trols of both *Lygodium* and *Mohria* grew well but none of them produced antheridia.

Spores of *Schizaea pusilla* germinated several weeks after inoculation and grew into long, rarely branched filaments or protonemata. This mode of growth is a characteristic of the genus (*24*). After many weeks of growth, scattered filaments developed antheridia. However, antheridia were also present in similar numbers in control flasks lacking gibberellin. Attempts are being made to obtain other species of the relatively primitive genus, of which *S. pusilla* is the only member which occurs north of the Tropic of Cancer (*17*).

Samples of several of the naturally occurring gibberellins (*25*) were tested for their influence upon the growth of *Anemia phyllitidis* and *Onoclea sensibilis*. The results are shown in Table 2. The minimum effective concentration to which the ferns responded varied greatly. Gibberellins A_4, A_7, and A_9 were particularly effective. The lowest concentration of gibberellin A_3 found to be effective was 10^{-7} g/ml, in agreement with the results of Schraudolf (*10*). Gibberellin A_7 was biologically active at concentrations as low as about 5×10^{-10} g/ml, a value similar to the lowest effective concentration (*5*) of pure antheridogen A.

Onoclea sensibilis did not respond to any of the gibberellins tested (Table 2). When the medium upon which this species had been grown was freed of gametophytes and reinoculated with *Anemia* spores, antheridia were formed on the *Anemia* gametophytes in every flask except the gibberellin-free controls. This eliminated the possibility that the gibberellins were inactivated in the medium to any appreciable extent by *Onoclea*.

Evidence has been presented by Voeller (*3*) that the seven gibberellins tested were not identical with the naturally occurring antheridogen A from *Pteridium* or antheridogen B from *Anemia phyllitidis*. The gibberellins and pure antheridogens were compared by thin-layer chromatography, the substances being detected by color tests and bioassay. The sensitivity of *Anemia phyllitidis* to the known gibberellins, and the rapidity and ease of growth of the fern suggest that it might be useful in qualitative assays of gibberellins in plant extracts.

The striking uniformity of the developmental stage of *Anemia phyllitidis*, when raised upon gibberellin-containing media, facilitates studies of the cytological and biochemical processes accompanying antheridium formation. The effects of various protein, nucleic acid and mitotic inhibitors upon these processes are being investigated. It is possible that the gibberellins or antheridogens are essential for several of the stages in antheridium development, rather than just in initiation of the organ. Such studies may help to elucidate the relationship between gene and hormonal action.

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14. Species which, at maturity, may show statistically greater numbers of antheridial prothallia than do controls, although controls also have antheridia. However, no antheridia were found up to the time of meristem formation.
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19. Collected by J. A. Small, Rutgers University, New Brunswick, N.J.
20. Collected by B. R. Voeller at the Rockefeller Institute.
21. All the species of *Anemia*, except *A. rotundifolia* and the two samples of *A. phyllitidis*, were collected by J. Mickel (Iowa State University, Ames) in Mexico. The genus is widely distributed in Latin America, Africa, and India.
22. Collected by B. R. Voeller at the Munich Botanical Garden.
23. Collected by H. B. Rycroft, National Botanic Gardens, Kirstenbosch, South Africa.
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Circadian Periodicity in the Concentration of Prolactin in the Rat Hypophysis

Abstract. *Prolactin activity in the hypophyses of rats maintained under standardized conditions was assayed in pigeons. The concentration of prolactin in the gland exhibited a circadian rhythm.*

A circadian periodicity in secretion of luteinizing hormone by the hypophysis of rats is indicated by the action of Nembutal, which blocks its release if administered at 2 P.M. but not when the same dose is given at 4 P.M. (*1*). Also, the concentration of corticotropin in the hypophysis of the mouse of strain C follows a circadian rhythm (*2*). In this study, a circadian periodicity in the concentration of prolactin in the hypophyses of rats was revealed by direct measurement.

Ninety-eight female Sprague-Dawley rats, each weighing 180 to 190 g, were kept for 1 to 2 weeks under standardized conditions: they were isolated in individual cages away from disturbances, exposed to light from 6 A.M. to 6 P.M., and kept at a constant temperature of 21°C. Groups of rats were killed at 2-hour intervals during the day and night, each group having been undisturbed for the preceding 48 hours. Within 1 to 2 minutes after removal from the standardized conditions, each animal was anesthetized with ether, the hypophysis excised, and the pars nervosa removed from it. The gland was frozen in liquid nitrogen and stored at −20°C. After thawing it was weighed, homogenized in 1.2 ml of distilled water, and divided into three 0.4-ml portions. Prolactin activity was measured by the method of Lyons as modified by Reece and Turner (*3*). One-tenth of a milliliter of homogenate was injected intradermally over the crop sac of pigeons, daily for 4 days, the homogenate from each rat being used for three pigeons. The pigeons were killed 5 days later and crop sac proliferation was estimated visually on a scale from 0 to 4 in increments of 0.25.

