SPECIAL ARTICLES

THE FATE OF ESTROGENIC METAHORMONES IN THE LIVER[1]

FULL evidence exists that inactivation of α-estradiol takes place in the liver. But there is still controversion as to the end-products of the metabolic changes ovarian estrogens undergo when metabolized in the liver (see the summary[2]). There is the fundamental problem whether urinary estrogens or metahormones, as estriol and others, are these end-products. We have undertaken a study of this question with two natural estrogens, as estriol and equilenin, and two artificial derivatives, as α- and β-dihydroequilenin. Our experiments, which are of long duration, give clear evidence that urinary estrogens, or their derivatives, are inactivated in the liver as ovarian estrogens are.

As has been shown in former work, abdominal fibroids similar to those induced with ovarian estrogens[3,4] can be elicited with the mentioned four estrogens when quantities sufficiently great are absorbed from subcutaneously implanted tablets during three months.[5] Will similar fibromatogenic quantities of urinary estrogens still elicit fibroids when absorbed from the spleen? Should estriol or equilenin be the end-products of hepatic antiestrogenic activity, these substances when again driven through the liver should invariably keep their estrogenic faculties, *i.e.*, they should still be fibromatogenic when absorbed from intrasplenic tablets as they are when absorbed from subcutaneously implanted ones.

Tablets of estriol, equilenin, α- and β-dihydroequilenin of about 5 mm in diameter and weighing 18 to 48 mgr were implanted into the spleen of 51 castrated female guinea pigs which were autopsied 90 days later. The results were compared with those formerly obtained with subcutaneously implanted tablets of urinary estrogens.[5] The fibrous abdominal reaction was classified according to units as explained in former papers (see especially[6]). Fibrous peritoneal strands or tumors of four different regions (uterus, mesosalpinx, digestive tract and abdominal wall, spleen) are arranged for each region separately in four different classes according to their size, and each region is characterized by the values 0.5, 1, 2 and 3; the sum of the values of the four regions is the "fibrous tumoral effect" (F.T.E.); the maximal number of units an animal can attain is 12. Our results in the two comparative series are given in Fig. 1. A F.T.E. of 8 to 10 was attained with the

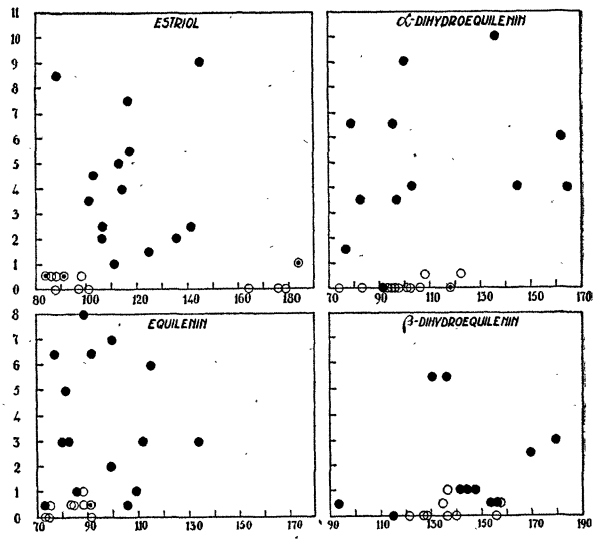

FIG. 1. Abscissa—quantities absorbed per day, in micrograms. Ordinates—units of F.T.E. ● Round black spot—animals with subcutaneously implanted tablets. ○ Circle—animals with intrasplenically implanted tablets. ⊙ Circle with dot in the center—animals with intrasplenically implanted tablets, with adhesions between spleen and abdominal wall.

absorption from subcutaneously implanted tablets of estriol, equilenin or α-dihydroequilenin; on the contrary, there was with the absorption of these substances from the spleen not a single animal with F.T.E. greater than 1, though the quantities absorbed were similar to and often greater than those which are fibromatogenic when absorbed from subcutaneously implanted tablets.

Our present work shows in a striking manner that the liver inactivates urinary estrogens even when enormous fibromatogenic quantities are absorbed from the spleen and administration is continued for several months.

As shown by the average uterine weight (see table) inactivation of urinary estrogens was not always a complete one; but the difference between the figures obtained with absorption from the spleen and with that from beneath the skin was enormous. There was

[1] Aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Rockefeller Foundation. Thanks are due for estrogens to Dr. Oliver Kamm, of Messrs. Parke, Davis and Co.

[2] J. Schiller and G. Pincus, SCIENCE, 98: 410, 1943.

[3] A. Lipschütz and R. Iglesias, *C. R. Soc. Biol.* (Paris), 129: 519, 1938.

[4] A. Lipschütz, *Cold Spring Harbor Symp. Quant. Biol.*, 10: 79, 1942.

[5] R. F. Mello, *Proc. Soc. Exp. Biol. and Med.*, 55: 149, 1944.

