Table 2. Comparison of agar and methyl cellulose as overlay; read 7 days after inoculation when plaques were 1 to 2 mm in diameter. PFC, plaque-forming cells.

Dilution	Plaque counts	Av.	PFC per ml
Methyl cellulose as overlay			
$10^{-3.5}$	*		
10^{-4}	42, 39	40.5	4.1×10^6
$10^{-4.5}$	22, 15	18.5	5.8×10^6
10^{-5}	5, 8	6.5	6.5×10^6
Agar as overlay			
$10^{-3.5}$	*		
10^{-4}	36, 47	41.5	4.2×10^6
$10^{-4.5}$	15, 22	18.5	5.8×10^6
10^{-5}	6, 6	6	6.0×10^6

* Too numerous to count in the two plates used at dilutions indicated.

These grew in size from 1 to 2 mm to 2 to 3 mm between the 7th and 10th days after inoculation (Fig. 1) but the counts remained relatively constant. A linear relationship between the concentration of cells inoculated and number of plaques observed was obtained (Table 1 and Fig. 2). The number of plaques counted represented 10 to 33 percent of the total cells (infected plus noninfected) seeded onto the monolayers. The plaques observed under agar became larger and were somewhat easier to count than the plaques seen under methyl cellulose. The counts were comparable, however (Table 2). This is in contrast to other experiments performed in this laboratory with herpes simplex virus where agar appears to inhibit plaque formation. Routine plaque counts are now being carried out 7 to 10 days after inoculation. Incorporation of 5-iodo-2-deoxyuridine completely inhibited the formation of plaques by cells infected with zoster virus. Serum from convalescent patients with varicella infection incorporated in the overlay did not inhibit plaque formation, although the serum contained zoster antibodies as measured by the immunofluorescent technique.

Under the same conditions cytomegalovirus-infected cells have not formed plaques; therefore, the technique described here may be useful in distinguishing these closely related viruses. The technique may also prove useful for other viruses for which difficulty is encountered in preparing cell-free extracts. Studies are now under way to determine the kinetics of the spread of zoster virus in human fibroblasts, to study plaque-purified lines of infected cells, and to obtain cell-free viable virus (*4*).

FRED RAPP
MATILDA BENYESH-MELNICK
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

References and Notes

1. T. H. Weller, *Proc. Soc. Exptl. Biol. Med.* **83**, 340 (1953).
2. ———, H. M. Witton, E. J. Bell, *J. Exptl. Med.* **108**, 843 (1958).
3. D. Taylor-Robinson, *Brit. J. Exptl. Pathol.* **40**, 521 (1959).
4. The technical assistance of Darlena Vanderslice is gratefully acknowledged. This work was supported by research grants CA-04600 from the National Cancer Institute and AI-05398 from the National Institute of Allergy and Infectious Diseases.

3 May 1963

Division of Labor among Primitively Social Bees

Abstract. *Australian bees of the genera* Allodapula *and* Exoneura *which commonly live in colonies of two to several individuals exhibit a division of labor among the adult females. Most foraging individuals are unfertilized and have slender ovaries, yet gather pollen and carry it to the nest; in contrast, fertilized egg-laying bees are not commonly foragers. Such castes are functionally similar to workers and queens found in some members of two other families of bees and represent a noteworthy example of physiological and behavioral parallelism in which the activities of different individuals are coordinated to form a functional unit.*

Among well known social bees (*Apis, Trigona, Melipona, Bombus,* all in the family Apidae) it is obvious that a division of labor exists between the female castes; the queens lay most of the eggs and the workers perform most other functions in the colony. Less extreme but similar division of labor among females occurs in some groups of a wholly unrelated family, the sweat bees or Halictidae (*1*) which usually live in colonies of very few (often less than five) individuals. In this family external morphological differences between the castes are absent or negligible. In the majority of bees there is no cooperative activity in nest construction or provisioning and no division of labor; each female makes and provisions her own cells, usually, but not always (*2*), in an independent nest. A small group of genera of the family Xylocopidae (*Allodape, Exoneura,* and their relatives) has long been known to care for larvae progressively and in this group two or more females are sometimes found in a single nest (*3*). It therefore seemed desirable to learn if there are workerlike females showing cooperative activity in this group as well as in Apidae and Halictidae.

