usefulness, $NaN_3$ could be of value in the preservation of anti-Rh serums. It possesses certain advantages over "compound I" in that it is active against fungi, and is effective in heavily contaminated materials. Like "compound I" the effectiveness of $NaN_3$ is not impaired by high concentrations of protein. In experiments now in progress it is being found that the bacteriostatic effectiveness of $NaN_3$ is not impaired in anti-Rh serums fortified with 20% bovine albumin. Through their action as specific metabolic inhibitors, compounds such as $NaN_3$ and "compound I" may find wider use in the preservation of diagnostic biologicals.

*Summary.* The effectiveness of $NaN_3$ in concentrations of 0.1% and 0.05% as a preservative for anti-Rh agglutinating serums has been investigated. It was found that in these concentrations the agent was markedly bacteriostatic, but had no apparent effect on the titer or avidity of the antiserum. Anti-Rh serum deliberately contaminated with common bacteria but not preserved with $NaN_3$ rapidly lost potency when stored at 37°C.

## 16544

## Effects of Ovarian Hormones on Certain Cytoplasmic Reactions in the Vaginal Epithelium of the Mouse.*†

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Since the classical work of Allen and Doisy[1] many investigators have studied the effects of the various gonadal hormones on the growth and differentiation of the vaginal epithelium. With the exception of the numerous studies on glycogen in the monkey and human and mucin in the rodent, little work has been directed toward the alterations in intracellular constituents. Recently Jeener[2] stated in a brief report that estrogen evoked a marked increase in both alkaline phosphatase and cytoplasmic ribonucleic acid in the vaginal epithelium of the mouse, but few cytological details were given.

In view of this paucity of information, the present study was designed to examine the effects of estrogen and progesterone on the amount and distribution of cytoplasmic ribonucleic acid, alkaline phosphatase and mucin in the vaginal epithelium of the mouse.

*Methods.* Twelve young adult mice of the Swiss albino strain were bilaterally ovariectomized. A control group of 3 animals received no further treatment and was sacrificed 3 to 4 weeks after operation. At this time the remaining mice were divided into 3 equal groups. One group received 1.0 μg of estradiol banzoate; the second, 1.0 mg of progesterone, and the third received simultaneously 1.0 μg of estradiol benzoate and 1.0 mg of progesterone daily for 3 to 5 days. Crystalline hormones were used. The daily dose was dissolved in 0.05 to 0.1 cc of sesame oil and was injected subcutaneously. The animals were sacrificed on the day following the last injection and were autopsied immediately. The vaginas from both the control and experimental groups were fixed in chilled 80% ethyl alcohol for at least 24

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† The estradiol benzoate used in this work was supplied through the courtesy of Schering Corporation, the progesterone by the Warren-Teed Products Company.

1 Allen, E., and Doisy, E. A., *J.A.M.A.*, 1923, **81**, 819.

2 Jeener, R., *Nature*, 1947, **159**, 578.

hours and were subsequently dehydrated and embedded in paraffin. Tissue sections were cut 7 microns in thickness.

Alkaline phophatase activity was determined by incubating sections in a medium containing 1% sodium glycerophosphate at pH 9 according to the technic of Gomori.[3] The original formula was altered slightly by the addition of 0.01 M magnesium sulfate which is an alkaline phosphatase activator. The tissue was incubated at 37°C for 1½ hours. Subsequent treatment results in the deposition of fine black particles of cobalt sulfide at the sites of enzyme activity. The sections were counterstained with eosin and were mounted in clarite dissolved in white gasoline.

Ribonucleic acid was demonstrated by staining sections in 0.01% toluidine blue in a citrate-phosphate buffer at pH 5 for one hour at 37°C. In order to ascertain the amount of staining due specifically to ribonucleic acid, occasional parallel sections were first incubated in a solution of crystalline ribonuclease (1 mg/cc buffered at pH 6.8 for 1 hour at 56°C) before staining in the above manner. The sections were rinsed in distilled water, dehydrated and mounted in clarite.

For the indentification of mucin, sections were first incubated with saliva for 5 minutes to remove any glycogen which might be present. The sections were then oxidized with chromic acid and treated with leucofuchsin according to the Bauer-Feulgen technique.[4] Mucin is stained a bright red by recolorized basic fuchsin. The nuclei were counterstained lightly with Weigert's iron hematoxylin and the sections mounted in the usual manner.

