conditions, those tested at 5 and 6 weeks of age were up to the optimum level.

(d) *The supplemented artificial diet deficient in all the components of the B complex except* $B_1$—there was a retardation in many mice, in that only 67 per cent. were resistant at 5 weeks as compared with 100 per cent. in the control group.

(e) *The supplemented artificial diet deficient in wheat germ oil (vitamin E)*—only 20 per cent. were resistant at 4 weeks of age. The 33 per cent. resistance observed at 6 weeks can not be regarded as significant until more mice have been tested.

(f) *The supplemented artificial diet deficient in the heat-labile components of the B complex*—the two weeks of nursing on properly fed mothers protected these mice sufficiently to prevent cannibalism and loss of life, but associated with increasing signs of vitamin $B_1$ deficiency, there was marked retardation in the development of resistance.

It is thus apparent that the presence or absence of certain factors either in the maternal diet during the nursing period or in the diet of actively growing young mice can promote, retard or inhibit the development of at least one type of constitutional barrier to involvement of the nervous system by a neurotropic virus. Not until the effect of adding the synthetic vitamins $B_1$, riboflavin or E to the respective, deficient diets has been studied will it be possible to state whether or not they, or other substances, are the factors which play a role in the development of this resistance. It should also be noted here that while inadequate nutrition could prevent or retard the appearance of this resistance in growing mice, it has not yet proved possible to break it down by the same means once it has been acquired by full-grown animals, even after they have developed signs of advanced vitamin $B_1$, E or riboflavin deficiencies.

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## EFFECT OF ESTROGENS AND ANDROGENS ALONE AND IN COMBINATION WITH CHORIONIC GONADOTROPIN ON THE OVARY OF THE HYPOPHYSECTOMIZED RAT[1]

IN 1928 Aschheim and Zondek[2] announced their discovery of a pituitary-like gonadotropin in the urine of the pregnant woman. Administered to immature female rats or mice, it caused follicular growth and luteinization in the ovaries. Subsequent studies showed that a typical A–Z response could not be secured in the hypophysectomized animal.[3,4,5]

Of the several theories advanced in explanation of the lack of effect of chorionic gonadotropin in the hypophysectomized animal, the one most generally accepted is that the pituitary of the normal animal contributes a "complementary" factor essential for the production of large follicles and corpora lutea. The secretion of this factor is believed to be mediated through the ovary, but the nature of this principle has not been definitely identified.

In connection with experiments designed to test the effects of estrogens and androgens administered alone and in combination with gonadotropins on the gonads of the normal animal, it was of interest to extend similar studies to the hypophysectomized animal. It was thought that the use of the hypophysectomized animal would obviate any modifying influences that would necessarily be introduced by the intact animal's pituitary. What follows relates to the results secured in the hypophysectomized series treated with diethylstilboestrol, estradiol dipropionate and testosterone propionate alone and in combination with chorionic gonadotropin.

The experimental material included 44 rats, 21 to 23 days old at the time of hypophysectomy. The crystalline hormones were compressed into pellets and inserted under the skin.[6] The gonadotropin was Antuitrin "S," labeled to contain 500 R.U. in each cc. Nineteen of the treated animals were allowed a seven-day regression period prior to implantation of the pellets. In twenty animals the pellets were embedded

TABLE 1

EFFECT OF DIETHYLSTILBOESTROL, ESTRADIOL DIPROPIONATE, TESTOSTERONE PROPIONATE GIVEN ALONE AND IN COMBINATION WITH CHORIONIC GONADOTROPIN ON OVARIAN GROWTH IN HYPOPHYSECTOMIZED IMMATURE RAT*

| Treatment | Number of animals | Average weight of ovaries—mg |
|---|---|---|
| None | 5 | 7 |
| Diethylstilboestrol | 8 | 28 |
| Estradiol dipropionate | 5 | 13 |
| Testosterone propionate | 3 | 8 |
| Antuitrin "S" | 9 | 14 |
| Diethylstilboestrol and Antuitrin "S" | 8 | 103 |
| Estradiol propionate and Antuitrin "S" | 3 | 21 |
| Testosterone propionate and Antuitrin "S" | 3 | 8 |

* The crystalline hormones were compressed into pellets and inserted under the skin. The average daily absorption (as determined by weighing the pellets at the time of implantation and on removal at necropsy) of diethylstilboestrol varied from 130 to 170 micrograms, and 40 to 63 micrograms for estradiol dipropionate. The total dose of chorionic gonadotropin was 75 R.U., distributed over three days with necropsy 96 hours after the first injection.

[1] Supported by the Christine Breon Fund for Medical Research.

[2] S. Aschheim and B. Zondek, *Klin. Wchnschr.*, 7: 8, 1928.

[3] F. L. Reichert, *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 28: 843, 1931.

