

2nd day menstruation	33
3rd " "	12
1st day after menses	12
4th " " "	15
5th " " "	18
7th " " "	30

In 2 cases of young adult women, who had never menstruated, and in whom sexual infantilism had been diagnosed, no hormone could be found in the circulating blood.

From our tables, it is seen that there is no sudden fall in the concentration of the hormone in the blood just preceding menstruation as reported by Frank and Goldberger and by Hirsch. Our tables do not support the assumption that menstruation is due to the sudden drop of this hormone in the blood.

Assuming an average of 7 liters of blood in the woman, we have computed at least 45,000 mouse units of hormone circulating in the blood stream, with no way of ascertaining the amount in storage in different organs. It appears to us, that to obtain clinical results in definite cases of hypofunction of the ovary, massive doses of the hormone must be given to approximate the amount found in a normally functioning woman.

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Reaction Between Antiseptics and Proteins and Antiseptic Activity of the Adsorbed Portion.

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It is a well know fact that the action of antiseptics upon bacteria is greatly diminished by the presence of proteins, blood serum, tissue juices and pus, but whether this is due to a chemical combination with the protein or to adsorption of the antiseptic on the surface of the protein, is not so certain.

Mathews,¹ Heidenhain,² and Chapman, Greenberg and Schmidt,³

* The experiments reported in this paper form the basis for a part of a Thesis presented by Harold N. Wright in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota (May, 1929).

¹ Mathews, A. P., *Am. J. Physiol.*, 1898, i, 445.

² Heidenhain, M., *Arch. f. d. ges. Physiol.*, 1902, xc, 115.

³ Chapman, L. W., Greenberg, D. M., and Schmidt, C. L. A., *J. Biol. Chem.*, 1927, lxxii, 707.

who advocated the theory of chemical combination, dealt with denatured lyophobic protein and not with protein in the natural lyophilic state; this has given rise to serious misconceptions. Ashley Cooper⁴ has advanced some evidence that proteins adsorb phenols and do not enter into true chemical combination with them. The experiments of Grollmann⁵ on the binding of phenol red indicate that the dyes are adsorbed, but the values (*i. e.*, $1/N$ approximating 1) obtained lay too close to agreement with the mass law equation also to make fine distinctions possible. Rosenthal⁶ also obtained an adsorption curve for the binding of rose bengal on a variety of proteins in acid solution.

If the reaction with protein is one of chemical combination, it should proceed in direct proportion to the reacting masses, while if it is an adsorption, it should follow the Freundlich adsorption isotherm ($X/N = KCl/n$) and give a logarithmic curve.

In order to study the reaction with proteins in their natural state, the protein (purified egg albumin) and dye were placed in a small wide-mouthed bottle containing, as a dialyzer, a segment of viscose sausage skin filled with water. The free crystalloid dye passes through the dialyzer until its concentration in the outside chamber is equal to that within, while any portion that is in colloidal form does not do so. We soon found that some of the antiseptic dyes like mercurochrome and acriflavine, which are generally supposed to be crystalloids, actually are partly in crystalloid and partly in colloidal form; and that physiological NaCl increases the amount of the colloidal form of these dyes, as well as converts a portion of the triphenyl methane dyes into colloidal suspensions, especially at pH 7 to 8.

We have found that egg albumin in 0.3 and 1.0% solutions at pH 7.5, adsorb crystal violet, brilliant green and malachite green, which show the following values for $1/N$: crystal violet 0.83, brilliant green 0.57, and malachite green 0.25. Crystal violet follows the adsorption isotherm only in the range (less than 50 mg. per 100 cc.) in which relatively little precipitation occurs. Mercurochrome and acriflavine were themselves too colloidal to permit of the use of this method of study.

The adsorption could be followed under the ultramicroscope, the soap-bubble-like appearance of the hydrated protein giving way to the star-like reflections of the larger and less hydrated aggregates.

⁴ Cooper, E. A., and Sanders, E., *J. Phys. Chem.*, 1927, **xxxi**, 1.

⁵ Grollmann, A., *J. Biol. Chem.*, 1925, **lxiv**, 141.

⁶ Rosenthal, S. M., *J. Pharmacol. and Exp. Therap.*, 1926, **xxix**, 521.

In studying the problem of antiseptics in the presence of protein, it is important to determine whether it is only the unadsorbed fraction of the dye which acts as an antiseptic, or whether the portion which is adsorbed on the protein also exerts this action. This was tested upon the amount of CO₂ production by yeast grown in sucrose solution. Taking our curves for antiseptic strength in water alone with the curve of antiseptics in the presence of protein, and comparing these with the adsorption curves, it is evident that not only the free crystalloid, but also the adsorbed fraction, exert antiseptic action. The adsorbed fraction, however, exerts only a part of the action that would correspond to the amount of dye present.

Thus, crystal violet in a concentration of 1/20,000 in the presence of 1% albumin should behave like a solution of 1/125,000 concentration if only the free dye is actively antiseptic. Experimentally it was found to behave like a crystal violet solution of 1/35,000 concentration.

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Is Schnitzer's "Interference" Between the Action of Two Chemotherapeutic Substances Due to Surface Reactions?

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Browning and Gulbransen¹ discovered the remarkable fact that although the injection of basic fuchsin cured rats infected with trypanosomiasis and acriflavine injections had the same effect, if animals previously fed basic fuchsin by mouth were infected with fuchsin-fast trypanosomes, and later acriflavine was injected, the therapeutic action of the acriflavine was prevented and the animals died of trypanosomiasis. They gave to this action the name of "interference phenomenon." These observations were confirmed by Schnitzer² (both with fuchsin-fast and normal trypanosomes) who showed that it applied also to other triphenyl methane dyes, *e. g.*, methyl violet and brilliant green, and to arsenicals, *e. g.*, arsphenamine and arsacetin.

*The experiments reported in this paper form the basis for a part of a Thesis presented by Harold N. Wright in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota (May, 1929).

¹ Browning, C. H., and Gulbransen, R., *J. Path. and Bact.*, 1922, **xxv**, 395.

² Schnitzer, R., *et al.*, *Z. f. Immunitätsforsch. u. exper. Therap.*, 1926, **xlvi**, 116; **xlvi**, 23; **xlvi**, 387, 393, 551; 1927, **liii**, 439; **liv**, 324.