

clear differentiation of the lids and the cornea; the anterior chamber is present in a rudimentary form only. In the normal eye the vitreous can be seen between the lens and the retina, while the same space in the abnormal eye is filled with connective tissue. The retina of the abnormal eye is folded and disorganized. In the closed abnormal eyes the lids are fused with the cornea and the anterior chamber appears as a linear space between the thick membrane thus formed and the lens. In

several specimens a cleft can be seen in the inferior part of the retina and a strand of connective tissue which penetrates this cleft, spreads out in the space between the lens and the retina. This cleft represents a coloboma of the retina.

As controls 62 females of the same strain were fed diet W supplemented with vitamin A. They had 410 young, none of which showed abnormal "open eyes."

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Effect of Dietary Hepatic Injury on Inactivation of Estrone.

REGINALD A. SHIPLEY AND PAUL GYÖRGY.

From the Departments of Medicine and Pediatrics, Western Reserve University School of Medicine, Lakeside Hospital, and Babies and Childrens Hospital, Cleveland, Ohio.

Golden and Sevringhaus¹ observed in the rat that when a functioning ovary was transplanted into the mesentery, estrus did not occur. They concluded that estrogen which was thus routed through the portal circulation had been inactivated by the liver. Failure of the liver to inactivate estrogen has been demonstrated by Biskind and Biskind² in rats maintained on a vitamin-deficient diet. These workers found that ovariectomized rats receiving a normal diet showed no vaginal cornification after estrone pellets were implanted in the spleen, while animals on a diet deficient in the vitamin B complex went into estrus within 1 to 3 weeks. The occurrence of estrus was interpreted as an indication of impaired liver function. Estrus could subsequently be made to disappear by the addition of yeast to the diet.

In the liver disorder produced by Biskind and Biskind, no anatomical changes in the organ were observed. The experiments were therefore concerned with functional changes resulting from the avitaminosis. It is easily possible to produce severe hepatic necrosis

and cirrhosis by the administration of diets low in protein and high in fat.³ The following experiments were performed in order to determine whether the hepatic lesions so produced would result in failure of estrogen inactivation and whether evidence of repair could be detected by the disappearance of estrus during treatment. In an additional series of rats, the experiments of Biskind and Biskind with B-complex-free diets were repeated.

Methods. Rats used in the cirrhosis studies were of Sprague-Dawley strain. Those that received the B-deficient diet were of 3 separate strains. The animals weighed approximately 200 g. About 10 days after ovariectomy, 2 ♂ of estrone were injected in order to test the reactivity of the animal. If reactive, a 5 mg pellet of estrone was implanted in the spleen.* More than half the rats went into estrus immediately thereafter but, in most cases, this lasted only from 2 to 4 days.

¹ Golden, J. B., and Sevringhaus, E. L., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 361.

² Biskind, M. S., and Biskind, G. R., *Endocrinol.*, 1942, **31**, 109.

³ György, Paul, and Goldblatt, Harry, *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 492; Webster, Graham, *J. Clin. Invest.*, 1941, **20**, 440; Blumberg, Harold, and McCollum, E. V., *Science*, 1941, **93**, 598; Lillie, R. D., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1941, **56**, 1255.

* We are indebted to Dr. D. K. Kitchen of Parke, Davis and Co. for the supply of Estrone (Theelin).

