development of the testes. They now appeared externally, increased gradually to normal size, and eventually descended. Microscopic examination at this time disclosed normally progressing spermatogenesis and normal epithelium in the seminal vesicles. Nevertheless, if males that had utilized completely their first dose of estrogen were given an additional supply in paraffin, the testes gradually decreased in size and reverted to an intraäbdominal position and a rudimentary functional state. Some observations on the temporary character of the testicular atrophy following treatment of males with estrogen have been reported by Deanesly,[14] and on reversible changes in females by Grumbrecht.[15]

Summary. An effective and simple method of insuring slow continuous absorption of estrogen in the rat is the incorporation of the crystalline hormone in paraffin (melting point 48°C), which after subcutaneous, intramuscular, or intraperitoneal injection in a liquid state, solidifies locally. As indicated by vaginal smear, the average time required for complete absorption and utilization of 5.0 mg estradiol dipropionate contained in 0.1 cc paraffin, introduced into animals 3 to 5 weeks of age, is 151 ± 5.9 days. In males the position and activity of the testes are comparable indicators of the utilization of the injected estrogenic hormone.

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An Enzyme Which Inactivates Estrone.*

BERNHARD ZONDEK AND JOACHIM SKLOW.

From the Hormone Research Laboratory, Hebrew University, Jerusalem, Palestine.

Zondek[1] was able to demonstrate that liver pulp of homothermal and poikilothermal animals as well as mash of hyacinth roots[2] inactivate estrogenic hormone *in vitro*. Cell-free liver extract yielded similar results. The inactivating factor in liver and hyacinth roots

14 Deanesly, R., *J. Endocrinology*, 1939, **1**, 36.

15 Grumbrecht, P., *Arch. f. Gynäk.*, 1940, **170**, 1.

* This paper has been aided by a grant of the Rockefeller Foundation.

1 Zondek, B., *Lancet*, 1934, **227**, 356; *Skandin. Arch. Physiol.*, 1934, **70**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd ed., Springer, Vienna, 1935, 124-162; *Genital Functions and Their Hormonal Regulations*, Williams & Wilkins, Baltimore, 1941, 147-175.

2 v. Euler, H., and Zondek, B., *Biochem. Z.*, 1934, **271**, 64.

was destroyed by boiling. This led to the conclusion that the inactivation of estrone is due to the action of an enzyme (estrinase).

Later experiments showed that estrone is also inactivated by potato juice, cauliflower, beetroots and bran[3] as well as by certain bacteria.[4] In every case boiling destroyed the active principle.

The nature of this enzyme is unknown. It is not certain whether the inactivation induced by substances so different as potato juice, liver and bacteria is due to the activity of one or of several different ferments. Heller[5] suggested that 2 different enzymes participate in estrone-inactivation by liver. According to this author an estrone-reducing enzyme contained in liver converts estrone into estradiol and the latter is then transformed by liver dehydrogenase into the inactive substance. Westerfeld[6] assumed, from experiments with mushroom extract, that tyrosinase was the inactivating principle. This was in contradiction to our own observations, in which estrone was found to be inactivated by tyrosinase-free cauliflower juice, yet was unaffected by tyrosinase-containing extracts of mealworm larvae.[3] Graubard and Pincus[7] hold laccase responsible for the inactivation of estrone. We cannot confirm this view, since laccase is present in nearly all plants while estrone-inactivation is produced only by a few plants.[3] In colorimetric determinations the presence of laccase may be masked by vitamin C. For determination of laccase we studied the oxydation of pyrogallol to purpurogallin, after having first destroyed hexuronic acid by oxydation in air at an alkaline reaction. It could thus be shown that juice of apples, tomatoes and onions as well as human saliva contain laccase but fail to inactivate estrone.

It was, therefore, concluded that estrone-inactivation is brought about by a specific enzyme, which is neither tyrosinase nor laccase.

Technic. The investigations reported below were concerned with the study of the properties of the estrone-inactivating enzyme contained in potatoes. The concentration of the enzyme in the press juice of potato peel is considerable and therefore such juice lends itself particularly well to the study of the estrone-inactivating factor.

