ACTH and not to the infections. To what degree the alteration of glucose resorption and other tubular functions as well as altered carbohydrate metabolism are related to sulfhydryl activity is a problem that deserves further attention.

Summary and conclusions. Glycosuria in the presence of normal blood sugar levels and normal glucose tolerance was demonstrated in a patient recovering from pneumococcal pneumonia during treatment with ACTH. The reducing substance in the urine was shown to be glucose and the glycosuria was accompanied by lowered blood glutathione levels. These observations indicate that renal tubular function may be affected by ACTH and that sulfhydryl activity may be of importance in tubular function.

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Hormone Replacement Therapy in the Aged Female—Estrogen Bioassay.* (17783)

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The practical importance of a "human unit" as opposed to a comparative animal unit in the clinical investigation and utilization of estrogen preparations is well recognized. A useful means by which such a unit may be derived lies in the comparative abilities of various forms of estrogen to produce withdrawal bleeding from the uteri of castrate women. The study presented here applies this method, described by Allen(1) to the determination of the relative effectiveness of pure alpha estradiol,† beta estradiol,‡ pure estrone,† and estradiol benzoate§ in causing withdrawal bleeding from the uteri of postmenopausal women.

Method of study and results. The group used for these determinations consisted of women between the ages of 60 and 86. Initially each member was given one milligram of estradiol benzoate three times per week intramuscularly and caused to bleed periodically by the withdrawal of this hormone. This manner of reactivation of the senile endometrium has been described in detail in previous publications(2,3). When a well stimulated and responsive endometrium had been achieved the bioassays were carried out as in Table I.

TABLE I.
Typical Estrogen Bioassay.
(9 patients in each series for 3 wk).

Drug	Dosage, mg/wk	Total dosage, mg	No. showing withdrawal bleeding
Estradiol benzoate	1 × 3	9	6
Alpha estradiol	1 × 3	9	2
Estradiol benzoate	1 × 3	9	5
Estrone	1 × 3	9	2
Estradiol benzoate	1 × 3	9	2
" "	1 × 3	9	6
Beta estradiol	5 × 3	45	0
Estradiol benzoate	1 × 3	9	1

It had become evident that in order to obtain uniform results, it was necessary to interpose between each 2 of the drugs being assayed "priming cycles" of an estrogen preparation known to be a good stimulator. By previous investigation it had been found that alpha estradiol benzoate when given in dosage of 1 mg 3 times per week was capable consistently of causing withdrawal bleeding in over half of the trials. Since this comes close to a theoretical human unit or bleeding dosage of the medication, estradiol benzoate was used as a control preparation to which the estrone, alpha estradiol, and beta estradiol

1. Allen, W. M., *Southern Med. J.*, 1944, v37, 270.

* The medication used in this study was generously supplied by † The Food and Drug Administration, ‡ Organon, Inc., § Schering Corp.

2. Masters, W. H. and Allen, W. M., *J. Gerontol.*, 1948, v3, 183.

3. Masters, W. H. and Magallon, D. T., *Am. J. Obst. and Gynec.*, press.

TABLE II.
Effects of Various Dosages of Estradiol Benzoate, Alpha Estradiol, Beta Estradiol, and Estrone in Induction of Withdrawal Bleeding in Postmenopausal Patients with Adequate Priming for 3 Weeks.

Drug	No. of patients	Dosage, mg/wk	Total dosage, mg	Fraction of total No. bleeding	%
Estradiol benzoate	12	1 × 3	9	7/12	
" "	12	1 × 3	9	5/12	
" "	9	1 × 3	9	6/ 9	
" "	9	1 × 3	9	6/ 9	
Total	42	1 × 3	9	24/42	57
Estradiol benzoate	12	1 × 2	6	3/12	
" "	12	1 × 2	6	10/12	
" "	12	1 × 2	6	5/12	
Total	36	1 × 2	6	18/36	50
Alpha estradiol	9	1 × 3	9	2/ 9	26
Beta "	9	5 × 3	45	0	0
Estrone	12	1 × 3	9	3/12	
"	9	1 × 3	9	2/ 9	
Total	21	1 × 3	9	5/21	24

TABLE III.
Treatment of Estradiol Benzoate Data Shown in Table II by the Behrens Method.

Dose	Actual No. cases bleeding	No. cases not bleeding	Behrens* No. cases bleeding	No. cases not bleeding	% bleeding
3 mg/wk	24	18	42	18	70
2 "	18	18	18	36	33⅓

* Behrens, *Arch. Exp. Path. Pharmak.*, 1929, v140, 237.