*Results.* As stated in the introductory remarks, numerous authors have dealt with the cytological changes in the vaginal epithelium due to the action of gonadal hormones. The writers have found the following classification of morphological patterns based on the rat (Freud[5]) to be most generally useful:

Type I. Epithelium 2 to 3 cells thick. The long axes of the cells in the most superficial layer tend to be perpendicular to the surface of the epithelium. Found in infantile, lactating and castrate animals.

Type II. Multilayered stratified epithelium without mucification or cornification.

Found in diestrus and proestrus and in the early stages of estrogen treatment in the castrate.

Type III. Multilayered stratified epithelium with mucification of all but the most basal cells.

Found in pregnancy and pseudopregnancy and in the castrate treated with estrogen and progesterone concurrently.

Type IV. Multilayered stratified epithelium with cornification of the more superficial layers. The deeper cells may be differentiated into a stratum germinativum and stratum granulosum.

Found in estrus and in the estrogen treated castrate.

The only variation from the descriptions of Freud which occurred in our series was in the estrogen-progesterone treated group and will be discussed below.

The present histochemical studies reveal that there is no alkaline phosphatase or mucin present in the vaginal epithelium of the ovariectomized mouse. Fine granules containing small amounts of ribonucleic acid are irregularly scattered throughout the cytoplasm of the epithelial cells. There is no change in the status of any of these cellular constituents after treatment with progesterone alone.

After treatment with estrogen, however, there is a marked increase both in alkaline phosphatase and ribonucleic acid. Heavy deposits of cobalt sulfide, indicating strong enzyme activity, are present in the nuclei and throughout the cytoplasm of the cells in the stratum germinativum. In the stratum granulosum the cytoplasmic enzyme activity tends to be concentrated in the periphery of the cells. In addition, there is a progressive diminution in the amount of phosphatase present from the deeper to the more superficial layers of the stratum granulosum. Little or no enzyme is present in the stratum cor-

[3] Gomori, G., *J. Cell. & Comp. Physiol.*, 1941, **17**, 71.

[4] Bauer, *Ztschr. mikr. anat. Forsch.*, 1933, **33**, 143.

[5] Freud, J., *Act. Brev. Neerland.*, 1938, **8**, 127.

neum. Cytoplasmic ribonucleic acid shows a parallel gradient in concentration but is more or less evenly dispersed throughout the cells in fine granules. The cytoplasm of the cells in the stratum germinativum is intensely basophilic. The depth of staining decreases progressively throughout the stratum granulosum. The stratum corneum is completely unstained by toluidine blue at the pH used in the present studies. No mucin is present in any of the cells of the vaginal epithelium in the estrogen treated animal.

As previously stated, the mucification response evoked by treatment with estrogen and progesterone concurrently was somewhat different than that described by Freud. In the present study, only the most superficial layer of epithelial cells has been markedly affected. The cells lining the vaginal lumen are of the cuboidal to low columnar type with basally situated nuclei. The cytoplasm of these cells is intensely stained throughout with basic fuchsin, indicating a high concentration of mucin. In scattered areas of the vaginal epithelium there is a partial mucification of the squamous layers immediately beneath the superficial mucous layer. This divergence from the results of Freud is due probably to differences in experimental conditions and hormone dosage.

The amount and distribution of alkaline phosphatase and cytoplasmic ribonucleic acid in the non-mucified layers of the estrogen-progesterone treated group is comparable to that seen in the stratum germinativum and deeper cells in the stratum granulosum in the estrogen treated animals. The gradient in the concentration of these substances is somewhat less pronounced in the former group. Unlike the cornified layer in the estrogen treated animal, the superficial mucified layer exhibits intense alkaline phosphatase activity in the apical cytoplasm of its cells. It is impossible to determine from our material whether these cells also contain small amounts of ribonucleic acid because of the intense cytoplasmic basophilia due to the presence of mucin.

*Discussion.* The present experiments show that the injection of estrogen into the ovariectomized mouse is followed by a marked increase in both alkaline phosphatase and cytoplasmic ribonucleic acid. These findings are in substantial agreement with the observations of Jeener.[2] In addition, it has been shown that progesterone alone does not have this effect. Injection of progesterone concurrently with estrogen neither augments nor diminishes the effect of the estrogen. Such combined treatment results in the eventual mucification rather than cornification of the superficial layers of the epithelium. It is interesting to note that estrogen and progesterone have similar effects on endometrial alkaline phosphatase (Atkinson and Elftman[6]) and cytoplasmic ribonucleic acid (Atkinson, unpublished data).