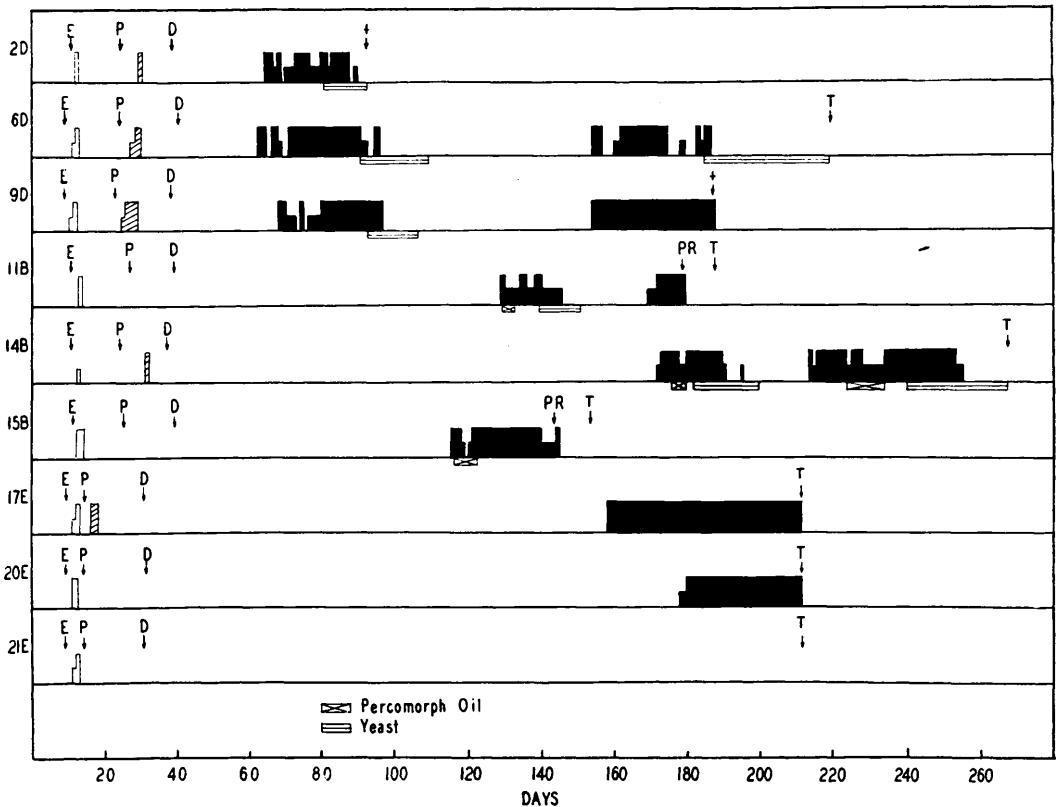


FIG. 1.

Series of rats receiving a diet low in protein and high in fat. The blocked areas represent estrus. Partial estrus is depicted by a reduction in height of the block. Symbols are as follows: E—test dose of estrone; P—insertion of pellet; D—diet started; PR—pellet removed; T—experiment terminated; +—death of animal. The rats were castrated at zero time.

If it persisted longer than 10 days, the animal was discarded. Of the rats originally ovariectomized, 60% fulfilled the necessary conditions to proceed with the experiment. The rate of absorption from the pellets average 11 γ a day.

The experimental diets were as follows:

Cirrhosis Producing Diet.

Casein	8%
Crisco	38%
Sucrose	48%
Cod liver oil	2%
Salt mixture	4%
Vitamin supplement—1 cc daily.†	

B-complex Deficient Diet.

Casein	18%
Peanut oil	8%
Sucrose	68%
Cod liver oil	2%
Salt mixture	4%

Results. Cirrhosis Diet. Nine rats received the diet. Eight went into estrus within 25 to 150 days, but one animal (21E) did not respond within the period of 180 days during which it was followed. The vaginal response was occasionally erratic during the first few days succeeding its onset, but usually remained constant thereafter. Percomorph oil, 3 drops daily (5000 I.U.), did not abolish the reaction but sometimes decreased it. After the estrus had persisted for 10 to 30 days, 5 rats were treated with Brewer's yeast (Anheuser Busch strain G), added to the basic diet in a concentration of 25%. In all cases, there was disappearance of estrus after an interval of 3 to 15 days. Rat 2D died of unexplained causes shortly after disappearance

† The supplement contained in each cc: Thiamine 20 γ , pyridoxine 20 γ , riboflavin 25 γ , Ca pantothenate 100 γ .

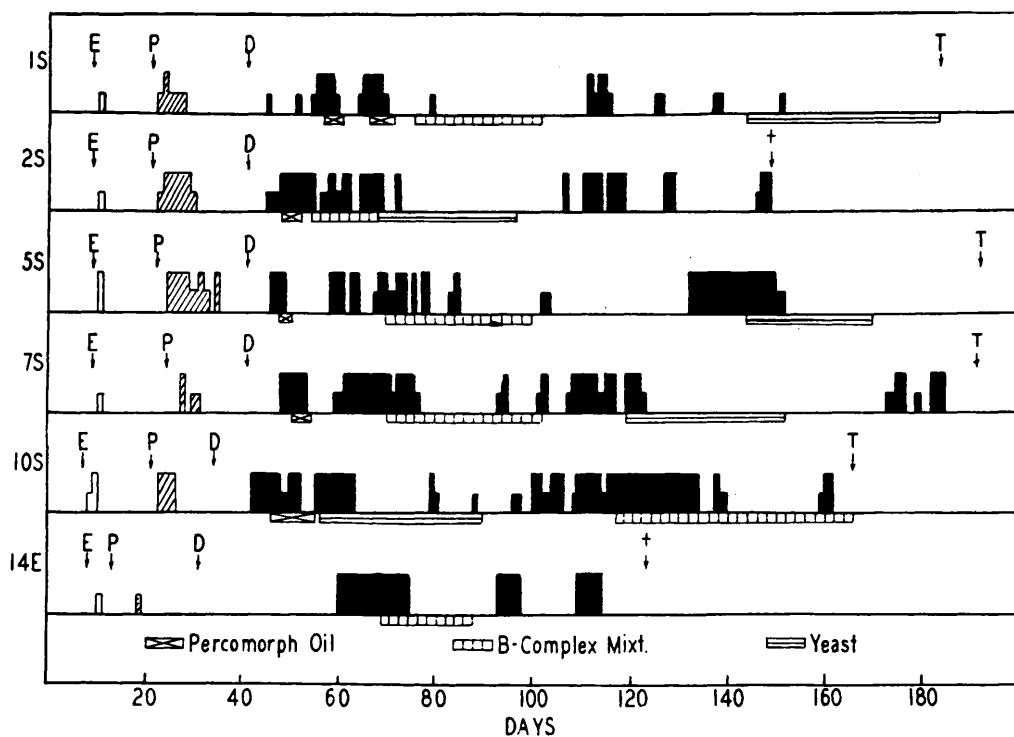


FIG. 2.