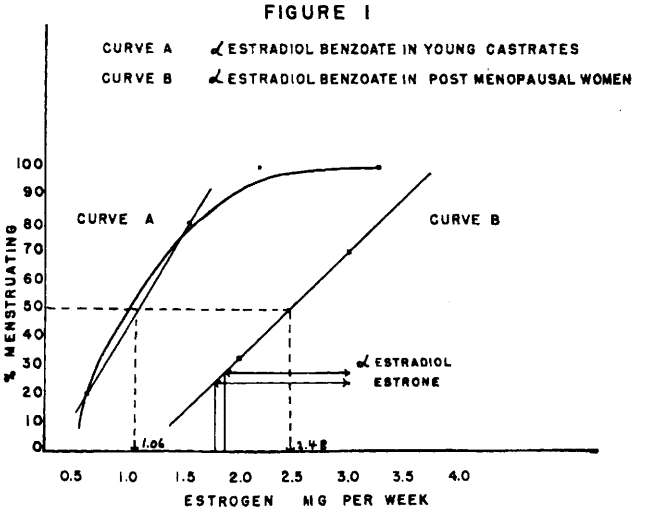
might be compared. The interposition of these priming cycles (estradiol benzoate 3 mg per week for 3 weeks) between each 2 of the other compounds under study also assured us that the uteri being tested remained responsive.

The bioassays were then carried out as shown in Table I. A 2 weeks rest period (7 days for estrogen withdrawal and 7 days for actual bleeding) was allowed between each 2 of the determinations. It will be noted that 2 priming cycles were run between the estrone and beta estradiol assays. The second priming cycle was considered necessary because of the poor response (2 out of 9 patients treated showing withdrawal bleeding) which immediately followed the comparatively ineffective estrone cycle.

Table II summarizes the data obtained on the effects of estradiol benzoate, alpha estradiol, beta estradiol, and estrone in the induction of withdrawal bleeding in postmenopausal patients with adequate priming. The term adequate priming implies that every trial represented in Table II was preceded by a priming cycle of estradiol benzoate following which withdrawal bleeding occurred in at least one half of the cases.

A total of 78 observations were made using estradiol benzoate at 2 different dosage levels. Treatment of these results by the Behrens method (Table III) offers a way in which the data may be augmented without actually increasing the number of experiments. In applying this method, devised by its author for deducing the minimum lethal dose of certain drugs, one assumes that a single observation with a given dose was made. In this instance 42 observations were made on a dosage level of 3 mg per week with 24 bleeding, and 36 observations were made using a dosage level of 2 mg per week with 18 bleeding. The assumption, then, is made that if all patients bleeding on the smaller dose (*i.e.* 18) had been given a larger dose they would surely have bled on that dose and that all patients who failed to bleed on a given dose would surely have failed to bleed on a smaller dose.

FIGURE 1



Curve B shows the percentage of cases menstruating following the use of alpha estradiol benzoate, estrone, and alpha estradiol in postmenopausal women. Curve A shows the percentage of cases menstruating following the use of alpha estradiol benzoate in young castrates.

Table III shows the actual figures obtained on the estradiol benzoate observations and these results treated by the Behrens method. An analysis of the data in this manner shows that in a significant number of individuals given 3 mg of estradiol benzoate per week 70% might be expected to show withdrawal bleeding, and on 2 mg per week only 33 1/3% might be expected to bleed. Thus a bleeding dose or a human unit (the minimal amount of estradiol benzoate necessary to produce withdrawal bleeding in 50% of the trials) might be expected to lie between 2 and 3 mg per week.

When these values are plotted as in Fig. 1 (curve B) the dose which should induce withdrawal bleeding in 50% of the trials, the calculated bleeding dose of estradiol benzoate, is found to be 2.48 mg per week. It is interesting to note by comparison of curves A and B of Fig. 1 (from data obtained by Allen in 1944) that the theoretical dose of estradiol benzoate required in young castrates for the induction of withdrawal bleeding in 50% of the cases is 1.06 mg per week, almost 1½ times less than that required in the postmenopausal or physiological castrates. One finds that the same holds true for estrone injected intramuscularly. Again, referring to Allen's observations in young castrates, it is found that 3 mg of estrone per week was adequate to induce withdrawal bleeding in 50% of those tested. In postmenopausal women 3 mg of estrone per week caused withdrawal bleeding in only 24% of the cases. When this data is projected on the estradiol benzoate curve B in Fig. 1 it may be predicted that the biological activity of 3 mg per week of estrone is equal approximately to that of 1.74 mg per week of estradiol benzoate. In a like manner, the activity of 3 mg of estradiol would be comparable to that of 1.8 mg of estradiol benzoate.

