Summary. The purified and polysaccharide-free lipoid A component of *E. coli* 0111: B4 endotoxin, upon intravenous injection into rabbits, strikingly alters dermal reactivity to epinephrine. Grossly, the lesions thus produced are red or brownish in appearance or show marked hemorrhages. Lipoid A is active in amounts as small as 2 μg/kg. The original lipopolysaccharide is approximately 10 times more potent than the lipoid A fraction obtained therefrom. Phenoxybenzamine hydrochloride inhibits the epinephrine reaction in rabbits injected with lipoid A.

1. Westphal, O., Lüderitz, O., *Angew. Chem.*, 1954, v66, 407.

2. Thomas, L., *Ann. Rev. Physiol.*, 1954, v16, 467.

3. Burrows, W., *Ann. Rev. Microbiol.*, 1951, v5, 181.

4. Springer, G. F., Editor, *Polysaccharides in Biology*, Josiah Macy, Jr. Foundation, New York, 1957.

5. Chase, M. W., *Fed. Proc.*, 1957, v16, 856.

6. Bennett, I. L., Jr., Cluff, L. E., *Pharmacol. Revs.*, 1957, v9, 427.

7. Westphal, O., Nowotny, A., Lüderitz, O., Hurni, H., Eichenberger, E., Schönholzer, G., *Pharm. Acta. Helv.*, 1958, v33, 401.

8. Westphal, O., Lüderitz, O., Bister, F., *Z. Naturforsch.*, 1952, v7b, 148.

9. Thomas, L., *J. Exp. Med.*, 1956, v104, 865.

10. Ribi, E., Hoyer, B. H., Milner, K. C., Perrine, T. D., Larson, C. L., Goode, G., *J. Immunol.*, 1960, v84, 32.

11. Westphal, O., Hammer, D., Lüderitz, O., Nowotny, A., Eichenberger, E., Goebel, F., *Z. Naturforsch.*, 1958, v13b, 572.

12. Lüderitz, O., Hammer, D., Goebel, F., Sievers, K., Westphal, O., *ibid.*, 1958, v13b, 566.

13. Zweifach, B. W., Nagler, A. L., Thomas, L., *J. Exp. Med.*, 1956, v104, 881.

14. Meyer, M. W., Ballin, H. M., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 288.

15. Gatling, R. R., *J. Exp. Med.*, 1958, v108, 441.

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Accumulation of Dermal Mucopolysaccharides in Animals Following Injection of Estradiol Benzoate.* (25670)

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It has been demonstrated that following administration of estrogenic hormones the amount of ground substance mucopolysaccharides can be increased in immature monkeys of both sexes(1,2). Similar effects were obtained by estradiol following local application of the hormone on skin of rhino and hairless mice(3), and in mice injected subcutaneously with this hormone(4). That part of this increase in dermal ground substance is due to hyaluronic acid or chondroitin sulfate was demonstrated by Schmidt(5). This paper presents the effect of a single subcutaneous inoculation of estradiol benzoate on dermal mucopolysaccharides of mice, rats, guinea pigs and hamsters.

Materials and methods. Male and female white Swiss mice weighing 12 to 15 g and 20 to 22 g respectively, female albino rats weighing 60 to 75 g, female Hartley line guinea pigs weighing 225 to 275 g and female Golden hamsters weighing 60 to 75 g were studied. Animals were obtained from local dealers. Mice, rats and hamsters were fed Purina lab chow while guinea pigs were fed Purina rabbit chow and lettuce *ad lib.* Animals had free access to water. Test animals received a single subcutaneous injection of 0.1 ml of a suspension of estradiol benzoate in sesame oil in inguinal region. Control animals received 0.1 ml of sesame oil. At 2 day intervals beginning with zero days and usually extending through the 21st, duplicate animals were sacrificed; mice by severing cervical cord, other animals by ether. Fur was initially removed

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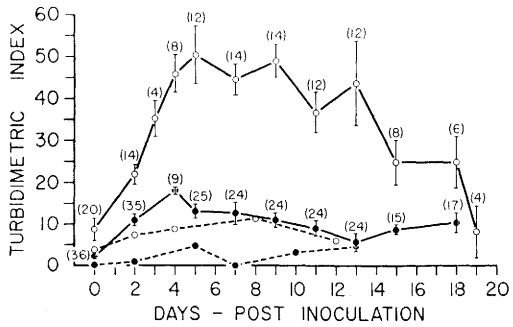

FIG. 1. Accumulation of mucopolysaccharides in skin of mice inoculated with 100 μg estradiol benzoate in sesame oil. Legend: No. in parenthesis is No. of mice used to determine point. Vertical lines indicate plus and minus one stand. error of mean which determines point. ●---● Abdomen control. ○---○ Back control. ●——● Abdomen treated. ○——○ Back treated.