The effect of Vitamin B-complex deficiency. The symbols have the same meaning as in Fig. 1.

of estrus. Four of the 5 rats were taken off yeast and estrus again appeared. It was made to disappear a second time in rats 6D and 14B by resumption of yeast therapy. The livers of the rats on the cirrhosis-producing diet showed the usual fatty changes along with necrosis and fibrosis.

B-complex Deficient Diet. A group of 7 hooded rats bred in the laboratory were fed the diet for 23 days without the appearance of estrus in any rat. Nine rats of Sprague-Dawley strain were followed until they either died or showed estrus. Seven out of this group responded with positive smears within 7 to 37 days; however, 3 did not react until they were extremely emaciated and near death. The 2 which failed to respond died after 42 and 56 days respectively. A group of 6 Sherman rats, kindly supplied by M. S. Biskind, promptly and uniformly went into estrus. All became positive within 5 to 14 days, and before any serious ill effects from the diet were evident.

In general, the estrus from avitaminosis B was not as solidly sustained as that following

the cirrhosis-producing diet but tended to be interrupted by periods of anestrus. In the rats which responded, estrus could usually be abolished by the administration of 3 drops of percomorph oil daily. It was at first suspected that this was indicative of a false estrus due to vitamin A deficiency. It was proved unlikely that such was the case, however, inasmuch as this dose of oil prevented estrus after the injection of 2 γ of estrone into several reactive castrate rats. Neither cornified nor nucleated cells appeared in the smears. Furthermore, a similar dose of the oil usually caused normal cyclic females to remain in diestrus. It has been reported that vitamin A in large doses prevents vaginal cornification and stimulates the formation of nucleated cells.⁴ In the series at hand, the effect was to maintain complete anestrus.

Administration of 1.8 g brewer's yeast daily to 5 rats which went into estrus resulted in total anestrus in 4 of them within 4 to 8 days.

⁴ Sherwood, T. C., Brend, M. A., and Roper, E. A., *J. Nutrition*, 1936, **11**, 592.

The fifth (10S) was treated for 37 days and during this time was for the most part anestrus although there were occasional days on which cornified cells in good number were encountered. Seven rats were treated with 1 cc of the B-complex mixture daily for 20 to 46 days. While on the supplement, these rats showed a tendency to remain in anestrus but the response was for the most part incomplete (Fig. 2). Fifty mg para-aminobenzoic acid daily in addition to the other B vitamins did not improve the response of one rat (10S) showing partial anestrus.

The livers of the untreated rats on the B-deficient diet showed no histological evidence of damage except in one case where a slight fatty change was demonstrable.

Discussion. The impairment of estrone inactivation which was observed in rats receiving a diet low in protein and high in fat may be assumed to have resulted from the accompanying injury to the liver. The onset of functional disability appeared after an interval when severe histological changes are known to make their appearance. It would appear that the method should be useful in the study of experimental cirrhosis. Reversal of the estrus reaction by the administration of large doses of yeast indicates that curative measures may be very nicely assessed in the living animal.

In confirmation of the work of Biskind and Biskind, vitamin B-complex deficiency was found to interfere with hepatic inactivation of estrone. The alteration produced by the

B-deficient diet, however, was neither so constant in occurrence nor so solidly sustained as that which followed the cirrhosis-producing diet and the effects of therapy were not so easily judged. There appeared to be a strain difference in response. Sherman rats were very susceptible while a local strain of hooded rats was relatively resistant. Sprague-Dawley rats were intermediate. The B vitamins known to be required by the rat were not adequate for the complete reversal of the effect when given in ordinary maintenance doses. Segaloff and Segaloff,⁵ however, in somewhat similar experiments recently reported that large doses of riboflavin and of thiamine were effective.

Summary. Liver damage produced in rats by diets low in protein and high in fat was consistently reflected in the living animal by impairment in ability to inactivate estrone. The curative effect of large doses of yeast could be demonstrated.

Vitamin B-complex deficiency was usually accompanied by a similar but less constant hepatic impairment and without anatomical damage to the liver. There appeared to be a strain difference in susceptibility. A mixture of known vitamins was only partially effective in abolishing the effect of the diet, while yeast was distinctly and more consistently effective.

We are greatly indebted to Miss Ethel Buchwald for technical assistance.

⁵ Segaloff, A., and Segaloff, A., *Endocrinol.*, 1944, **34**, 346.

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Spasmolytic Activity of Diethylaminoethyl Esters of Substituted α -Thienylacetic and α -Thienylglycolic Acids.

A. M. LANDS AND V. LORAIN NASH.

From the Pharmacological Research Laboratory, Frederick Stearns and Company, Detroit, Mich.

During recent years a number of reports have appeared which described the pharmacologic activity of a variety of synthetic products which were developed in an effort to obtain an effective antispasmodic substance that would

be relatively free of the undesirable side actions of atropine. These substances may be conveniently divided into two groups: (1) amines, such as Perparine, Cyverine, or Sestron, and (2) basic esters of arylaliphatic