[6] A. Lipschütz and M. Maass, *Cancer Research*, 4: 18, 1944.

TABLE 1

Estrogen used	Number of animals*	Absorbed per day µgr	Uterine weight Average† gr	Uterine weight Range gr
		Intrasplenic		
Estriol	10	115	1.6	0.6–2.7
Equilenin	12	80	0.9	0.5–2.8
α-dihydro-equilenin ...	10	97	1.1	0.3–4.4
β-dihydro-equilenin ...	10	137	0.8	0.4–2.2
		Subcutaneous		
Estriol	15	111	5.4	3.7t.§–15.0t.
Equilenin	15	97	6.8	3.8 –15.5t
α-dihydro-equilenin ...	12	111	7.5	3.6t. –15.1t.
β-dihydro-equilenin ...	12	136	5.9	3.4 –10.5t.

* Animals with adhesions of the spleen to the abdominal wall were discarded; see circles with dot in the center in the figure.

† 0.3 to 0.5 gr is the uterine weight of a castrated guinea pig; 1 gr is the approximate uterine weight of an adult virginal female.

§ Uterine weight including subserous or mesometric tumors.

in the present series also an estrogenic action on the vaginal mucosa in about two thirds of the animals, though a weak one in most cases.

Our experiments leave no doubt about the statement that the liver is able to inactivate great quantities of estriol and equilenin. Though these urinary estrogenic metahormones derive from the intrahepatic conversion of ovarian estrogens, they are not the end-products.[7] On the other hand, the question remains open about the special changes the metahormones undergo in the liver.

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THE FIRST STAGE OF ANTIGEN-ANTIBODY REACTION IN INFECTIOUS MONONUCLEOSIS

ONE of the problems posed following the description of the pathogenesis of erythroblastosis fetalis was the failure to demonstrate anti-Rh agglutinins in about 50 per cent. of the Rh – mothers of erythroblastotic infants.[1] It was assumed that in these individuals the antibodies are fixed to the tissue cells of the reticulo endothelial system.[1, 2] This view is no longer tenable because Race[3] and Wiener[4] independently described antibodies which unite with Rh + blood but fail to cause visible agglutination.[5] Presumably the Rh + cells are specifically coated with the antibody so that they are no longer susceptible to the agglutinins in anti-Rh sera. These immune substances are referred to as blocking or incomplete antibodies. These significant observations were amply confirmed by the writers.

TABLE 1

THE SERUM USED IN THESE TESTS WAS DERIVED FROM A PATIENT SUFFERING FROM INFECTIOUS MONONUCLEOSIS

Blood	Agglutination titer	Hemolysis with complement*	Absorption for sheep blood†	Absorption for goat, type 3†	Blocking antibody‡
Goat, type 1	1:20	1:400	almost complete	none	strong
Goat, type 2	1:100	1:400	complete	complete	very strong
Goat, type 3	1:4000	1:400	complete	complete	
Sheep	1:2000	1:400	complete	none	

* Fresh guinea pig serum 1:10 was added. The values given indicate complete hemolysis.

† The agglutinating serum was diluted 1:20 and absorbed with one-half volume washed sediment of each of the four bloods. The absorbed fluids were tested with sheep blood and goat blood, type 3.

‡ Sheep blood was added after the initial readings were made.

It is known that the vast majority of the sera of patients suffering from infectious mononucleosis agglutinate sheep blood (Paul-Bunnel reaction). Exceptionally, however, this diagnostic reaction can not be demonstrated in otherwise typical cases.[6] Apparently, this state of affairs is quite analogous to that existing in erythroblastosis fetalis.

Accordingly, a search was made of sera of patients in whom a diagnosis of infectious mononucleosis was established. A number of sera containing potent agglutinins for sheep blood were collected and one serum was found with almost complete lack of action on sheep red blood cells.[7] This serum, however, contained antibodies which coated the surface of sheep

[7] It has been shown in the meantime that also equilin is inactivated when injected into the spleen of the castrated rat. A. Segaloff, *Endocrinol.*, 33: 212, 1943.

[1] P. Levine, L. Burnham, E. M. Katzin and P. Vogel, *Am. Jour. Obst. and Gyn.*, 42: 925, 1941.

[2] A. S. Wiener, *Arch. Path.*, 32: 227, 1941.

[3] R. R. Race, *Nature*, 153: 771, 1944.

[4] A. S. Wiener, *Proc. Soc. Exp. Biol. and Med.*, 56: 173, 1944.

[5] A similar effect with bacterial anti-sera was observed by A. F. Coca and M. F. Kelley, *Jour. Immunol.*, 6: 87, 1921.

[6] N. Rosenthal and G. Wenkebach, *Klin. Woch.*, 12: 499, 1933.

[7] For this specimen, I am indebted to Dr. J. H. Scherer, of the Medical College of Virginia.