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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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

these components were antigenically deficient compared with the Bence Jones protein. Figure 1*b* shows the isolated Bence Jones protein of Gr as a single homogeneous arc extending from the application well toward the cathode. However, in the crude urine (Fig. 1*a*) the same antiserum reveals an additional arc which extends further to the cathode. This component is antigenically deficient as compared to the Bence Jones protein. A similar picture was observed in many other urines, and antigenically deficient components were detected with a variety of antiserums to heterologous as well as to the homologous Bence Jones protein. The homologous antiserums, however, proved more useful for recognizing these components, particularly when patients excreted λ-type Bence Jones proteins. In Fig. 1*d*, for example, one antiserum (to Bence Jones protein Ne)

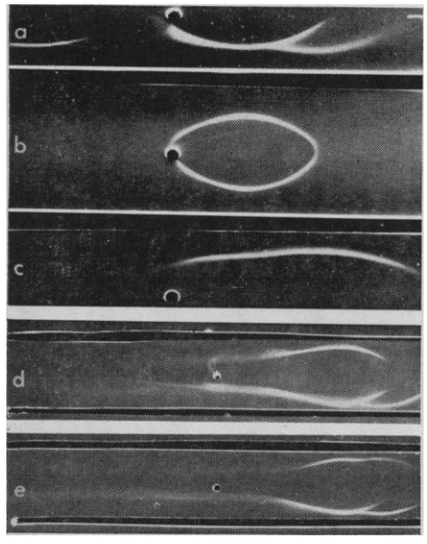

Fig. 1. Immunoelectrophoresis of the low-molecular-weight components in the urines of two different patients with Bence Jones proteinuria. The anode is to the left. (*a*, *b*, *c*) Preparations from patient Gr (κ-type) detected with antiserum to Ro in the troughs. The crude urine (*a*) shows the deficient component on the cathode side of the Bence Jones protein. The isolated Bence Jones protein (*b*) and the isolated deficient components (*c*) are shown for comparison. (*d*, *e*) Crude urine from patient Da (λ-type). In (*d*) the upper trough contains heterologous antiserum (antiserum to Ne), which detects the Bence Jones protein but fails to detect the deficient component, the lower trough with homologous antiserum (to Da) clearly shows the deficient component. In (*e*) the lower trough again contains the antiserum to Da while the upper trough contains this antiserum absorbed with a heterologous Bence Jones protein.

shows only one component, the Bence Jones protein, in the urine of patient Da. On the other hand, antiserum to Bence Jones protein Da reveals an additional component which is antigenically deficient compared with the Bence Jones protein.

Vacuum dialysis of urine through Visking tubing of varying permeability (*4*) provided information on the relative molecular size of the antigenically deficient components. Fresh desalted urine was "ultrafiltered" through 8/32 Visking tubing at 660 mm-Hg. The urine was concentrated approximately 100-fold, and the dialyzate was lyophilized. The Bence Jones protein dimers and most of the monomers were retained by this membrane (*5*). The antigenically deficient components were markedly concentrated in the dialyzate although still contaminated primarily by Bence Jones protein monomers. Both types of protein were contained within reinforced 23/32 Visking tubing under 660 mm-Hg pressure (*6*).

Density-gradient ultracentrifugation provided additional evidence that the antigenically deficient components were smaller than Bence Jones proteins. Crude urine samples as well as material obtained in 8/32-Visking dialyzates were applied to a sucrose gradient (5 to 20 percent) and centrifuged at 36,000 rev/min for 18 hours. In immunoelectrophoresis (Fig. 1), the patient's Bence Jones protein was present in the lower portion of the gradient (Fig. 1*b*). In contrast, the antigenically deficient component was present in the uppermost portion of the gradient (Fig. 1*c*). Gradient studies of the urines of other patients always showed that the antigenically deficient components sedimented more slowly than the Bence Jones protein.

The low-molecular-weight components from the urine of patients (Gr, Ha, and Di) who excreted κ-type Bence Jones proteins were isolated and studied in detail. The isolation was carried out initially by vacuum dialysis of the urine through 8/32 Visking tubing. Further purification of the components in the dialyzate was achieved by gel filtration or by zone electrophoresis. In gel-filtration experiments (Sephadex G-100 in 5*M* guanidine) the antigenically deficient components were eluted after the Bence Jones protein. In some instances the urinary components were purified further by density-gradient ultracentrifugation. Agar-diffusion analysis (Fig. 2, top) shows that the isolated low-molecular-weight proteins (wells 2, 4, 6) are antigenically deficient compared to the isolated Bence Jones proteins (wells 1, 3, 5). Analytic ultracentrifugation of the isolated antigenically deficient components from patient Gr revealed an observed sedimentation coefficient of 1.2*S*.

The isolated antigenically deficient components showed electrophoretic heterogeneity, particularly so in the material isolated from patient Gr. Starch-gel electrophoresis of the urine (Fig. 2, lower pattern) showed several protein bands cathodal to the origin as well as a heavy band in the anodal portion of the gel. This heavy band corresponded to the isolated Bence Jones protein (Fig. 2, middle gel pattern). The upper gel pattern of the isolated small urinary protein shows two bands in the cathodal region. These two components were isolated from each other by zone electrophoresis. They had similar gel-filtration properties and gave a reaction of identity with different antiserums.

