Table 1. Values of constants.

Constant	Water	Acetic acid	
		0.1M	0.5M
V_0^2	0.29	2.17	3.30
V_∞^2	0.72	4.00	5.42
$2\alpha k$	0.0018	0.0025	0.0025

of the equilibrium volume V_∞ on the parameters A and α.

$$V_\infty = (A/\alpha)^{1/2} \quad (2)$$

When Eq. 1 is integrated and solved explicitly for V^2, Eq. 3 is obtained.

$$V^2 = V_\infty^2 - (V_\infty^2 - V_0^2)e^{-2\alpha kt} \quad (3)$$

If measurements of V^2 are made at sufficiently short times so that $e^{-2\alpha kt}$ can be approximated by $(1 - 2\alpha kt)$, then Eq. 4 is formed.

$$V^2 = V_0^2 + (V_\infty^2 - V_0^2)2\alpha kt \quad (4)$$

The following is an application of this theory to the swelling data of collagen in water and dilute acetic acid (0.1 to 0.5M).

One hundred milligram samples of hide powder collagen (*2*) were placed in 15-ml graduated centrifuge tubes. Then, 10 ml of water or dilute acetic acid was added. The tube was shaken for 2 to 3 minutes and spun at 3000 rev/min for 10 minutes to pack the swollen fibers. The volume of the sediment was then read to the nearest 0.1 ml, and the mixture was shaken again for 2 to 3 minutes. It was allowed to settle until the next volume was read. This process was repeated at 30-minute intervals during the initial stages of swelling but later at longer intervals. Water and aqueous solutions of acetic acid (0.1 to 0.5M) were used to swell collagen at temperatures of 23° to 25°C. The force and time at which the samples were centrifuged determines the volume of the sediment. However, both of these factors were constant throughout the study.

In Fig. 1 the volume of collagen is shown as a function of time for swelling in water and in 0.1 and 0.5M acetic acid at 23° to 25°C. The experimental data were plotted according to Eqs. 4 and 5 and were in conformity with the theoretical requirements except at zero time. The latter points always deviated from theoretical. The interpretation is given that collagen swells in two steps. Initially, the fibers imbibe solvent at a rapid rate until they reach the volume V_0. Thereafter, they swell from V_0 to V_∞ by the proposed osmotic mechanism. Table 1 shows the values of the constants determined for the swelling of collagen in water and dilute acetic acid. The influence of solvent on V_∞ is probably due to a reduction in α by the more active acetic acid. Since $2\alpha k$ showed only slight alteration with solvent, then, the specific rate constant k must have been elevated in acetic acid in comparison to water. V_0 presumably is also a measure of collagen solvation and its swelling by a particular solvent. In going from water to 0.5M acetic acid, the greater V_0 could be due to a discharge of carboxylate-basic salt-links. The lower pH of the acetic acid solutions would free the ionic attraction of chains previously bound by such bridges.

An analysis of the sorption of water by rubber (*3*) similarly was based on the osmotic pressure gradient of a solvent in the matrix as presented by Testor. If V_∞ were considerably greater than V_0, the rubber swelling equation would be identical with that for collagen. It appears then that processes other than Fick diffusion mechanisms determine the swelling and sorption in some types of polymers.

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16 July 1958

Measurement of Precipitin Reactions in the Millimicrogram Protein-Nitrogen Range

Abstract. Exploitation of newer instrumentation and a dye-binding method for protein measurement made possible reduction in volume down to 1/1000 that commonly used, with no greater error. The procedure was tested at two levels, 1- and 10-μl volumes, with human gamma globulin and rabbit antiserum. Of the dyes tested, bromsulfalein proved best for the protein estimation.

The value of a method which would require a few drops to less than one drop of blood for replicate measurements of the precipitin reaction, particularly in pediatric practice and for studies with small laboratory animals, is obvious. Such a method should also be applicable to the microsamples involved in certain quantitative histo- and cytochemical studies. Exploitation of newer instrumentation (*1*) and a dye-binding method for determination of protein in millimicrogram amounts (*2*) made it feasible to modify and scale down the commonly used procedure, involving the so-called "micro" Kjeldahl analysis of the antigen-antibody precipitate (*3*), to the level required. With the method herein described, interaction of 10 μl of antigen solution with 10 μl of antiserum provides over 1 ml of colored solution for final measurement. Since this measurement can be carried out on less than 10 μl [for example, with the Beckman DU spectrophotometer adapted to microcuvettes (*1*)], it should be possible to use volumes of antigen and antiserum down to 0.1 μl, although in this study volumes down to only 1 μl were used. A comparison of four acid dyes previously employed for protein measurement or dye-protein binding studies (*2, 4*) was included in this investigation (*5*).