Relatively little is known concerning the specific functions of ribonucleic acid and phosphatase in cellular metabolism. In a recent extensive review, Caspersson[7] has presented considerable evidence to support the view that cytoplasmic ribonucleic acid is essential to the synthesis of proteins. The role played by alkaline phosphatase in the hydrolysis of phosphoric esters (*e.g.*, nucleotides, glycophosphates, phospholipids, etc.) makes it potentially important in a wide variety of physiological processes. One possible function of particular interest (Moog,[8]) is the suggestion that phosphatase activity parallels the synthesis of fibrous proteins.

The presence of large amounts of cytoplasmic ribonucleic acid and alkaline phosphatase in the estrogen and estrogen-progesterone stimulated vagina is compatible with the views of Caspersson and Moog in view of the concomitant rapid growth and elaboration of keratin or mucin. The significance of the intracellular distribution of these substances and the gradients in their concentrations is entirely speculative at the present time.

*Summary.* The amount and distribution of cytoplasmic ribonucleic acid, alkaline phos-

[6] Atkinson, W. B., and Elftman, H., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 148.

[7] Caspersson, T., *Symp. Soc. Exp. Biol.*, 1947, **1**, 127.

[8] Moog, F., *Biol. Rev.*, 1946, **21**, 41.

phatase and mucin was studied in the vaginal epithelium of ovariectomized mice and in castrates injected with estrogen and progesterone. In the untreated castrate the vaginal epithelium contains negligible quantities of all these cellular constituents. Injection of estrogen, and estrogen with progesterone concurrently, results in a marked increase in both phosphatase and ribonucleic acid. Mucification occurs only when the two hormones act simultaneously. Progesterone alone has no effect on any of the substances studied.

## 16545

## Age and Susceptibility to Convulsions.*

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Although the great susceptibility of children to convulsive disorders is well established, little experimental work has been done on the relationship of age to convulsive reactivity. Froehlich and Mirsky[1] showed that 75 to 100% of 7- to 17-day-old rats convulse when injected with 0.5 to 1.0 mg acid fuchsin per gm subcutaneously. Adult rats however did not show convulsions even after injection with 3 mg per g, but these animals show a high incidence of convulsions after treatment with theophylline. The latter drug seems to increase the permeability of the blood-brain barrier according to experiments of Froehlich and Zak mentioned in this paper (apparently unpublished). This interpretation is supported by Aird[2,3] and collaborators who found that the percentage of convulsions induced by several convulsive drugs (strychnine, picrotoxin and cocaine) decreased after pretreatment with Brilliant Vital Red or Trypan Red and that the amount of cocaine present in the brain was greatly reduced by the dye although the concentration of cocaine in the blood remained unchanged. Moreover, experiments of Behnsen[4] on the mouse and Dölter and Kruse[5] on human infants have given evidence of a greater permeability of the blood-brain barrier at an early than at a later age. For this reason experiments involving parenteral injection of convulsive drugs cannot be used for the investigation of the question of whether the convulsive reactivity of the brain itself depends on age.

The proper method for studying this question seems to be the application of electrical stimuli adequate to produce convulsions. No experimental studies on normal material seem to have been reported but observations by Watterson[6] on schizophrenics suggest that the duration of convulsive threshold current increases with age (investigated for the interval of 19 to 49 years). In view of the fact that this study was limited to clinical cases in which the disease process may have contributed to the convulsive reactivity and since the younger age group which is known to be particularly susceptible to convulsions could obviously not be included it was decided to investigate the effect of electrically induced convulsions on rats of varying age.

*Method.* The experiments were performed on 24 hour fasted Sprague-Dawley rats†

* Aided by a grant from the Office of Naval Research.

1 Froehlich, A., and Mirsky, I. A., *Arch. Neurol. Psychiat.*, 1942, **47**, 30.

2 Aird, R. B., *Arch. Neurol. Psychiat.*, 1939, **42**, 700.

3 Aird, R. B., and Strait, L., *Arch. Neurol. Psychiat.*, 1944, **51**, 54.

4 Behnsen, G., *Anat. Anzeiger*, 1926, **61**; *Erg. H.*, 179, and *Z. Zellforschung*, 1927, **4**, 515.

5 Dölter and Kruse cf. Gellhorn, E., *Das Permeabilitätsproblem, Berlin* (Springer), 1929.

6 Watterson, D. J., *Neurol. Neurosurg. Psychiat.*, 1945, **8**, 121.