induces spine production [D. D. Whitney, *Biol. Bull.* **31**, 113 (1916)] is of questionable validity because several authors have been unable to repeat the work [P. de Beauchamp, *Bull. Biol. France Belg.* **62**, 51 (1928); K. Kikuchi, *J. Fac. Sci. Univ. Tokyo Sect. 4* **2**, 163 (1931); and J. J. Gilbert, in preparation].
10. Data on this subject have been obtained both by me and, more recently, by J. Waage, whose work was supported by NSF undergraduate summer fellowship GE-6247.
11. Supported by USPHS postdoctoral fellowship (IF2 GM-20, 171-01) and NSF grant GB-3166.

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Low-Molecular-Weight Proteins Related to Bence Jones Proteins in Multiple Myeloma

Abstract. *Urinary proteins distinct from Bence Jones proteins, but sharing antigenic determinants, were found in the urine of a number of patients with multiple myeloma. These components were smaller in size and antigenically deficient compared with Bence Jones proteins. They were best detected with antiserums to the homologous Bence Jones proteins and, in some cases, were related to the variable portion of the Bence Jones protein molecule.*

Considerable interest has been focused recently on Bence Jones proteins because of their potential significance with regard to the structure of antibodies. Several types of heterogeneity of Bence Jones proteins from single individuals have been described (*1*).

Recently, several investigators have noted the presence of a low-molecular-weight protein, related to Bence Jones protein, in the urine of single patients with multiple myeloma (*2*). Observations on another such patient, followed over a period of years, stimulated more detailed studies of other patients with multiple myeloma. It was found that such low-molecular-weight proteins occurred frequently in the urines of these individuals.

Daily urine samples were obtained in bottles containing NaN_3 and stored at 4°C. The urines were filtered through Whatman No. 12 paper and desalted either by passage through G-25 Sephadex columns or by dialysis in 23/32 Visking tubing against distilled water. Antiserums to isolated κ- and λ-type Bence Jones proteins were prepared in rabbits. Immunoelectrophoresis, agar-diffusion analysis, and ultracentrifugation were performed as described (*3*).

Immunoelectrophoresis analysis of the urine of one of the patients studied (Gr), revealed several patterns of precipitation lines which depended in part on the particular antiserum to Bence Jones protein employed. Some antiserums showed only the Bence Jones protein as a single, relatively homogenous component. Other antiserums showed additional components, in the same urine specimen, which, however, gave a reaction of identity with the patient's Bence Jones protein. Still other antiserums revealed that