for sodium. In this connection it should be noted that the elution characteristics of the column we are now using differ slightly from those depicted previously(2).

Conclusion. A new method for the determination of specific activity of sodium in bone is presented. The method consists of placing a solution of bone ash on a cation exchange column. Elution with acid results in the quantitative recovery of both stable and radioactive sodium in a certain fraction of the eluate; this fraction is free from P, K, Mg and Ca and can be easily analyzed for both sodium and radiosodium.

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Oestrogenic Activity of 16 Epi-Oestriol.*† (21975)

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The isolation of 16 epi-oestriol (oestra-1:3:5-triene-3:16B:17B-triol) from the acid hydrolyzed urine of pregnant women was described by Marrian and Bauld(1). Since the yield of this new Kober Chromagen was 0.1 mg/l and since this substance seems identical with 16 epi-oestriol as prepared by Huffman(2) considerable impetus was given to the present investigation in an effort to ascertain its activity on the growth potential of the uterus of the ovariectomized rat. Vaginal smears were also studied quite extensively. Moreover, since practically no biological information exists in the scientific literature on this naturally occurring oestrogen it seemed of particular significance at this time to elaborate its biological activity.

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Procedures. Virgin, albino rats, approximately 100 days of age, were used in these experiments. They were started on treatment 7 days after bilateral ovariectomy. The 16 epi-oestriol was dissolved in U.S.P. propylene glycol, and injected subcutaneously once daily for 3 days. Vaginal smears were taken daily, and 72 hours after the first injection necropsies were performed. The uteri were removed quickly and slit longitudinally on bibulous paper, thus removing excess uterine luminal fluid. The uteri were then weighed to the nearest 0.2 mg on a Roller-Smith torsion balance. After the wet weights were determined, the uteri were placed in an oven at 110°C for dry weight determinations. A standard period of 24 hours at 110°C was used uniformly for drying each uterus. After this period the weight of each uterus was determined on a Roller-Smith torsion balance.

Results. A. Effect of 16 epi-oestriol on vagina of the ovariectomized rat. These results clearly indicate that 16 epi-oestriol has the ability to induce oestrogenic changes in the vagina of the ovariectomized rat (Table I). A demonstrable effect can be observed with 1.0 μg of this substance. Additional

TABLE I. Influence of 16 Epi-oestriol on Vagina of the Ovariectomized Rat.

Daily dose of 16 epi-oestriol, μg	Vaginal smear 24 hr after 1st inj.	48 hr after 1st inj.	56 hr after 1st inj.	72 hr after 1st inj.
.00 (1 cc propylene glycol)	L+++E	L+++++	L++++	L++++
.01	L+++C+	L++E+C+	L++E+C+	L++E+C+
.1	"	L++C+	"	L+E+C+
1	L++E+C+	L+E+C+	L+E++C+	L+E+C++
5	L+C++	L+E++C+	"	LE++C++
10	L+EC++	LE+C++	L+EC++	LEC++
25	L+E+C++	"	"	LE+C++
30	"	L+C++	L+C+++	L+C+++
40	L+C+++	LC+++	LC+++	C++++
50	L+EC+++	LE+C++	"	LC++++
100	L+E+C++	"	"	"
.1 oestradiol 17β	L++E+	L+E+C++	L+EC++	C++++

L = Leucocytes; E = Nucleated epithelial cells; C = Cornified cells.
The pluses indicate the ratio of cell types found in the vaginal smear. For example: L+E+C++ = considerably more cornified cells than leucocytes and nucleated epithelial cells.

doses of 16 epi-oestriol produced a more definitive oestrous pattern, whereas 3 daily doses of 40 μg effected a full-blown estrous pattern in the vagina. Comparatively, this material is a very weak oestrogen, especially when one compares it with the vaginal cornifying effects of the various oestrogens(3,4). Of particular significance is the fact that 72 hours were required for the induction of a full vaginal oestrous smear (Table I).

B. Influence of 16 epi-oestriol on uterus of the ovariectomized rat. That this substance is a very weak oestrogen is further borne out by the information obtained from the wet and dry uterine weights. A slight, but statistically significant, increase in both the wet and dry uterine weights was observed in those animals receiving 1.0 μg of this material (Table II). A further increase in uterine weight was observed in the group of rats given 10 μg, and the maximum response was observed when 40 μg were administered daily for 3 days. Interestingly enough, parallel results were obtained with the various dosages of 16 epi-oestriol for both the vaginae and uteri (Tables I and II).

