parison. The solution of lot 4 as prepared by the methyl alcohol method was given intramuscularly in the dose of 0.28 to 0.36 gm. in 5 children, and the sulphate preparation of the same lot, in the dose of 0.36 gm. in 4. All children were known to have been intimately exposed to measles in their brothers or sisters and none had had measles before. The age of the children and the number of days of exposure before the prophylactic injection was given are comparable in the 2 groups. Either modified measles or complete protection was the result in all the cases.

Conclusions. A dry form of placental globulin extract could be prepared by means of methyl alcohol precipitation. When dissolved, this preparation was found to be as potent as the preparation from ammonium sulphate precipitation with regard to neutralization of Dick toxin and prophylaxis against measles.

8360 C

Vaginal Cornification Induced by Swabbing and Its Bearing on the Rat Unit of Estrogenic Substance.

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In the course of a series of assays on one lot of human pregnancy urine extract, employing the method of Coward and Burn,[1] we have encountered findings which were difficult to explain. The recent report of Wade and Doisy[2] directed our attention to the effect of swabbing on the presence of cornified cells in the vaginal smear together with its bearing on the definition of the biological unit of estrogenic substance.

Sexually mature, ovariectomized, albino rats were employed in the experiments. The animals were kept in scrupulously clean cages, each containing 2 or 3 animals. They were fed with a diet in which the supply of vitamin A was adequate. Estrogenic substance was prepared by extracting human pregnancy urine with butyl alcohol by the method described in a previous report.[3] Injections of this extract in olive oil were given by the subcutaneous route.

1 Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

2 Wade, N. J., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 707.

3 Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.

When cornified cells were present in small numbers widely distributed in the smear, or when a few clumps of them were found, the result was marked as C_1 (+). A C_1 reaction is to be interpreted as borderline positive. Correspondingly greater numbers of cornified cells were graded as C_2, C_3, or C_4 (++, +++, or ++++). The number of leucocytes and nucleated epithelial cells was graded in a similar way. As a rule, the appearance of cornified cells was preceded by a decrease of leucocytes, and followed by a sudden increase of leucocytes, usually with the admixture of nucleated epithelial cells. Full cornification with entire absence of the other cellular elements was of infrequent occurrence. This phase of reaction is at times very transient even when the estrogenic substance is given in oil. Some workers[4] consider the mere disappearance of leucocytes from the smear to indicate a positive reaction, while others[5] require cornification like that of the full estrous smear of the normal animal in which there is a complete absence of leucocytes.

The effect of swabbing alone, carried out 3 or 4 times a day, is manifest in the following observations. Three swabbing experiments were performed on 2 sets of rats. The first set of 24 animals had been previously employed in a series of assays. The second set of 25 animals was used before any estrogenic substance was injected into the animals. The latter group was tested twice with the usual 2 weeks intervening. The results of the 3 experiments were similar enough for combined consideration. Sixty to 88% (average: 78.5%) of the animals gave at least a C_1 reaction. Thirty-eight, or 51.3%, of the animals showed a reaction greater than C_1.

In a second series of experiments 20 spayed female rats were tested with decreasing doses of the same lot of urine extract. The peak of reaction (the time of appearance of the maximum number of cornified cells) following the injection of 0.5 cc., 0.5 cc., and 0.25 cc. respectively of a 0.5% solution of the extract in olive oil occurred within 40 hours of the injection. Injection of 0.12 cc. or 0.05 cc. of the extract in olive oil, or injection of 0.5 cc. of pure olive oil had their peaks more than 50 or 60 hours after the injection. Actual figures showed that the peak reaction for animals swabbed 3 or 4 times a day without injection of any kind occurred at an average of 46.4 hours after the first swabbing. However, following injection of either estrogenic substance or plain olive oil, a period of about 17 hours elapsed before the first swabbing was made. In order to make

[4] Laqueur, E., and de Jongh, S. E., *J. Pharm. and Exp. Therap.*, 1929, **36**, 1.
[5] Marrian, G. F., and Parkes, A. S., *J. Physiol.*, 1929, **67**, 389.

the different sets of figures comparable, this figure of 17 hours should be added to that of the swabbed untreated animals. The corrected peak reaction would thus be at 63.4 hours. Delayed appearance of cornified cells in the vaginal smears of animals injected with estrogenic substance may therefore indicate a false positive reaction, since swabbing alone causes cornification.

The mechanism of cornification of the vaginal epithelium as induced by swabbing is not clear. Although swabbing apparently stimulates the process of cornification it does not prolong it indefinitely. The cornified cells usually disappear from the vaginal smear within 3 or 4 days after the first swabbing. In general, when epithelial proliferation and cornification follow local irritation, the process is progressive within limits and tends to be chronic.

Summary. Swabbing alone produces a significant cornification of the vaginal epithelium in a high percentage of ovariectomized rats. The changes thus induced come later than those following injections of estrogenic substance, and are transient, even though swabbing is continued. The reaction to swabbing materially affects the results of biological assay.

8361 P

Photodynamic Action of Methylene Blue on Bacteria.

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The effect of the prolonged use of photodynamically active dyes on a number of bacteria was summarized by Reitz.[1] Workers mixed the dyes and the cultures and let them stand for days exposed or unexposed to light. It was found that some dyes, including methylene blue, were more active in the presence of light than other dyes, such as fluorescein. It was also found that *B. diphtheriae* was more susceptible to such action than *B. typhosus.* Since then, however, very few reports have appeared. In view of the recent interest in the study of photodynamic action on viruses, bacteriophage,[2] and toxins,[3] it seems worthwhile to restudy this problem systematically.

[1] Reitz, Adolf, *Zentralb. f. Bakt.*, 1908, **45**, 270, 374, 451.

[2] Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. London*, 1933, **112**, 277, 288.

[3] Shortt, H. E., and Mallick, S. M. K., *Ind. J. Med. Research*, 1935, **22**, 529. Lippert, K. M., *J. Immunol.*, 1935, **28**, 193.