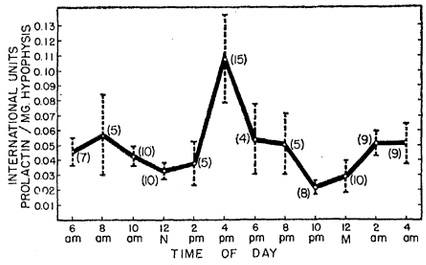

Fig. 1. Circadian variation in the concentration of prolactin in the hypophysis. The figures in parentheses represent the number of rats used at each point. The vertical bars represent standard errors of the mean.

The product of the mean response of the three pigeons multiplied by 3 was considered equivalent to the total activity of prolactin in the gland. In most instances, the pituitary homogenates were injected on one side of the crop sac and various concentrations of a prolactin preparation of known activity (*4*) on the opposite side. From the observations made with the prolactin preparation, a regression equation was obtained by the method least squares. Calculations of unknown concentrations were made from the equation

$$\log Y = -1.724 + 0.608X$$

where Y is the log of the dose, and X is the pigeon response.

The highest concentration of prolactin in the hypophysis occurred at 4 P.M. (Fig. 1). The concentration had decreased significantly by 10 P.M. ($p < .02$) and by midnight ($p < .05$). Also, the concentration at 4 P.M. was considerably higher than that at noon ($p < .05$). Except for the peak at 4 P.M., significant changes in the concentration of prolactin were not detected at other times of day. When data from rats killed at various times of day were grouped, the concentration at 4 P.M. was higher than the mean concentration for all rats taken from 6 A.M. to 2 P.M. ($p < .05$) and for those from 6 P.M. to 4 A.M. ($p < .05$). The concentration of prolactin in individual rats bore no consistent relationship to the period of estrus as determined from microscopic sections of the vaginal wall, rats in most stages of the cycle being represented in each group.

Thus, a circadian periodicity in the concentration of prolactin in the hypophysis was demonstrated, with a 325-percent increase occurring between 12 noon and 4 P.M. and the concentration reaching its lowest point at 10 P.M.

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Hybridization of Half Molecules of Rabbit Gamma Globulin

Abstract. *Specifically purified rabbit antiovalbumin and normal γ-globulin labeled with I-131 were dissociated into half molecules by reduction and acidification. When a mixture of the two preparations was neutralized, a large proportion of mixed molecules having active combining sites and the same sedimentation coefficient as the original γ-globulins was formed. Since the sulfhydryl groups were inactivated after reduction, the recombined subunits appear to be linked by noncovalent bonds.*

It was recently reported (*1*) that molecules of 6.2*S* rabbit γ-globulin are split into two subunits of molecular weight 75,000 to 80,000 by reduction with 0.1*M* mercaptoethylamine followed by acidification to *p*H 2.5 in 0.1*M* sodium chloride. Supporting evidence included the agreement between the weight and *Z*-average molecular weights; the fact that the subunits migrate almost entirely as a symmetrical peak in the ultracentrifuge; and that the splitting can be accomplished without proportionate release of free "B chains" (*2–5*). The results indicated that each subunit contains an "A chain" and a "B chain." The molecular weights of the chains are 55,000 and 20,000 to 25,000, respectively (*6, 7*). After neutralization, most of the combining sites of an antihapten antibody were shown by equilibrium dialysis to be intact. No appreciable formation of insoluble material was associated with these procedures.

After neutralization of the acidified protein, the major component has a sedimentation coefficient of 6*S*. Smaller amounts of a 4*S* component and some faster-moving material are ordinarily present. The $s_{20,w}$ value of the major component has been found in numerous experiments to agree within 3 percent with that of the untreated γ-globulin, which strongly suggests that most of the half molecules recombine in pairs at neutral *p*H. Since the −SH groups are inactivated with *p*-chloromercuribenzoate (CMB) after reduction, the recombined molecules are evidently held together by noncovalent bonds, as is the case prior to acidification. More than 90 percent of the protein can be redissociated, to give a product having a sedimentation coefficient characteristic of half molecules, by dissolving a recombined preparation in 0.025*M* NaCl, *p*H 2.4 (*8*).

Since each of the subunits obtained by reduction and acidification appears to contain an "A chain" and a "B chain," the possibility was suggested that they are univalent (*1*). The evidence reported here provides support for this hypothesis. The data also indicate that the subunits of different γ-globulin molecules associate to form 6*S* molecules after neutralization of a mixture of two reduced, acidified preparations.

A pool of antiserum obtained from several rabbits hyperimmunized with ovalbumin was specifically purified by partial dissociation of a specific precipitate at *p*H 2.5 and fractionation at this *p*H with sodium sulfate (*9*). The product had a sedimentation coefficient, $s_{20,w}$, of 6.1*S*; approximately 8 percent of faster moving material (about 10*S*) was also present (*10*). An optimal concentration of ovalbumin precipitated 89 percent of the protein. The method used for estimating amounts of antigen in precipitates near equivalence has been described (*11*). In view of the slight solubility of antigen in the presence of excess rabbit antibody, contamination of the purified antibody by antigen was probably very small.

A γ-globulin fraction was prepared from the serum of a nonimmunized rabbit by three precipitations with sodium sulfate (*12*), followed by passage through DEAE-cellulose (diethylamino-