The small urinary components were

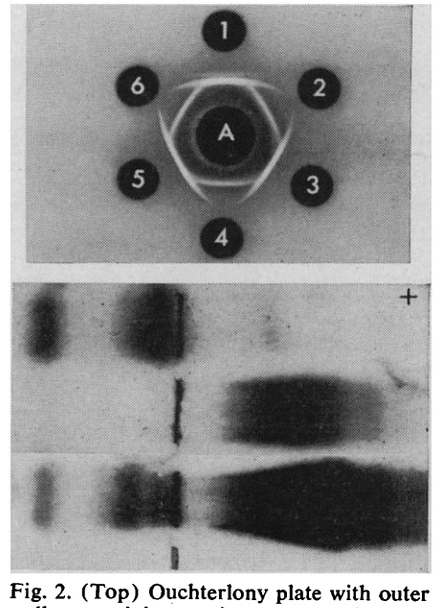

Fig. 2. (Top) Ouchterlony plate with outer wells containing various preparations of isolated Bence Jones proteins (wells 1, 3, 5) and isolated low-molecular-weight components (wells 2, 4, 6). Center well (A) contains antiserum to Ro Bence Jones protein. (Bottom) Starch-gel electrophoresis (glycine buffer, *p*H 8.8) of preparations from patient Gr. The crude urine at high concentration is shown at the bottom with the isolated low-molecular-weight components at the top and the isolated Bence Jones protein in the middle. The anode is to the right.

always found to be antigenically deficient when compared with Bence Jones proteins. No antiserums showed that these components form spurs over Bence Jones proteins. Additional immunoelectrophoresis experiments were performed with antiserums to Bence Jones protein that were absorbed with heterologous Bence Jones proteins. In one such experiment (Fig. 1*e*) the absorbed antiserum (upper trough) no longer shows the spur of Bence Jones protein over the second component; there is a line of identity between the two proteins. Antiserum to Bence Jones protein Ro proved of particular value after absorption with Bence Jones protein Cu. These proteins, intensively studied by Hilschmann and Craig (*7*), are homologous in the COOH-terminal half and show extensive differences in the NH_2-terminal half. The deficient component in the urine of Ro and of a number of other patients as well, continued to react with antibody to Ro absorbed with Cu; but the spur of the Bence Jones protein was no longer evident. It appeared that, in these instances, the deficient components were related to the variable portion of Bence Jones protein molecule in the NH_2-terminal half (*7*, *8*). However, two urines gave quite different results, and various similar absorption experiments always removed the line for the deficient component with only a diminution of the Bence Jones line.

The small components related to Bence Jones proteins were clearly detected in the urine of 9 out of 24 patients excreting κ-type, and 7 out of 22 patients excreting λ-type Bence Jones proteins. The detection of these smaller proteins in approximately one-third of the patients probably represents the minimum incidence in that, in some patients, not enough material was available and antiserums to each patient's Bence Jones protein were not available. The amounts of these proteins found in urine were usually quite low, in the range of a few percent of the quantity of Bence Jones protein; the largest amount was noted in patient Gr where it represented 10 to 15 percent of the Bence Jones protein.

These small components were detected in freshly voided urine and, in the case of Gr, were found at similar concentration in 20 different fresh urine samples taken over extended periods of time. In order to find out whether these proteins represented products of degradation in vitro of Bence Jones protein, isolated I^{131}-labeled Bence Jones protein (from patient Gr) free of small components was added to the urine which was then processed over a period of 72 hours. Starch-gel electrophoresis of the purified material revealed no radioactivity in antigenically deficient components. However, evidence was obtained for some increase in similar small components in other, older urine preparations, and such preparations were excluded from the study. These appeared to be enzymatic degradation products because incubation of concentrated urine preparations at 37°C, particularly at low *p*H, produced various new antigenic components in certain specimens.

The significance of the low-molecular-weight components remains obscure. They were readily separated from the dimer and monomer forms of Bence Jones protein and could be repeatedly demonstrated in fresh urine specimens from the same individual. However, it became evident that a number of precautions in the handling of the urine specimens was necessary to avoid loss of these components in the process of isolation of the Bence Jones proteins as well as to avoid production of low-molecular-weight material through degradation. It remains to be determined whether the deficient components might be synthesized independently or represent catabolic breakdown products of Bence Jones proteins.

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5. Solutes of lower than 28,000 molecular weight are not or only partially retained under these conditions (*4*).
6. Solutes of greater than 10,000 to 15,000 molecular weight are retained under these conditions (*4*).
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Flavin Mononucleotide Control of Glycolic Acid Oxidase and Photorespiration in Corn Leaves

Abstract. *Enzyme preparations from young corn shoots lacked the coenzyme, flavin mononucleotide, that is required for glycolic acid oxidation. When the coenzyme was added to the shoots, the rate of carbon dioxide production during photosynthesis increased. Shoots of wheat or oats did not lack the coenzyme.*

In air and in light of high intensity, detached leaves of tobacco, wheat, and oats can lower the concentration of carbon dioxide in a closed container to about 50 parts per million (ppm) by photosynthesis (*1*, *2*). This equilibrium is the carbon dioxide compensation concentration. It varies as a linear function of the concentration of oxygen around the leaf (*3*). On the other hand, young corn shoots have a carbon dioxide compensation concentration of less than 5 ppm in air (*2*, *4*). Apparently corn shoots produce little if any carbon dioxide during photosynthesis, so that the equilibrium value is very low, while the amount produced by leaves from other plants changes with the concentration of oxygen. The sensitivity of carbon dioxide compensation to high concentrations of oxygen and the known effect that a change in oxygen concentration has on glycine and glycolic acid metabolism in photosynthesis (*5*) suggest that glycolic acid oxidase (GAO) is important in carbon dioxide production during photosynthesis. The enzyme is present in high concentration in leaves (*6*). It has a low affinity for oxygen, which might be expected from the cofactor requirement of flavin mononucleotide (FMN) (*7*).

Taken together, these conclusions lead to the prediction that the glycolic acid oxidation pathway is not active in young corn shoots. There are conflicting reports on the GAO activity in corn shoots (*6*, *8*, *9*), and I report now on rates of oxygen consumption