Included in Table II is the effect of injections of oestradiol 17β on the uterus of the ovariectomized rat. A dramatic uterine response, far above that obtained with 400 times (on a weight relationship basis) the amount of 16 epi-oestriol, was achieved with oestradiol. The increase was quite marked regardless of the vehicle used, sesame oil or propylene glycol (Table II).

Discussion. These results show quite conclusively that 16 epi-oestriol is the weakest of all known naturally occurring oestrogens described thus far(3-5). The exact function of this oestrogen is not known at this time, but it would not be too conjectural to think of this substance as a possible link in the metabolism of the oestrogens. Furthermore, while the recent results of Hisaw, Velardo, and Goolsby (5) indicated that oestriol had the ability to markedly restrict the action of oestradiol 17β on the growth of the uterus of the ovariectomized rat, preliminary results at hand seem to suggest quite strongly that this compound (16 epi-oestriol) shares in common with oestriol the property of restricting the uterine growth promoting action of oestradiol 17β. Additional research on this phase of our work is now in progress.

Summary. The oestrogenic activity of 16 epi-oestriol was determined. Virgin, adult albino rats were ovariectomized, and 7 days later were put on experiments. A dose-response (0.01 to 100 μg) of this substance was determined utilizing the uterus and vagina as end points. The results from these experiments indicate that 1.0 μg of this oestrogen produces a slight effect on the vagina as well

TABLE II. Influence of 16 Epi-oestriol on Uterine Growth in Ovariectomized Rats.

Treatment	No. of animals	Uterine wet wt (mg)	Uterine dry wt (mg)
Control, 10 full days after ovariectomy	25	115.5 ± 3.3*	22.4 ± .65
.1 μg oestradiol 17β in Sesame oil	36	227.3 ± 6.1	42.3 ± 1.8
.1 " in prop. glycol	15	236.0 ± 1.0	44.4 ± 2.5
.01 16 epi-oestriol	8	143.0 ± 7.2	27.0 ± 2.1
.1	15	126.4 ± 5.2	27.2 ± 1.5
1	8	150.0 ± 8.6	26.1 ± .8
5	8	142.7 ± 6.8	25.8 ± 1.7
10	9	171.4 ± 8.7	28.5 ± 1.6
25	9	164.3 ± 5.1	30.5 ± 1.1
30	7	167.2 ± 8.3	32.8 ± 2.0
40	7	181.6 ± 8.0	33.0 ± 2.0
50	7	175.1 ± 8.1	32.6 ± 1.9
100	6	180.2 ± 7.3	30.6 ± 1.5

* Avg ± stand. error.

as on the uterus. Three daily doses of 40.0 μg of 16 epi-oestriol produced a maximal effect on the vagina (full cornification) and the uterine weight. This substance seems to be the weakest of all the known naturally-occurring oestrogens described thus far.

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Pentose Dependence of *Lactobacillus gayonii* and Related Species for Early Growth.* (21976)

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Requirements for early growth of *Lactobacillus gayonii* and the closely related species, *buchneri, lycopersici, fermenti, mannitopoeus* and *brevis* have been investigated recently in the authors' laboratory because preliminary screening experiments showed this group of organisms to be particularly well suited for studies of D-α-hydroxy fatty acid and related lipid metabolism. The latter investigations, which were prompted by the finding that D-α-hydroxy fatty acids are essential in the nutrition of *Lactobacillus casei* 280-16(1,2), are to be described in subsequent reports. The effects of pentoses were studied because it had been shown previously that these sugars promote better growth and acid production than does glucose for this particular group of microorganisms(3), and it has now been found that although glucose may be utilized adaptively, an appropriate pentose is essential for early growth of most of these species.

Methods. The sugars tested were D-glucose (C.P. dextrose, J. T. Baker Chemical Co.), L-arabinose (Pfanstiehl Chemical Co.), D-arabinose, L-xylose, D-xylose, and D-ribose (products of Nutritional Biochemicals Corp.). Sugar solutions of appropriate concentration were added to 13 x 100 mm soft glass test tubes together with sufficient water to make 1.0 ml

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