A purified enzyme preparation was obtained in the following manner: Potatoes were thoroughly cleaned with tap water and the peel together with the outer portion of the potato grated on a glass

3 Zondek, B., and Sklow, J., *Endocrinology*, 1942, in press.

4 Zondek, B., and Sulman, F., *Endocrinology*, 1942, in press.

5 Heller, C. G., *Endocrinology*, 1940, **26**, 619.

6 Westerfeld, W. W., *Biochem. J.*, 1940, **34**, 51.

7 Graubard, M., and Pincus, G., *Proc. Nat. Acad. Sci. U. S.*, 1941, **27**, 149.

grater. The resulting pulp was pressed through gauze and the juice centrifuged. The sediment, which consisted mainly of starch, was discarded. To the supernatant a saturated solution of ammonium sulphate (SAS) was added until the juice was 0.6 saturated with ammonium sulphate. The precipitate which contained the ferment was then centrifuged and suspended in water to its original volume. The fraction that failed to dissolve was discarded and the solution once more precipitated with SAS as before. The second precipitate was separated by centrifuging, dried in the exsiccator and ground to a fine powder. This material which could be preserved in the exsiccator for a considerable time without loss of potency was used in the following experiments.

Before each experiment 200 mg of powder were shaken for 30 minutes with 5 cc of diluted NaOH (pH 9-9.5) at room temperature (20°). The centrifuged extract was then dialyzed against tap water in a rocking dialyzor for 30 minutes. Owing to loss of alkali the solution became turbid and its pH was between 7.5-7.8. At least 0.02 mg estrone were inactivated by 0.5 cc of the dialyzed solution. The concentration of estrinase in this solution was 4 times higher than in the original potato juice. For the inactivation of 0.02 mg estrone 5.6 mg dry powder of crude potato juice were needed, while an equal effect was produced by only 1.5 mg of dry powder of purified juice. The purified enzyme solution still contained tyrosinase and laccase.

In order to gauge the activity of the purified potato enzyme solution, 0.5 ml was shaken with 1.5 ml phosphate buffer pH 6.4 and 0.02 mg estrone† for 5 hours at 37°. The mixture whose pH was 6.7 was then heated in a boiling water bath and tested for its estrone content on spayed female mice.

To each of the experiments reported below (Nos. 1-7) 40 mice were used, making a total of 280 animals. The test dose was given to 5 groups of 8 animals each, subcutaneously, in 6 injections distributed over a period of 48 hours. Vaginal smears were taken at 12-hour intervals between the 48th and 96th hour after the experiment was begun. The quantity of estrone which produced full cornification (Allen-Doisy Test) was considered to be 1 MU. In our mice, 1 MU is equal to 2 IU. The estrinase unit was defined

† We are indebted to Dr. Breant, Roche-Organon, Inc., Nutley, N.J., for kindly supplying the estrone. The estrone solution was prepared by adding 1 mg estrone to 0.2 ml of 96 % alcohol and 50 ml n/100 NaOH. The alcohol was then allowed to evaporate in vacuum. The alkaline estrone solution contained 0.02 mg = 200 IU = 100 MU estrone in 1 ml.

as that quantity of enzyme which completely inactivates 2 IU = 1 MU of estrone.

Results. The following properties of the estrone inactivating enzyme solution of potatoes were noted:

1. The activity is not affected by standing in the open air for 20 hours.

2. All activity is destroyed on heating to 70°C for 45 minutes at pH 7.2. At a more alkaline or more acid reaction destruction takes place even at a lower temperature. At pH 5.5 or 8, heating to 65°C for 45 minutes is sufficient to destroy the active principle.

3. Activity is optimal between pH 6-7 and present in the range between 5-9.

4. In a 2% H_2O_2 solution the enzyme is destroyed within 1 hour.

5. In m/500 NaCN solution the enzyme is ineffective.

6. Ultraviolet irradiation of the solution (distance 50 cm) for one hour causes a 20% loss of activity.

7. Dialysis in the rocking dialyzer for one hour does not lessen its potency.

The properties of potato estrinase are thus seen to be closely related to those of tyrosinase, though to some extent different as regards the effect of pH. Tyrosinase is inactive at pH 5, while estrinase retains 50% of its potency at this reaction. The two enzymes are, however, not identical since cauliflower, which contains no tyrosinase, inactivates estrone, whereas the tyrosinase-rich extracts of mealworm larvae do not affect it.

The NCN estrinase reacts similarly to oxidase. A decrease in the estrone-inactivating potency of liver pulp on exposure to m/500 solution of NaCN has also been reported by Heller.[5]

Summary. The properties of estrinase, an estrone inactivating enzyme contained in potatoes, are described.