to increase after maximum intravenous doses of reserpine and guanethedine considered safe for human subjects may indicate that tissue depletion did not occur. Indirect data favoring this possibility as regards guanethedine have recently been published by Cohn(6) who found persistence of pressor responses to ephedrine and tyramine after both acute intravenous and prolonged oral administration in man. If depletion did occur the data reported here are consistent with the hypothesis that this results not from release of catecholamines from tissue stores, but by interference with synthesis of or by blocking storage of these substances.

The measurement of urinary VMA rather than NE and E offers several advantages: 1) more precise measurement is possible; 2) 50-100 times larger amounts for measurement are excreted; and 3) it would be possible more accurately to determine the release from stores if release were taking place slowly enough for enzymatic degradation to keep pace with it.

The possibility that a change in VMA excretion might take place later than 24 hours after drug administration and thus have been missed in the present study must be considered. Against this possibility is the fact that catecholamine tissue levels in animals have always decreased within a matter of a few hours after doses comparable to those used here.

Summary. The effect of intravenous reserpine and guanethedine on urinary excretion of VMA in man has been studied. No remarkable or consistent changes occurred after either drug. Significance of these findings is discussed.

The authors wish to acknowledge the technical assistance of Miss Billie S. Bush and the assistance of Dr. James E. Grizzle in statistical evaluation of data.

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Received June 5, 1963. P.S.E.B.M., 1963, v114.

Effect of Selenium on Muscular Dystrophy in Vitamin E-Deficient Rats and Guinea Pigs.* (28600)

E. BONETTI AND F. STIRPE (Introduced by K. Schwarz)
Istituto di Patologia Generale dell'Universitá di Siena, Siena, Italy

Schwarz and Foltz(1,2,3,4) have shown that selenium together with Vit. E are among the protective factors against dietary necrotic liver degeneration. Experimental procedures for reproducing this lesion in the rat by feeding diets without Vit. E and deficient in selenium with modifications in quantity and quality of protein, are well known(5,6). Selenium, however, does not prevent all the lesions produced by Vit. E deficiency in different animal species. It prevents exudative diathesis(7,8,9), but not encephalomalacia (10) in Vit. E-deficient chicks. It does not affect the reduced hatching nor high death rate of chickens hatched from the eggs of Torula-fed hens(11), nor sterility due to foetal resorption in rats(12). Selenium prevents multiple lesions in the liver, kidney, heart and skeletal muscle of the mouse on Torula diet(13) and muscular dystrophy in the chick(14), but not muscular dystrophy in the rabbit(15,16) or in the guinea pig(17). Muscular dystrophy in lambs and calves is also prevented and cured by selenite administration. Muth *et al*(18,19) were able to cure with selenite, and not with Vit. E,

* Supported by grant from Consiglio Nazionale delle Ricerche, Roma.

