Estrogen-Inactivation by the Liver as Modified by Dietary Protein.* (17578)

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One of the functions of the liver is to inactivate both endogenous and exogenous estrogens. This phenomenon has been amply confirmed in both *in vitro* and in the intact animal.(1-5) The inactivation mechanism becomes impaired in the presence of hepatic damage in the experimental animal and in man.(6-9) Golden and Sevringhaus(10) implanted ovaries into a site drained by the hepatic portal system and demonstrated that if the liver is the first organ in contact with the estrogen, the latter is completely inactivated. Biskind and Marks(11) confirmed this observation by implanting pellets of estrone into the spleen. Biskind and Biskind (12) carried out further studies to show that when rats are placed on diets deficient in the components of the vitamin B complex the liver loses its ability to inactivate the estrone absorbed from the implanted pellet. Segaloff and Segaloff (1944)(13) claimed that the important components of the B complex in this respect were thiamine and riboflavin. Drill and Pfeiffer(14) and Jailer(15) although employing different methods, have demonstrated that the concomitant inanition which accompanies vitamin B deficiency is the important factor in the loss of ability of the rat liver to inactivate the estrogens in these experiments.

The purpose of this study was to ascertain whether the protein content of the diet can modify the role of inanition in the inactivation mechanism of estrogens by the liver.

Methods and Materials. Adult female rats of the Wistar strain were ovariectomized and pellets of α-estradiol weighing 1-3 mg were subsequently inserted under the capsule of the spleen. Only those animals whose vaginal smears reverted to the diestrus type within eight days were used as experimental animals. About one-fourth of the animals were discarded because the vaginal smears remained cornified. Vaginal smears were done daily throughout the course of the investigation and at the conclusion of the experiment the animals were sacrificed, the uteri dissected clean of connective tissue and weighed on a torsion balance.

Two different categories of experiments were performed. In the first, the animals were placed on iso-caloric diets deficient in the components of the vitamin B complex containing either 5, 15, or 50% casein. These rats were allowed to eat *ad libitum* and the

* Aided by a grant from the Schering Corporation and from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

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TABLE I.
Composition of Diets Used.

	5% protein	15% protein	50% protein
Casein	5	15	50
Sugar	81	72	38
Vegetable oil	8	8	8
USP salt mixture	4	4	4

amount of food consumed daily was recorded. The constituents of the diet are presented in Table I. Under this regimen the daily food intake gradually declined so that after 3 weeks the amount of food consumed was less than one-third that of the control period. In the second experiment, the same diets were employed, however the various components of the B complex (Vifort-0.3 ml every other day) were added to the drinking water. In this experiment the food intake was limited to 3 g daily.

Results. Vitamin deficiency experiments. In general, the vaginal smears in the animals on the 5 and 15% protein diets began to show signs of cornification on about the twentieth day while those on the 50% protein diet remained atrophic. On the twenty-seventh day after the institution of the diet the animals were sacrificed. The results are presented in Table II. The average uterine weights of those animals on the 50% casein diet showed practically no evidence of stimulation, while the uteri of the rats in the other 2 groups showed definite signs of estrogen activity. All 3 groups of animals consumed about the same quantity of food and lost about the same percentage of the initial weight on the vitamin B deficient regimen (Table II).

Inanition experiments. Although the intake of the components of the B complex was adequate, the results of this experiment were similar to those of the previous one (Table III). Here again, those animals which were on the 50% casein diet exhibited lower uterine weights than those on the 5 and 15% diets. This is indicative of a more efficient estrogen inactivation mechanism. It is also clear that in the 5 and 15% casein group there is some inactivation of the estrogen absorbed from the splenic pellet since the control group with intramuscular pellets showed still higher uterine weights (Table II). However, sufficient estradiol escapes inactivation by the liver so that there is approximately a 100% increase in uterine weight over those of the castrate controls.

Discussion. The data presented confirm the previous observations of Drill and Pfeiffer (14) and Jailer (15) that the inanition which accompanies vitamin B deficiency is an important factor in the inability of the liver to inactivate estrogens. There was no difference in the group of animals exhibiting avitaminosis B and the group on an inanition diet but receiving adequate amounts of the various components of the B complex. The animals in both groups of experiments were fed iso-caloric diets, consumed approximately the same amount of food and lost about the same amount of weight. Yet, the livers of those animals which received the 50% protein could inactivate practically all the estradiol absorbed from the pellet which had been implanted into the spleen. Only those rats which were maintained on the 5 and 15% protein diets, however, showed increased uterine weights, indicating that their livers have lost the ability to inactivate all the estradiol absorbed. Although the livers were not analyzed for their protein content, it is probable that the protein concentration varies directly with the protein content of the diet. The enzymes responsible for the degradation of estradiol or estrone have not been isolated as yet, but it would appear that they are proteins or at least the prosthetic groups may be attached to a protein. It is entirely possible that during inanition this enzyme cannot be synthesized by the liver, except in those animals which were consuming the 50% protein diet.

In both experiments the caloric intake of the animals was approximately one-third of that consumed by control animals allowed to eat *ad libitum*. Therefore, the animals consuming the 50% casein diet are getting the same absolute amount of protein as do the control animals (15% casein) although the total caloric intake is reduced. This appears to be a sufficient amount of protein to synthesize the enzymes responsible for the inactivation of estradiol. Unna *et al.* (1943) (16) have previously shown that liver slices of rats maintained on a low protein diet were

TABLE II.
Vitamin B Deficient Diet. Food Intake Unrestricted.

% protein diet	No. of rats	Wt, g		Food intake, g		Uterine wt, mg
		Start	Finish	Total	Protein	
5	6	166	104	159	8.0	99 ± 19
15	5	173	118	149	22.4	130 ± 27
50	6	148	109	159	79.4	66 ± 3
Castrate control	3					63 ± 3
Intramusc. pellet	4					157 ± 23

TABLE III.
Food Restricted to 3 Grams Daily. Vitamin B Complex Added.

% protein diet	No. of rats	Body wt, g		Uterine wt, mg
		Start	Finish	
5	7	186	112	118 ± 9
15	7	189	116	93 ± 9
50	8	186	117	70 ± 5
50 (No pellet)	5	209	125	50 ± 1

not as effective in inactivating estradiol *in vitro* as slices from the control rats. Hepatic damage whether surgically, chemically or nutritionally induced, interferes with the hepatic mechanism concerned in the degradation of the estrogens. György(9) has also shown that when rats are placed on a low protein diet the liver loses its ability to inactivate estrogens, the type of injury involved in his experiments is different from the ones reported here. His animals were fed a diet low in protein but high in fat which ultimately results in fatty infiltration and finally hepatic cirrhosis. Visible hepatic damage is present at the time the estrogen inactivation mechanism is impaired. In these experiments, on the other hand, there is no visible evidence of hepatic damage.

Summary. The liver of rats maintained on a 50% casein diet retains its ability to inactivate estradiol in spite of avitaminosis B or inanition. However, animals on a 5 or 15% diet under the same conditions cannot inactivate estradiol.

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Received November 22, 1949. P.S.E.B.M., 1950, v73.