Table 1. Ovarian and spermathecal conditions of females collecting pollen.

Individuals observed	Spermatheca without sperms	Ovaries slender	Month
	Allodapula spp. (*5*)		
24	22	14	Various
	*Exoneura bicolor**		
22	19	20	November
	Exoneura variabilis †		
26	21	25	February
	Exoneura robusta †		
3	1	3	October
	Exoneura hackeri †		
2	0	2	December

* From the New England highlands of New South Wales. † From localities in southeastern Queensland.

In nonsocial bees, or any kinds of bees in which there are no workers and each female makes and provisions her own cells, females collecting pollen from flowers have enlarged ovaries and sperm cells in the spermathecae; as soon as she has provisioned a cell, such a bee lays an egg in it. Pollen collecting and transport by bees with slender ovaries is evidence of worker-like activities, especially if the bees are also unfertilized.

Data based on studies of Australian species of *Allodapula* and *Exoneura* (Table 1) show that a high percentage of the pollen-collecting individuals are unfertilized and do not have enlarged ovaries. These data indicate not only the activity of workers in foraging but the inactivity of queens. Studies of nests of *Exoneura variabilis* showed that pollen was being carried into the nests and was presumably fed to larvae by workers. When such bees were present in a nest, the queen was not seen to carry pollen. Sometimes there was only one worker in a nest, in addition to the egg layer. In other nests two or three probable workers were recognized on dissection. No external morphological differences between egg layers and workers were found.

In these xylocopids, as in many halictids, a division of labor has arisen even in the very small colonies in which they live. Seemingly in all bees whose

females regularly cooperate in producing and feeding the young, division of labor among these females exists. Such division of labor involves a variety of modifications of the physiology and behavior of nonsocial ancestors. For queens this is shown by long life, reduction of foraging activities, and continued egg laying, for workers by the relatively short life, frequent failure to mate, and frequent failure of ovarian enlargement, and for both castes, by their cooperative nesting activity. It seems probable that among bees (superfamily Apoidea) there must be a tendency of some sort toward acquisition of these features, hence the noteworthy physiological and behavioral parallelisms which are coordinated to form a functional unit by the individuals of any one colony (*4*).

CHARLES D. MICHENER
Department of Entomology, University of Kansas, Lawrence

References and Notes

1. C. D. Michener, *Proc. Intern. Congr. Entomol. 10th, Montreal, 1956* **2**, 441 (1958); ——— and A. Wille, *Univ. Kansas Sci. Bull.* **42**, 1121 (1961); C. Plateaux-Quénu, *Annee Biol.* **35**, 325 (1959).
2. C. D. Michener and R. B. Lange, *Ann. Entomol. Soc. Am.* **51**, 155 (1958).
3. S. F. Sakagami, *Insectes Sociaux* **7**, 231 (1960).
4. Facilities provided by the University of Queensland through the courtesy of Mr. F. A. Perkins are acknowledged. Studies in Australia were possible thanks to a research grant from the Fulbright program of the United States government. Dissections and the preparation of this paper were aided by a grant (G 11967) from the National Science Foundation.
5. C. D. Michener, *Insectes Sociaux* **9**, 355 (1962).

21 June 1963

Crystalline Low Molecular Weight γ-Globulin from a Human Urine

Abstract. *A basic protein with a molecular weight near 17,000, related serologically to the normal serum γ_2-globulins, has been isolated in crystalline form from the urine of a patient with multiple myeloma. Proteins of this size along with the Bence-Jones molecules whose molecular weight is about 35,000 can provide subunits for studies of the structure of the serum γ-globulins.*

There are relatively low-molecular weight proteins with serological properties of γ_2-globulins in normal human urine (*1–3*) and in plasma (*3*). Their molecular weights have been reported to be between 10,000 and 40,000 and they have been generally designated as γ_L-globulins (*1*). Recently Berggård and Edelman (*4*) have indicated that these proteins resemble the so-called light (L) chains of normal human 7*S* γ-globulins and that they may be normal counterparts to Bence-Jones proteins.

These γ_L-globulins have been difficult to obtain in sufficient amount and of a degree of purity adequate for physical-chemical characterization. Relatively large amounts have now been obtained from the urine of certain myeloma patients; one of these proteins has been crystallized; the method of preparing this crystalline form and some of its properties are described in this report.