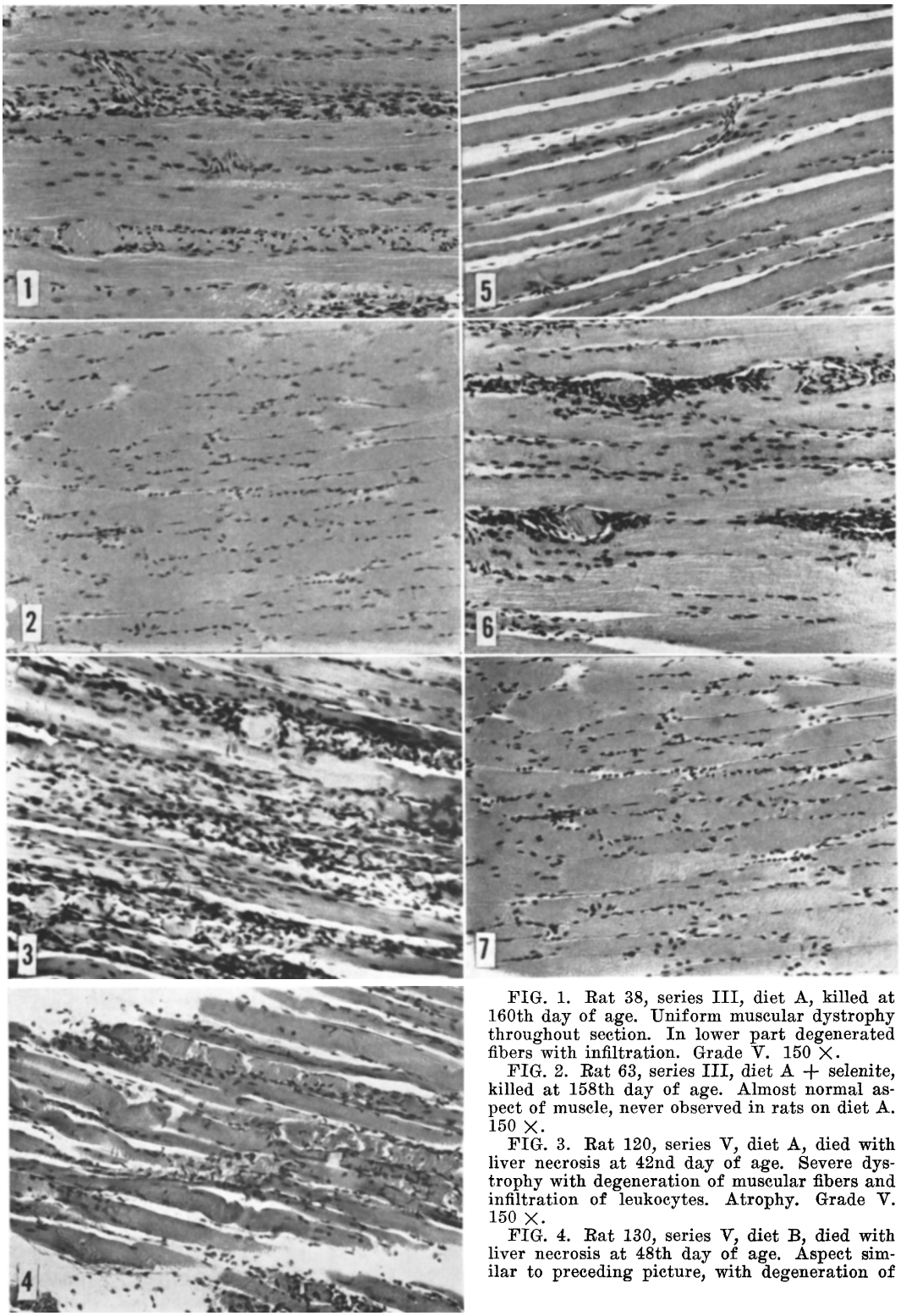

FIG. 1. Rat 38, series III, diet A, killed at 160th day of age. Uniform muscular dystrophy throughout section. In lower part degenerated fibers with infiltration. Grade V. 150 ×.

FIG. 2. Rat 63, series III, diet A + selenite, killed at 158th day of age. Almost normal aspect of muscle, never observed in rats on diet A. 150 ×.

FIG. 3. Rat 120, series V, diet A, died with liver necrosis at 42nd day of age. Severe dystrophy with degeneration of muscular fibers and infiltration of leukocytes. Atrophy. Grade V. 150 ×.

FIG. 4. Rat 130, series V, diet B, died with liver necrosis at 48th day of age. Aspect similar to preceding picture, with degeneration of

muscular dystrophy (white muscle disease) reproduced experimentally in lambs born from mothers fed hay from areas in which the illness was spontaneous. According to these authors, white muscle disease is not caused only by deficiency of Vit. E. Hartley *et al*(20) prevented congenital muscular dystrophy in lambs by administration of selenite to mothers during pregnancy, and also cured with selenite the late manifestations in lambs. Comparable results were obtained by Welch *et al*(21), Proctor *et al*(22), Kuttler and Marble(23), Drake *et al*(24) and by Lagage (25) who observed a lowered or disappeared serum glutamic-oxaloacetic transaminase activity after treatment with selenium. Sharman treated muscular dystrophy in calves with Vit. E or with selenium(26). Selenium increases body weight of calves(26), lambs(24) and rats(27).