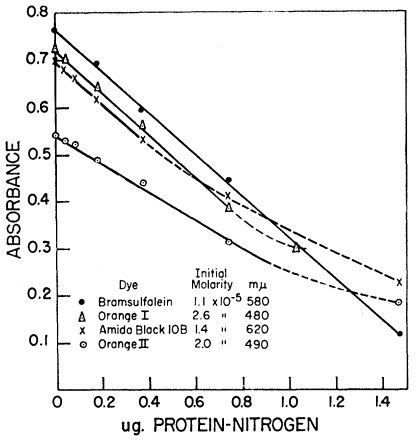

Fig. 1. Dye-binding of bovine serum albumin in 1 mg per 100 ml of bromsulfalein (Hynson, Westcott, and Dunning) and other dyes (Hartman-Leddon), at pH 2.

For the dye preparations used, maximal absorbance was given at the wavelengths shown in Fig. 1, and maximal binding to bovine serum albumin (Armour, fraction V) was obtained at pH 2 in 0.1M citric acid-sec phosphate buffer. Readily available albumin was used, since the dye-binding is comparatively nonspecific. The data in Fig. 1 were obtained by mixing 50 μl of buffered dye solution (12 mg/100 ml) with 20 μl of albumin solution and letting the mixture stand for 15 minutes, centrifuging it at 3000g for 5 minutes, adding 60 μl of the clear supernatant to 0.5 ml of distilled water, and measuring the absorbance. A blank was prepared by replacing the albumin solution with water. The dye concentrations listed in Fig. 1 are those of the blank solutions. Commercial dye preparations were used without further purification, since this sufficed for the practical purposes at hand. Dye-binding was linear at low albumin concentrations in all cases, but only bromsulfalein maintained linearity over the whole range tested; in fact, under the conditions of tissue analysis, its linearity extends to 2.25 μg of protein-nitrogen, with a reduced slope (*2*). Although both Amido

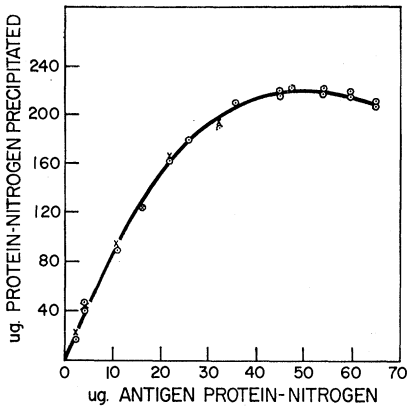

Fig. 2. Comparison of the Kjeldahl (×) and bromsulfalein (⊙) methods for measuring precipitate as a function of quantity of antigen (human gamma globulin) in the precipitin reaction with rabbit antiserum. Values from the Bromsulfalein method were multiplied by 100 to make them comparable to the Kjeldahl data.

Black 10B and Orange I exhibited approximately the same change in absorbance per unit protein over their linear ranges as bromsulfalein, their linearity was confined to a short range, making bromsulfalein preferable for analytical work.

The agreement between the Kjeldahl method for determining the amount of antigen-antibody precipitate and the bromsulfalein method is apparent in Fig. 2. The procedure for the latter method, which follows, differed from that for the former in the use of volumes 100 times smaller. Ten microliters of rabbit antiserum to human gamma globulin was placed in each of a series of glass tubes 27 mm long and of 4-mm inner diameter. Ten microliters of the gamma globulin in 0.9-percent sodium chloride (10 dilutions were used over the range 4.3 to 47 mμg/μl) was added to each tube and mixed. Each concentration of globulin was set up in triplicate, and blanks were included in which 0.9-percent sodium chloride was substituted for the globulin solution. The tubes were held at 37°C for 1 hour and transferred to a refrigerator for 7 days' storage at 2°C, during which time the precipitates were carefully resuspended once each day by gently tapping the tubes so as to prevent precipitate from lodging on the walls above the liquid. The tubes were then centrifuged at 2°C for 1 hour at 3800*g* in a microcentrifuge (*6*). The supernatant fluid was withdrawn by a micropipette and discarded, 15 μl of cold 0.9-percent sodium chloride was added, and the precipitate was resuspended by "buzzing"—that is, vibrating the vessel by touching its side near the bottom to a flattened nail spinning in the chuck of a high-speed hand drill—and then recentrifuged as before. A second washing of the precipitate was carried out in the same manner, and then the precipitate was used for protein-nitrogen measurement. A check of the procedure revealed that a third washing was without influence.