with clipper. To remove remaining stubble depilatory was applied. After removing excess depilatory with tap water, pieces of skin were removed and placed in hot air oven (60°C) overnight. The effect of estradiol benzoate on accumulation of mucopolysaccharides in skin of test animals was assayed by 2 different methods: (A) turbidimetric assay employed for titration of hyaluronic acid and (B) chemical determination of hexosamine. For turbidimetric determinations samples were removed from oven the following day, weighed, ground in mortar and pestle and suspended in 10 ml of 0.85% saline solution. This suspension was then kept at 5°C overnight. After centrifugation at 1500 rpm for 5 minutes, the supernatant fluid was tested for mucopolysaccharides which are attacked by hyaluronidase in accordance with turbidimetric method described previously(6). An index of mucopolysaccharide accumulation was calculated by taking the difference between optical density of an aliquot of solution before and after depolymerization with 150 USP units of bovine testicular hyaluronidase, then dividing this value by weight of sample in mg and multiplying quotient by 100. For determination of hexosamine in skin samples, the procedure was similar through the drying stage. Thereafter, oils were removed by cutting skin samples into narrow strips and shaking them in acetone on shaking machine for 30 minutes. Three to 4 washings were usually sufficient. Samples were then treated by the method of Rimington(7) with the modification of protein hydrolysis maintained for 24 hours using 3N HCl as opposed to 4 hours using N HCl.

Results. Turbidimetric determinations for mucopolysaccharides which are depolymerized by hyaluronidase and hexosamine assays revealed that evidence of increased ground substance accumulation could be demonstrated in dorsal skin of the mouse only (Fig. 1, Tables I, II). A slight increase of ground substance was noted in abdominal skin to fourth day after inoculation. However, values fell almost to control levels subsequently (Fig. 1). Since the mouse appeared unique with respect to increased ground substance accumulation, it was necessary to determine whether method of sacrifice was responsible for disparity in response. Therefore, several mice as well as other species were sacrificed with ether. Turbidimetric analysis revealed no differences between mice sacrificed with ether or by severing the cervical cord.

An arbitrary standard was derived from Fig. 1 to evaluate other variables. Since peak of estradiol activity occurred from 4th through 13th day post-inoculation, values for 4th to 13th day were averaged. This value was used as index to measure effect of variables.

Data summarized in Table II demonstrate

TABLE I. Glucosamine in Skin of Mice Inoculated Subcutaneously with Estradiol Benzoate in Sesame Oil.

Age of mice (wk)	Estradiol benzoate (μg)	Skin from	Glucosamine, mg/100 g* Time after inoc. (days) 0	2	5	7
3	100	Abdomen	313	481	484	674
3	1000		397	447	551	632
3	Control		388	558	455	514
3	100	Back	391	663	527	1054
3	1000		432	614	788	942
3	Control		316	520	422	380
8	100	Abdomen	330	388	499	488
8	1000		415	318	489	496
8	Control		451	421	385	543
8	100	Back	312	495	745	754
8	1000		509	543	720	699
8	Control		406	357	405	548

* Each value represents mean of samples from each of 2 mice.

TABLE II. Mucopolysaccharides in Skins of Animals after Subcutaneous Inoculation of Estradiol Benzoate in Sesame Oil.

Animal	Sex	Age or wt	Skin from	Drug and/or procedure	Dose	Avg turbidity index of ground substance from 4-13 day*	No. of animals	Range of individual determinations
Mouse	♂	3 wk	Dorsum	Estradiol	10 μg	35.5	10	9.2– 11.2
	♂	8			"	33.0	"	4.8– 60.5
	♂	3			100	50.1	"	8.0– 62.0
	♂	8			"	33.5	18	6.9– 54.5
	♂	3			"			
				Estradiol + cortisone	4.8 mg	42.8	8	17.4– 62.5
	♂	3		Cortisone	"	10.0	8	4.5– 14.3
	♂	3		Castration + estradiol	100 μg	39.8	8	13.6– 63.7
	♀	3		Estradiol	"	63.4	4	22.4– 90.9
	♀	8			"	51.4	40	18.5–104.2
	♀	3			1000	40.8	4	22.2– 61.4
	♀	8			"	54.0	4	29.2– 91.9
	♀	3		Sesame oil	.1 ml	6.8	4	3.9– 10.9
	♀	8			"	6.2	8	3.7– 17.8
Rat	♀	60– 75 g	Abdomen	Estradiol	100 μg	9.6	10	2.7– 13.3
	♀	60– 75	Dorsum		"	9.8	"	1.0– 14.9
	♀	60– 75	Abdomen		1000	10.1	12	3.2– 21.5
	♀	60– 75	Dorsum		"	10.9	"	1.6– 18.0
Guinea pig	♀	225–275 g	Abdomen		"	1.6	10	.0– 4.5
	♀	225–275	Dorsum		"	4.8	"	2.7– 8.3
Hamster	♀	60– 75 g	Abdomen		"	6.0	"	.0– 15.0
	♀	60– 75	Dorsum		"	3.2	"	.0– 6.8

* Since deposition of ground substance shows a plateau between 4th and 13th day (Fig. 1), in order to compare effect of different variables, avg value of turbidity index within these 2 time intervals was chosen.

that response of the mouse to estradiol is independent of age, sex or dose. Three and 8-week-old mice responded similarly to estradiol benzoate. In addition when varying doses of estradiol benzoate were inoculated *i.e.*, 10 μg, 100 μg, and 1000 μg, no significant differences were found in amount of increased dermal ground substance or in length of time excess material remained. Males as well as females responded the same way as far as dermal ground substance was concerned. Furthermore, male mice castrated at 3 to 4 weeks of age did not appear to differ in response from normal male or females.