Goettsch(27) has denied recently any effect of selenium in preventing muscular dystrophy in Vit. E-deficient rats. We have reported(28) that a supplement of selenite reduced the severity of muscular dystrophy in rats fed necrogenic diets. This paper concerns results obtained by administration of selenite to rats and guinea pigs fed diets without Vit. E and with different protein contents.

Material and methods. Wistar-Glaxo rats, inbred in this laboratory, were used. Litters of 6 male rats were left with each mother; if a litter contained an insufficient number of males, it was supplemented by males from other mothers 3 to 4 days after birth. At this time, feeding of the 20% casein diet A(28) with or without the addition of sodium selenite (0.15 mg per kg diet) was initiated. After weaning (24th to 26th day), the young rats were maintained on this diet for ca 20 days. On the 45th day, one group was fed the 5% casein diet B(28), plus or minus selenite, while the other group continued on diet A, plus or minus selenite. Controls received 10 mg α-tocopherol (Ephynal-Roche) in 0.1 ml olive oil, given orally twice a week after weaning.

Male guinea pigs from our colony were placed on a Vit. E-deficient diet used in a preceding study(29), with the following composition, in per cent: fat-extracted casein 15, rice starch 50, sucrose 10, dried brewer's yeast 10, Osborne and Mendel's salt mixture 3, cod liver oil 3, lard 4, cellulose powder (Whatman "B quality") 5, vitamin mixture(30) 2, choline chloride 0.2. Menadione (5 mg per kg diet) was dissolved in the cod liver oil. Each guinea pig received 20 mg ascorbic acid in water 3 times a week. The animals were fed the basal diet alone, or the basal diet plus a dose of 15 mg α-tocopherol 3 times weekly, or the basal diet supplemented with selenite (0.15 mg per kg diet) (Table III).

Hemolysis induced by dialuric acid was determined according to the method described by Rose and György(31). For histological examination, specimens of skeletal muscle (psoas, gastrocnemius), liver, myocardium and testis were fixed in Bouin or in formalin buffered at pH 7.2. Sections were stained with hematoxilineosin.

Results. a) Rats. The effect of selenium on muscular dystrophy may be evaluated by comparing quantitatively, as far as possible, the severity of dystrophic lesions in selenium supplemented and non-supplemented animals. The dystrophic pattern of muscles is far from constant, with quantitative differences after the initial lesions. Severity depends upon length of dietary treatment, composition of the diet and time of year, and upon other individual factors.

An arbitrary scale for evaluating the severity of dystrophic lesions may be proposed as follows: 1. Moderate atrophy of muscular fibers, with slight nucleosis without degenera-

muscular fibers and infiltration of leukocytes observable uniformly throughout the section. Grade V. 150 ×.

FIG. 5. Rat 123, series V, diet A + selenite, killed at 39th day of age. Well preserved muscular fibers, moderate atrophy. Very few degenerated muscular fibers observable in other fields. Grade II. 150 ×.

FIG. 6. Rat 134, series V, diet B + selenite, killed at 61st day of age. The microscopic field shows the most severe muscular lesion observed in the slide and in all rats of this dietary group. Compare with Fig. 5. 150 ×.

FIG. 7. Rat 136, series V, diet B + selenite, killed at 46th day of age. Muscle is almost normal. Grade I. 150 ×.

TABLE I. Extent of Muscular Damage in Vit. E-Deficient Rats Fed a 20%-Casein Diet (Diet A) or a 5%-Casein Diet (Diet B), With or Without Selenite Addition During Different Periods of Year. Rats were put on diet B at 45th day of age. All times counted from birth of animals.

