

TABLE I.
Separation of Pyridoxine and Pseudopyridoxine by
Means of Lloyd's Reagent.

	Filtrate, γ/cc	Eluate, γ/cc
Pyridoxine		
<i>Sacch. carlsbergensis</i>	0.0000	0.0016
Pseudopyridoxine		
<i>L. casei</i>	0.16 equivalents	0.31 equivalents

brought to pH 3.0 with sulfuric acid. 0.5 g of Lloyd's reagent was added to absorb the pyridoxine. The pyridoxine was eluted with 15 cc (three 5-cc portions) of 1/10 N NaOH. This eluate was neutralized and diluted to 25 cc. Both the solution left after the Lloyd's reagent absorption (concentrated to 25 cc) and the eluate were analyzed for pyridoxine

and for pseudopyridoxine. The results are given in Table I.

From this table it can be seen that while about two-thirds of the pseudopyridoxine follows the pyridoxine in its absorption as found by Snell *et al.*,¹ the filtrate does contain considerable pseudopyridoxine and no pyridoxine. Thus some separation was secured and again the yeast was unable to utilize the pseudopyridoxine.

Summary. Pyridoxine has been separated from pseudopyridoxine by using the yeast *Saccharomyces carlsbergensis* and the bacterium *Lactobacillus casei*. It has been shown that *Sacch. carlsbergensis* assays and utilizes only pyridoxine while *L. casei* assays and utilizes only pseudopyridoxine.

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Ovarian Tumors in Adult Rats Following Prepuberal Administration of Estrogens.*

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Estrogens injected into lactating rats have been shown to be transmitted to the suckling young inducing changes in urogenital organs of both sexes.¹ The effects are of such a nature that it seemed advisable to make further observations on similar animals, at intervals after cessation of treatment, in order to determine the permanency of the condition and the course of any subsequent changes. It has been reported that female rats given prepuberal injections of androgen^{2-4,6} or estro-

gen⁵⁻⁷ have severe reproductive deficiencies as adults. Because of variations in methods of administration, hormones used, and length of treatment, the present study brings out differences thought to be of significance.

Methods. We have observed that in untreated female littermates of immature animals given daily injections of diethylstilbestrol, the vaginas open at the same time as in injected animals. This has been confirmed repeatedly with large litters containing only one injected animal. Similar changes have been observed in litters confined to cages in which stilbestrol was sprayed upon shavings used as nesting material. Injection of cagemates or contamination of nesting material with stilbestrol may therefore be used as alternative methods of administering hormone to immature animals.

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¹Weichert, C. K., and Kerrigan, S., *Endocrinology*, 1942, **30**, 741.

²Wilson, J. G., Young, W. C., and Hamilton, J. B., *Yale J. Biol. and Med.*, 1940, **13**, 189.

³Wilson, J. G., Hamilton, J. B., and Young, W. C., *Endocrinology*, 1941, **29**, 784.

⁴Greene, R. R., Burrill, M. W., and Ivy, A. C., *Anat. Rec.*, 1942, **83**, 19.

⁵Wilson, J. G., *Anat. Rec.*, 1943, **86**, 341.

⁶Greene, R. R., and Burrill, M. W., *Proc. Am. Physiol. Soc.*, 1941, 114.

⁷Turner, C. D., *Proc. Am. Physiol. Soc.*, 1941, 283.

Five groups of rats, totalling 154 animals, were treated with estrogen for various lengths of time, the initial treatment being given at various times between birth and the 28th day of life. Animals of Group I were suckled by mothers injected with .1 mg stilbestrol daily; in those of Group II the mothers received .5 mg estrone daily. Group III received direct injections of .01 mg stilbestrol, while those of Group IV were affected by stilbestrol injected into littermates given .01 mg daily. Group V consisted of animals exposed to small quantities of environmental stilbestrol on nesting material. Periods of treatment for the first 4 groups ranged from 1 to 14 days, for Group V, 21 or more days. Vaginal smears were taken routinely. Representatives from each group were autopsied either at the end of treatment, at intervals following treatment, or at mature ages ranging from 4 to 15 months.

Results. Abnormalities appeared in the smear histories in all groups. The degree of abnormality was related to the age at which treatment was begun since it was less pronounced and appeared only after 4-9 months among animals treated at the later ages. Characteristically, these animals tended to remain in vaginal estrus for extended periods. In many, a peculiar type of smear, which often persisted for months, contained both large epithelial cells and leucocytes in quantity. Since this was maintained over extended periods it cannot be regarded as a transitional stage but would seem to be the result of continued estrogenic stimulation associated with some other unknown condition.

The majority of these prepuberally treated animals failed to breed. In many, however, copulations occurred repeatedly. Spermatozoa were often noted in the vaginal smears, and sperm plugs were also observed. The few animals which did produce young, in almost every case, came from litters in which treatment was begun late. Some of these subse-

quently failed to breed and either developed irregular cycles or permanent vaginal estrus. Some animals exposed to stilbestrol sprayed repeatedly on shavings between days 8 and 20 maintained regular cycles for over 400 days while caged with males but, nevertheless, failed to become pregnant.

Few gross changes were observed in reproductive organs of animals under 120 days of age, but after this time marked changes began to appear in ovaries, uteri, and Fallopian tubes. The most pronounced alteration was the formation of roughly spherical or multilobular cysts in one or both ovaries, which in some cases were larger than the stomach. These cysts contained a great amount of yellowish caseous material of a semi-fluid consistency. Gross sections, made after fixation, showed a firm outer wall enclosing a relatively large granular mass. In animals less than 6 months old the enlargement was generally of a slighter degree. In a few cases ovaries were atrophic. Some of these were taken from animals after 40 to 60 days of persistent vaginal estrus. A considerable amount of tissue often connected the enlarged ovaries to the viscera and to the body wall, which in 2 cases became necrotic. Histological and bacteriological studies are being carried on and will be reported later. The following abnormal conditions have been identified: pyo-ovarium, pyometra, pyosalpinx, and uterine metaplasia. No typical ovarian tissue has been seen in the greatly enlarged ovaries studied thus far.

Our results differ from those of other investigators⁵⁻⁷ who have failed to report a cystic ovarian condition in rats following similar treatment. In size and gross characteristics, these ovaries resemble those of animals given large amounts of testosterone propionate or estrone over prolonged periods.⁸

⁸ Shay, H., Gershon-Cohen, J., Paschkis, K. E., and Fels, S. S., *Endocrinology*, 1939, **25**, 933.