In other experiments, after having received estradiol benzoate, 3-week-old male mice were inoculated *I.M.* with 1.2 mg of cortisone acetate every other day for a week for a total of 4.8 mg. Despite this regimen, no reversal of ground substance accumulation could be demonstrated (Table II).

Discussion. These data suggest that when estradiol benzoate is inoculated into rodents such as mice, rats, guinea pigs and hamsters in doses previously described, only the mouse responds with accumulation of excess dermal ground substance. If other animals responded to inoculation it was not with any substance detectable by our assay methods. Furthermore, the response seemed confined usually to skin of dorsal region. Although accumulation of ground substance occurred exclusively in the mouse, wide variation in response was noted (Table II). It was thought that estrous cycles in the mice might account in part for this variation. To elucidate this one possible controlling mechanism, random samples of female mice were autopsied and gross examinations of uteri were made. However, no consistent correlations between engorged or normal uterus and degree of activity of estradiol in effecting accumulation of ground substance could be made.

The amount of ground substance mucopoly-

saccharide in skin can be the steady state of 2 opposing rates: rate of synthesis and rate of depolymerization. Whether estradiol exerts its effect by increasing rate of synthesis while rate of depolymerization remains the same or whether accumulation results from slowing of rate of depolymerization while rate of synthesis remains constant is an interesting speculation on which we have no evidence.

Although we did not succeed in modifying the effects of estradiol on mucopolysaccharides we confirmed an observation of Schmidt's(5) that cortisone had no effect on accumulation of mucopolysaccharides in mouse skin.

Since some tumors(8-10) contain large amounts of hyaluronic acid, it was of interest to study the effects of estradiol on their growth *in vivo* and *in vitro*. Preliminary studies were initiated to determine whether the accumulated ground substance could act to retard or at least contain growth of these tumors. Studies have also been started to determine whether the accumulated mucopolysaccharide might also cause local accumulation of antibodies in skin and hence act as a deterrent to local infection.

Summary. Accumulation of mucopolysaccharides in response to estradiol benzoate inoculation in the skin of various rodents was studied. Only white Swiss mice responded with increased accumulation. Rats, guinea pigs, and hamsters did not show this response. Neither age, sex of the animal, nor dose of estradiol had any effect on amount of mucopolysaccharide accumulated. This activity of estradiol was neither effected by castration of males nor reversed by administration of cortisone.

1. Duran-Reynals, F., Bunting, H., van Wagenen, G., *Ann. N. Y. Acad. Sci.,* 1950, v52, 1006.

2. Zuckerman, S., van Wagenen, G., Gardiner, R. H., *Proc. Zool. Soc.,* London, 1938, Series, A108, 385.

3. Selye, H., *Arch. Dermatol. Syphilol.,* 1944, v50, 261.

4. Duran-Reynals, F., *Ann. N. Y. Acad. Sci.,* 1957, v68, 430.

5. Schmidt, A., *Acta. Pharm. et Toxicol.,* 1958, v14, 350.

6. Warren, G. H., Gray, J., *J. Bact.,* 1954, v67, 167.

7. Rimington, C., *Biochem. J.,* 1940, v34, 931.

8. Pirie, A., *Brit. J. Exp. Path.,* 1942, v23, 277.

9. Warren, G. H., Williams, E. C., Alburn, H. E., Seifter, J., *Arch. Biochem.,* 1949, v20, 300.

10. Warren, G. H., Horenstein, E., Gray, J., *Arch. Biochem. Biophy.,* 1953, v44, 107.

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Blood Glucose Distribution in the Domestic Fowl. (25671)

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It has been demonstrated(1) that in adult laying hens the circulating blood glucose is transported almost exclusively in the plasma. In this respect these birds are similar to the adults of many subprimate mammals(2). Blood glucose has been found to be almost equally distributed in the blood of the fetus and of the newborn in swine, sheep and cattle (2,3). A gradual shift in distribution occurs within the first few months of life at which time almost all of the sugar is in the plasma. Plasma of the newborn foal contains essentially all of the glucose at birth(2). It has been suggested that the changes in swine and ruminants are associated with the change-over from fetal to adult erythrocytes and differences in glucose metabolism observed between the 2 types of red cells(4,5). Foal blood contains only adult cells at birth. In the chicken, replacement of fetal erythrocytes by adult types is said to be completed by the third week after hatching(6). This study deals with distribution of circulating glucose in the blood of the chicken from time of hatching until maturity.

Materials and methods. Eighty-five chickens were used. With the exception of 24 Rhode Island Red chicks, ages 2, 3 and 4