Series and period of year	No. of rats	Diet	Liver necrosis		Survivors killed at days	Body wt, g	Wt of testes, g	Severity of muscular dystrophy					
			No.	At days				0	I	II	III	IV	V
I Oct.–	6	B	2	59, 61	62	108	—					3	3
Dec.	8	" + Se			61	99	—		3	3	2		
II Oct.–	6	A	2	57, 75	57– 90	—	—			1	3	2	
Dec.	7	" + Se			48– 71	—	—	2	3	1	1		
III Oct.–	9	A			160–167	285	2.91				1	5	3
March	9	" + Se			158–168	330	2.85	1	2	2	4		
IV Jan.–	5	B	5	54.7 (52–56)		130	1.73			4	1		
April	7	" + Se			60	135	2.13	6	1				
	3	" + Vit. E			60	132	—	3					
	6	A			59	180	2.13	1		3	2		
	6	" + Se			59	185	2.09	2	4				
	6	" + Vit. E			59	190	2.23	5	1				
V May–	10	A	6	40.0 (38–42)		—	—				1	2	7
July	3	" + Se			32– 47				1	2			
	6	B	6	47.0 (46–48)		117	1.38					1	5
	6	" + Se			46– 61	122	2.02		1	1	3	1	
	5	" + Vit. E			61	141	2.13	5					

tion of the fibers. 2. More severe atrophy and nucleosis; occasional degenerated fibers; proliferation of the muscle nuclei. 3. Two to three degenerated fibers per microscopic field with masses of homogeneous wax-like material and consequent infiltration (neutrophil granulocytes, proliferating nuclei of the sarcolemma) and atrophy. 4. Large areas of degeneration affecting several fibers per microscopic field and infiltration. 5. The same picture as in Fig. 4 qualitatively, but more severe and extended, with lumps of waxy degeneration and necrosis of muscle fibers and marked infiltration. This picture may be similar to that observed in dystrophic muscles of rabbits and guinea pigs.

Results, and distribution of animals into various classes of muscular dystrophy, are reported in Table I. The selenium supplement caused an increase of body weight only in rats fed diet A for a long period. No variations were observed in groups of animals supplemented for shorter periods. The dialuric acid-induced hemolysis was always 100% in all Vit. E-deprived animals, regardless of selenium supplement.

Average weight of testes was not substantially different in unsupplemented rats, as compared with Vit. E- or selenium-supplemented animals killed at the same age. In those groups on diet B in which mortality due to liver necrosis was more severe and accurred at various ages, the testes did not reach maturity and consequently weighed less. Histological examination confirmed the low grade or even absence of lesions due to Vit. E deficiency in testes of rats killed at about 60 days of age. Lesions were very moderate and always inferior to Grade 2 of Mason's scale, with reduced spermatogenesis and agglutination of spermatozoa. Selenium supplement was without effect. Only one animal which died at the 75th day showed very severe lesions (Grade 4 of Mason).

The severity of muscular dystrophy was expressed quantitatively by the distribution of rats within the classes described above. Supplementation of selenite did not completely prevent the occurrence of muscular dystrophy. It attenuated the dystrophy to varying extents, in some animals prevention being almost complete. The effect of selenite seemed to be inversely proportional to the grade of dystrophy of unsupplemented rats: the more severe the dystrophic lesions, the less effective selenite appeared to be, as estimated by histological examination, and the distribution of rats remained shifted toward the classes of higher severity.

The severity of dystrophic lesions was re-

TABLE II. Severity of Muscular Dystrophy in Rats on Diet A With or Without Liver Necrotic Degeneration During Different Periods of Year.

Period	No. of rats	Age at death, days	Liver necrosis	Severity of muscular dystrophy I	II	III	IV	V
Oct.–April	2	66.0	+				1	1
	15	65.2*	—	1	8	4	2	
May–Sept.	21	47.2	+		1	2	3	15
	6	62.0*	—				3	3

* Killed.

duced during winter (Series II, III and IV); selenium in this period almost completely prevented dystrophy, and muscles appeared almost normal, which was comparable with that of Vit. E-supplemented rats. This occurred in groups on diet A (Series II and III) as well as in those on diet B (Series IV). But when muscular dystrophy was much more severe, as in summer, the action of selenite was not sufficient to maintain a normal histological aspect of muscles.

