

subcutaneous, intranasal or intracerebral routes, of a St. Louis virus freshly isolated from mosquitoes, failed to produce clinical disease. It did produce inapparent infection resulting in the presence of virus in the blood, in 2 instances in which suitable tests were made, and in all 4 inoculated animals induced a high titer of serum antibody. We conclude that in horses, inapparent infection occurs frequently in many widely scattered areas of the United States.

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Effect of Estradiol-Benzoate on Serum Lipid of Rats Consuming a High Fat Diet.

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The ability of estrin to induce a lipemia in the male fowl was demonstrated by Lorenz, Chaikoff, and Entenman.¹ This was confirmed by Zondek and Marx,² who not only showed a marked lipemia but also the accumulation of fat in the internal organs, particularly the liver. Very large doses were employed of the order of 40,000 I.U. (4 mg estradiol-benzoate) given on 2 successive days. Their results with mammals were negative. They could not induce lipemia in rabbits, rats, and man with large doses of estrin. The work reported below is part of a larger experiment and is presented, primarily, to show that an increase of serum lipid in rats is possible with estradiol-benzoate.

Methods. Rats were taken at weaning time and placed on low fat diets for 4 weeks. During the subsequent 8 weeks which comprised the experimental period the special diets and estrogenic hormone were administered. One group received the fat-free, high carbohydrate diet 550-B;³ a second group received the balanced ration containing 20% lard, diet 560-B;³ and the third group was

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¹ Lorenz, F. W., Chaikoff, I. L., and Entenman, C., *J. Biol. Chem.*, 1938, **126**, 763.

² Zondek, B., and Marx, L., *Arch. Int. Pharmacodyn.*, 1939, **61**, 77.

³ Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

given a high fat diet, 580-B,⁴ with the fat (71%) supplied as hydrogenated coconut oil (no unsaturated fatty acids were included in the diet). The same supplements were provided with all 3 diets. Estradiol-benzoate[†] was administered only to male rats receiving diet 580-B, as follows: In subgroup 1 each rat received subcutaneously 300 μ g administered in 100 μ g doses[‡] (1000 I.U.[§]) on alternate days during the last week of the experimental period. Those in subgroups 2 and 3 were given 30 μ g (300 I.U.) and 5 μ g (50 I.U.), respectively, every day except Sunday during the last 4 weeks of the experimental period (24 injections for each animal in each subgroup). All of the rats were anesthetized with ether 24 hours after the last injection and blood was taken from the abdominal aorta. The blood obtained from each group of 3 animals was pooled. After centrifugation the serum was removed and analyzed for total lipid^{||} by means of the oxidative method described by Boyd.⁵

Results. Animals receiving the high fat diet showed a higher average value for serum lipid than those on the balanced and fat-free rations. With rats in subgroup 1, which received large amounts of the hormone over a relatively short period, the value for serum lipid did not exceed that observed in the controls. This is contrary to Zondek's observation on cocks in which equivalent amounts of hormone over a short interval caused a large increase in the blood lipid value. However, subgroups 2 and 3 which received smaller doses over a longer interval showed a definite, though moderate, increase in serum lipid. The negative result in subgroup 1 indicates that rats are much more resistant to the estrogen effect than fowl. Animals in subgroup 3, although they received in all less hormone (120 μ g) than those in subgroup 1 (300 μ g) exhibited the lipemic response.

Whether the positive estrogen effect is due to the nature of the diet or the deficiency of essential fatty acids, or possibly both, can not be ascertained from the data at hand. Zondek and Marx² report that in cocks, at any rate, the effect of estrogenic hormone on the blood lipid level is not significantly influenced by the composition of the diet.

⁴ Loeb, H. G., Ph.D. Thesis, University of Minnesota Library, 1940.

[†] The authors wish to thank Dr. R. J. Floody (Roche-Organon, Inc.), who kindly supplied the estradiol-benzoate.

[‡] Each dose in every case was contained in 0.05 cc of olive oil.

[§] International unit for benzoylated forms of pure estrogens = 0.1 μ g of α -estradiol-3-monobenzoate. *Quart. Bull. Health Organization of League of Nations*, 1935, **4**, 618.

^{||} i. e., total fatty acids plus total cholesterol.

⁵ Boyd, E. M., *Am. J. Clin. Path.*, 1938, **8**, 77.

TABLE I.
Serum Lipid in Rats Receiving Estradiol-benzoate (mg%)

Diet	Group	Total estradiol-benzoate (μg)	Total* lipid (mg%)	Avg
580-B	Controls ♂	—	363 403	351
	” ♀	—	386 251	
580-B	Sub-group 1 ♂	300 (3)†	366 301	334
	” ” 2 ♂	720 (24)	578 553	566
	” ” 3 ♂	120 (24)	521 541	531
560-B	♂	—	251	252
	♀	—	252	
550-B	♂	—	213	246
	♀	—	278	

*Each figure was obtained on the serum from 3 pooled samples of blood. Figures refer to total fatty acids plus total cholesterol.

†Figures in parentheses represent the number of injections.

Summary. A moderate lipemia was induced in male rats with subcutaneous injections of estradiol-benzoate. The animals were maintained on a diet rich in fat (71% hydrogenated coconut oil) but deficient in essential fatty acids.

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Physiological Properties of the Reynals Testicular Diffusion Factor.

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We have previously described¹ some chemical properties of the diffusion factor from mammalian testicle. This substance, discovered by Duran Reynals,² can be demonstrated by injecting a

*Commonwealth Fund Fellow in Biochemistry.

¹ Aylward, *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 477.

² Duran Reynals, *Compt. rend. Soc. Biol.*, 1928, **99**, 6.