The urine was dialyzed against distilled water and then lyophilized. The proteins recovered were made up into a 5 percent solution in tris-HCl buffer (0.02 ionic strength, *p*H 8.1 ±0.1) and passed over a column of DEAE-cellulose (*5*) previously equilibrated against the same buffer. The γ_L-globulin passed through the column unretarded whereas the more acidic components were retained on the column. When the γ_L-component is present in the urine in minute amounts it may be concentrated first by passing a solution of the urinary proteins in phosphate buffer (*p*H 6 ±0.1, 0.01 ionic strength) over a CM-cellulose (*5*) column equilibrated against this buffer. The column is washed with the same buffer after the addition of the protein until the effluent has an extinction $\left(E^{1\ cm}_{280\ m\mu}\right)$ of less than 0.100. The protein is then sharply eluted by passing a solution of 0.5 to 1.0*M* NaCl over the column. The eluted protein is then dialyzed against tris-HCl buffer (0.02 ionic strength, *p*H 8.1 ±0.1) and then passed over a column of DEAE-cellulose in tris-HCl buffer (0.02 ionic strength, *p*H 8.1 ±0.1). The γ_L-globulin fraction passes unretarded through this column. It is concentrated by lyophilization and a 2- to 5-percent solution was dialyzed against 0.001*M* tris-HCl buffer, *p*H 8. Crystallization usually occurred within a few hours. When crystallization failed to occur the presence of acidic proteins could usually be demonstrated by starch-gel electrophoresis (*6*). These impurities were removed by passage of a 2- to 5-percent solution of the γ_L-globulin in 0.2*M* NaCl at *p*H 5.5 to 7.5 over a column of G-75 Sephadex equilibrated against 0.2*M* NaCl. The γ_L-globulin fraction obtained could then be crystallized at *p*H 8 as described. The crystals have a solubility of between 1 and 2 mg per ml at 1° to 3°C under these conditions. The crystalline γ_L-globulin readily dissolves at *p*H 5 to 6 in 0.1*M* salt and can be recrystallized by dialysis against buffers of low ionic strength at alkaline *p*H. A photomicrograph of the crystals obtained is shown in Fig. 1.

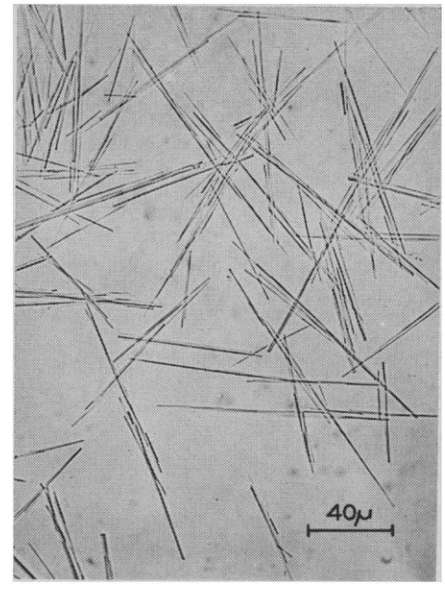

Fig. 1. Twice crystallized γ_L-globulin.

The protein gives a single boundary in the ultracentrifuge and has a sedimentation constant of 1.85*S* in potassium phosphate buffer (*p*H 7.4, 0.2 ionic strength). A velocity sedimentation diagram is shown in Fig. 2. The partial specific volume (0.74) was determined by the method of Drucker (*7*). The molecular weight was determined by the approach to sedimentation equilibrium method (*8*). Average values of 16,900 and 17,700 were obtained at the top and bottom of the cell respectively. These give an average molecular weight of 17,300. The close agreement of the molecular weight determined at the top and bottom of the cell indicate that the γ_L-globulin was essentially homogeneous.

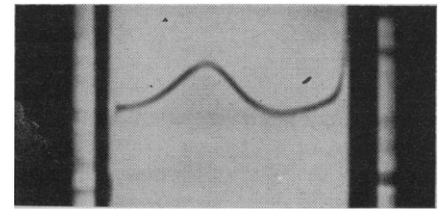

Fig. 2. Sedimentation diagram of crystalline γ_L-globulin after 280 minutes centrifugation at 59,780 rev/min. (The direction of sedimentation is toward the right).