Effect of dietary protein level and of seasonal variations on severity of muscular dystrophy. Histological examination showed that the low protein diet caused an increased frequency of severe dystrophy as compared with the adequate protein diet. Another lesion, hepatic necrosis, was always present in rats on the low protein diet, and might have an aggravating effect on muscular dystrophy. Muscular lesions seemed to be more severe in summer than in winter in rats fed diet A as well as diet B. On the other hand, in summer a higher frequency of liver necrosis could be observed also in rats fed the adequate protein diet A. A parallelism between occurrence of liver necrosis and severity of muscular dystrophy is shown in Table II.

b) Guinea pigs. Selenite supplementation did not prevent the dialuric acid-induced hemolysis which, as in rats, was completely absent only in the Vit. E-supplemented group.

Selenite-supplemented rations did not prevent or alleviate muscular dystrophy, which was particularly severe in guinea pigs irrespective of age. However, selenite administration prolonged life and enhanced body weight (Table III).

Discussion. Numerous observations have been reported on the effects of dietary selenium. In some cases the results are quite similar to those obtained with Vit. E; in other cases, where some other lesions or different animal species are concerned, selenium is inactive. In preventing muscular dystrophy in rats in the present experiments selenite was not equal to Vit. E. Also, it did not prevent hemolysis induced by dialuric acid, or the moderate histological damage to the testes. Selenite, however, prevented, to some extent, muscular dystrophy in rats fed low- or adequate-protein diets for varying lengths of time. When the dystrophic lesions were not severe, complete protection was afforded.

It was impossible to detect histologically any effect in guinea pigs; selenite seemed only to prolong survival time and to improve growth, as reported by Siedel and Harper 17). Muscular damage in guinea pigs and rabbits (in which selenite is equally ineffective) (15,16) was among the most severe and rapidly fatal lesions produced by Vit. E deficiency in the various species studied.

Our observations on the protective action of selenite on muscular dystrophy in the rat agree with unpublished results of Hove and

TABLE III. Survival Time of Guinea Pigs Fed the Vitamin E-Deficient Diet With or Without Selenite Addition.

Series	No. of guinea pigs	Diet	Initial body wt, g	Survival time,* days
A	4	Basal	180-320	60.8 (55- 69)
	4	" + Se	180-280	81.5 (55-104)†
	2	" + Vit. E	240-270	104‡
B	3	Basal	90-177	46.3 (39- 61)
	4	" + Se	108-185	66.2 (48- 77)

* From beginning of feeding with experimental diet.
† The last animal was killed.
‡ Killed.

Schwarz (quoted by Hove *et al.*(16) and personal communication), and also with those on the mouse reported by DeWitt and Schwarz(13), but not with those of Goettsch (27). The lesions observed in our animals were never as severe as those described by Goettsch in the younger rats used in her experiments. Those animals were more severely pre-depleted of Vit. E, their mothers being fed the deficient diet even during pregnancy; furthermore, at weaning they were immediately put on the low-protein, Vit. E-deficient diet. This condition is confirmed by the mortality occurring during the suckling period and by the symptoms of paralysis observed at weaning. Furthermore, a comparison between our histological picture and those of Goettsch is difficult, because the rats examined by her are the few survivors of groups of animals destroyed almost completely by liver necrosis. The dystrophic lesions observed by Goettsch may be too severe to be prevented by selenite, as far as can be evaluated by histological examination. However, some beneficial effect of selenium is indicated in Goettsch's experiments by the prolonged survival time and by improved growth rate of rats receiving selenite.

The higher frequency of severe muscular lesions during the summer in animals on diet A is suggestive of a correlation between severity of muscular dystrophy and liver necrosis, which occurred in this period of the year even in animals on diet A. It is likely that Vit. E deficiency is more severe in this season, causing either the frequency of hepatic necrosis or the greater severity of muscular damage. Liver necrosis is sudden and rapidly fatal, and therefore is unlikely to aggravate muscular dystrophy, which is a slow process, at least under our experimental conditions. Otherwise, one would suppose that selenium would be effective by the indirect mechanism of preventing liver necrosis, thus eliminating an aggravating condition. Opposed to this argument is the observation of the effectiveness of selenium even in animals on diet A without liver necrosis. Furthermore, the severity of muscular dystrophy showed seasonal variations even in rats on diet B, which is constantly necrogenic. This leads to exclusion of hepatic necrosis as an aggravating factor in muscular dystrophy.