[4] Y. Noguchi, *Jap. Jour. Med. Sci. and Pharm.*, 5: 104, 1931.

[5] J. B. Collip, *et al.*, *Nature*, 131: 56, 1933.

[6] The author is grateful to Dr. G. Biskind, Mount Zion Hospital, San Francisco, who generously prepared the sterol pellets.

in the subcutaneous tissues in the region of the neck at the time of hypophysectomy; the remaining five served as controls. Three to seven days subsequently, the animals were divided into groups and treated as indicated in Table 1.

It will be noted that both diethylstilboestrol and estradiol dipropionate treatment led to considerable ovarian enlargement. This occurred even after the ovaries had undergone considerable involution following hypophysectomy. The average ovarian weight was 28 mg following diethylstilboestrol treatment and 13 mg following estradiol dipropionate, compared with 7 mg in the untreated hypophysectomized controls.[7] Testosterone propionate, although shown to be slightly estrogenic in both normal and hypophysectomized rats,[8,9] was without effect.

The most striking difference in ovarian growth, however, occurred in the animals implanted with diethylstilboestrol pellets and subsequently injected with chorionic gonadotropin. The average ovarian weight of the animals receiving the combined treatment was 103 mg as compared with 14 mg with chorionic gonadotropin alone. Estradiol dipropionate, although markedly estrogenic and prolonged in its action,[10] yielded ovaries weighing but 21 mg, a value not significantly greater than that secured with the sterol alone. In this respect, testosterone propionate in combination with chorionic gonadotropin also proved ineffective.

Exceedingly interesting were the microscopic findings resulting from the different types of treatment. The ovaries of the diethylstilboestrol treated animals consisted of medium-sized follicles packed tightly together and markedly reduced interstitial tissue. Estradiol dipropionate, though causing some ovarian stimulation, was not as effective as diethylstilboestrol. The ovaries of the testosterone propionate treated animals showed no significant changes.

The most pronounced effect was secured in the animals implanted with diethylstilboestrol and subsequently injected with chorionic gonadotropin. The follicles were enlarged, many corpora lutea were present, and in two instances hemorrhagic follicles were also found. Similar treatment with estradiol dipropionate and testosterone propionate in combination with chorionic gonadotropin failed to give the ovarian development secured with diethylstilboestrol.

A partial explanation for the discrepancies in the results secured with diethylstilboestrol and estradiol dipropionate in combination with chorionic gonadotropin may perhaps be found in the amount of material absorbed. The average daily absorption for diethylstilboestrol (as determined by weighing the pellets at the time of implantation and on removal at necropsy) varied from 130 to 170 micrograms as compared with 40 to 63 micrograms for estradiol dipropionate. This suggests that the estrogen level necessary to enhance the effect of chorionic gonadotropin must be relatively high. The difference in behavior of the two sterols is now under further investigation.

The significance of the experiments just described and their broader application to hypophyseal-ovarian physiology will be discussed elsewhere.

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[7] P. C. Williams, *Nature*, 14: 388, 1940, also reports ovarian enlargement in hypophysectomized rats implanted with diethylstilboestrol.

[8] A. Butenandt and H. Kudszus, *Hopper-Seyl. Z.*, 237: 75, 1935.

[9] A. S. Parkes and S. Zuckerman, *Jour. Physiol.*, 93: 16P, 1938.

[10] K. Miescher, *Biochem. Jour.*, 32: 725, 1938.

# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## MICROFILM WITH THE 35-MILLIMETER CANDID CAMERA

THE use of microfilm is becoming of increasing interest as is evidenced by the number of libraries offering microfilm service and the fact that at least three journals are devoted mainly to this subject. Many advantages of microfilm have been summarized by Seidell.[1] The purpose of the present note is to call the attention of scientists to the ease with which an inexpensive 35-mm camera may be used to make their own microfilm for short runs of a few pages. Such film may be read without undue eyestrain, using the Seidell hand reader costing $1.50. The price ranges of microfilm cameras and projection readers have been reported[2] as varying from $50.00 to $5,750 and from twenty to several hundred dollars, respectively. English,[3] in *American Photography*, has given details for building a complete outfit including projection reader for about $50.00.

It has been stated[4] that photostats are more useful for short runs of a few pages and microfilm is better adapted for long runs. This is undoubtedly true on the basis of cost where the service charge is an important part of the total. Nevertheless, microfilm for short articles is of great potential use to any one who has a 35-mm camera available. By means of such a camera and a stand made from laboratory

[1] A. Seidell, SCIENCE, 89: 32–4, 1939.

[2] V. D. Tate, *Jour. Documentary Reproduction*, 1, No. 3, Part 2, 6 and 36, 1938.

[3] F. L. English, *Amer. Photography*, 32: 825–8, 1938.

[4] H. H. Fussler, *Jour. Documentary Reproduction*, 2, No. 1, 3–4, 1939.