The "micro" Kjeldahl analysis of protein-nitrogen was conducted in the standard fashion. The dye procedure was carried out as follows. The precipitates in the tubes were dried in a vacuum desiccator. The protein was dissolved in 20 μl of 1*N* sodium hydroxide by mixing, letting the mixture stand for 30 minutes, and again mixing. Fifty microliters of bromsulfalein reagent (1 ml of 5-percent dye plus 100 ml of 1*N* hydrochloric acid plus 50 ml of 1*M* citric acid plus distilled water to a final volume of 250 ml) was added and mixed. The tubes were centrifuged at room temperature for 5 minutes at 3800*g*. Sixty microliters of supernatant was withdrawn and added to 1 ml of 0.1*N* sodium hydroxide. After mixing, the absorbance was measured at 580 mμ, and the protein-nitrogen was calculated as described earlier (*2*).

To test the precision of the dye method, the entire procedure was repeated nine times; a single gamma globulin solution was used (43 mμg/μl), and all volumes were reduced to 1/10 the volumes used in the earlier tests—that is, 1 μl of antiserum and 1 μl of gamma globulin were used. Tubes 27 mm long and of 2.5-mm inner diameter were employed. A mean value of 233 mμg of protein (standard deviation and error, 10.4 and 4.7 percent, respectively) was obtained.

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5. This study is No. LV in the Studies in Histochemistry series; it was supported by research grants from the U.S. Public Health Service [RG3911, H2028, H2085], the Louis W. and Maud Hill Family Foundation, and the American Heart Association.
6. The microcentrifuge used in these experiments was provided by the Microchemical Specialties Co., Berkeley, Calif.

4 August 1958

Hypothalamic Lesions and Sexual Behavior in the Female Rat

Abstract. This study reports selective effects on the mating pattern of the female rat of partial destruction of the hypothalamus. Independent neural control of the ovarian cycle and of the mating response are demonstrated. Both depressed and augmented female sexual activity are reported.

Several studies have suggested the importance of the hypothalamus in the regulation and control of sexual behavior (*1–5*). Most previous work reports diminution or abolition of mating behavior following interference with hypothalamic structures. The effect is usually ascribed to disturbances in the production of the gonadotrophins.

This study (*6*) indicates that sexual behavior is differentially affected by small lesions in discrete hypothalamic regions; some lesions augment sexual activity. Moreover, the effect is not wholly hormonal; central neural mechanisms are immediately involved.

Forty-five female rats surviving all operative procedures were divided among two major groups, spayed and nonspayed. Each group consisted of four subgroups: animals with lesions in the anterior, central, and posterior hypothalamus (designated "operated" subgroups), and animals without lesions. Lesions were stereotactically placed 1 mm lateral to the midline and 1 mm dorsal to the ventral surface of the brain, and at Krieg coordinates 58 and 59 (anterior), 56 and 57 (central), and 54 and 55 (posterior). Direct current of 1.5 to 2.0 ma, lasting for 10 to 15 seconds, was used to make the lesions. Locations of lesions were subsequently verified histologically.

Estrus was induced in the spayed rats once weekly for 3 weeks by treatment with diethylstilbestrol and progesterone in sequence (*7*).

Females were tested for mating responses with male rats preselected as vigorous maters. Tests were conducted daily for 10 minutes for each female. Tests eliciting no male response were repeated the same day with another test male. All tests were conducted in transparent cages, which served as the male's home cage.

Female mating behavior was defined in terms of the characteristic stereotyped lordosis response, a concave arching of the back upon approach and mounting by the male. Lordosis frequencies were tallied and appear as "responses" in Table 1.

Daily vaginal smears were taken. Smears were dichotomized into diestrus (leukocytes only) and estrus (presence of epithelial cells). This unconventional,