Summary. Administration of sodium selenite reduced the severity of muscular dystrophy in rats fed Vit. E-free diets, with adequate or low protein content. The effect of selenite was more evident when dystrophic lesions were not severe. Selenite was without effect on muscular dystrophy in Vit. E-deficient guinea pigs.

The technical assistance of Sig.ri M. Bellini and G. Papi is gratefully acknowledged. Ephynal was a generous gift of Prodotti Roche S.p.A., Milan.

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Received April 29, 1963. P.S.E.B.M., 1963, v114.

Phloretin, A Weak Estrogen and Estrogen Antagonist. (28601)

LEONARD J. LERNER, A. ROBERT TURKHEIMER AND ALECK BORMAN
Squibb Institute for Medical Research, New Brunswick, N. J.

Weak estrogens can partially antagonize the uterotrophic effect of strong estrogens. Hisaw *et al.*(1) and Velardo and Sturgis(2) demonstrated that such potent estrogens as estradiol and estrone could be partially prevented by estriol or 16- epiestriol from exerting their full effect upon uterine weight. Nonsteroidal weakly estrogenic compounds such as *p*-hydroxypropiophenone pyridine(3), di-p-hydroxyphenylalkanes and alkenes(4,5), clomiphene(6,7) and dimethylstilbestrol(8) have also been reported to have selective anti-estrogenic activities. The anti-estrogenic properties of dimethylstilbestrol have been extensively investigated and reviewed by Emmens *et al.*(8). At least one compound, 1-(*p*-2-diethyl-aminoethoxyphenyl)-1-phenyl-2*p*-methoxyphenyl ethanol (MER-25) has been reported to inhibit the response of a number of target tissues to several estrogens in several species of animals(10,11,12). This compound, while not demonstrating estrogenicity by the typical cornified vaginal smear or increase in uterine weight with relatively large dosage, nevertheless has shown subtle evidence of estrogenicity(11,12,13,14) and is structurally related to non-steroidal estrogens such as trianisylchlorethylene.

Phloretin (β-(*p*-hydroxyphenyl) 2,4,6-trihydroxy-propiophenone), a compound structurally related to the diphenolic estrogens, has been reported to have weak estrogenic activity(15). It was of interest therefore to investigate this substance for estrogen antagonism as well as for gonadotrophin inhibition and effect on fertility.

Materials and methods. Uterotrophic and anti-estrogenic activities. Immature female Swiss Albino mice weighing 6-8 g were employed in these studies. Estradiol benzoate and phloretin were each dissolved or suspended in sesame oil and administered subcutaneously once daily in volumes of 0.1 ml for 3 days. Animals were sacrificed on the fourth day and their uteri were dissected. The quantity of intrauterine fluid was estimated, the fluid was expressed and the uteri were weighed. *Estrogenic and anti-estrogenic activities.* Mature castrated female Sprague-Dawley rats of approximately 200 g which responded to estradiol benzoate with fully cornified vaginal smears were used in this study. Estradiol benzoate and phloretin were dissolved or suspended in an aqueous vehicle and administered in a single subcutaneous dose. Vaginal smears were made at 24, 48, 64 and 72 hours post treatment and graded as positive for estrogenicity only if cornified or nucleated epithelial cells were present in the absence of leucocytes. *Gonadotrophin inhibition and uterotrophic activity in parabiotic rats.* Immature female litter mate Sprague-Dawley rats were made parabiotic by the method of Bunster and Meyer(16). Estradiol benzoate and phloretin were dissolved or suspended in sesame oil and administered subcutaneously once daily for 10 days to the ovariectomized partner. The animals were sacrificed on the eleventh day and the