Beta estradiol has been considered for some time to be relatively inactive. In these studies a dosage of 15 mg per week for a period of 3 weeks was given to 9 adequately primed women as shown in Table II. None of the 9 demonstrated withdrawal bleeding. The complete ineffectiveness of beta estradiol in a total dosage of 45 mg as far as stimulation of the endometrium is concerned is supported by the fact that the estradiol benzoate priming cycle which immediately followed caused only one out of 9 patients to bleed by withdrawal.

Vaginal smears taken daily and stained by

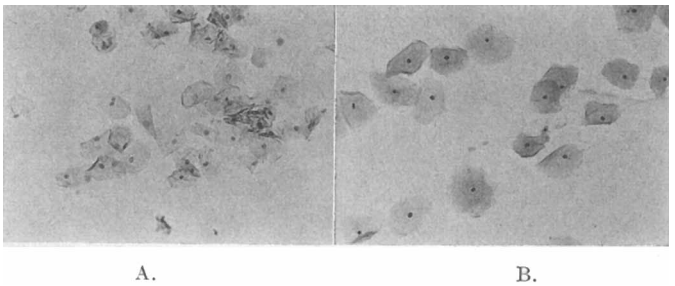


FIG. 2.
A demonstrates a vaginal smear composed of relatively unstimulated epithelial cells obtained after 17 days of beta estradiol therapy (5 mg given 3 times per week). Note the small cells with vesicular nuclei and the absence of cornified cells. B shows a vaginal smear made up of well stimulated epithelial cells taken from the same individual after 17 days of estradiol benzoate therapy (1 mg given 3 times per week). Note the large, clear, flat cells with small pyknotic nuclei. About half of the cells in this smear show precornification or full cornification.

TABLE IV.
Effects of Estradiol Benzoate and Alpha Estradiol in Induction of Withdrawal Bleeding in Postmenopausal Patients Without Adequate Priming. Dosage 1 mg × 3 weekly for 3 weeks. Total dosage, 9 mg.

Drug	Preceding cycle	No. patients	Fraction of total No. bleeding	%
Estradiol benzoate	Alpha estradiol	9	5/ 9	
" "	Estrone	9	2/ 9	
" "	Beta estradiol	9	1/ 9	
Total		27	8/27	29.6
Alpha estradiol	Estrone	12	1/12	8.3

the Shorr technic showed that the vaginal epithelium remained almost totally unstimulated. Fig. 2 compares the vaginal smear taken from a patient on the seventeenth day of a 21 day beta estradiol cycle (A) with a smear taken from the same individual on the seventeenth day of a 21 day estradiol benzoate cycle (B). Although the variation in color is not clearly demonstrated in the black and white illustrations, the difference in morphology of the vaginal epithelial cells after use of the 2 compounds is quite evident.

Fig. 2 (A) (beta estradiol) shows a relatively unstimulated vaginal smear consisting of small cells with comparatively large vesicular nuclei. No cornified cells are present. By contrast, Fig. 2 (B) shows a smear which demonstrates the characteristics of good estrogen stimulation. The cells are large, flat, and have very small pyknotic nuclei. About half of the cells in this photomicrograph are "pre-cornified" or "fully cornified", the color varying from a light purple to a clear deep pink.

It was noted early in this study that the amount of recent priming to which the uteri had been subjected played a definite part in the degree of response that might be expected from the subsequent use of various preparations. Table IV shows the diminished effects of estradiol benzoate and alpha estradiol in the induction of withdrawal bleeding when priming was not adequate. Estradiol benzoate is little more than half (53.6%) and alpha estradiol about one third (31.8%) as effective in poorly primed uteri.

A method such as that described above by which the relative potencies in the human female of the various forms of estrogen may be determined somewhat accurately finds practical importance in the following ways. First, it serves to pick out the more potent pure

4. Shorr, E., *Science*, 1941, v94, 545.
5. Shorr, E., *J. Mt. Sinai Hosp.*, 1945, v12, 667.

or relatively pure drugs that may be used therapeutically not on the basis of results in the animal, but rather on the basis of response actually seen in the human uterus. Secondly, such observations may provide a fairly reliable index for clinicians in their use of mixed estrogens. It becomes apparent that such drugs may have a wide range of biologic potency milligram for milligram depending entirely upon content of the more active estrogenic substances. In addition, a rational basis is provided for establishing therapeutically effective dosage levels of the various estrogenic preparations and the modification of these levels under certain circumstances, depending upon whether one is dealing with recently or remotely stimulated uteri.

Summary. Estradiol benzoate (as a standard), alpha estradiol, estrone, and beta estradiol preparations were bioassayed using withdrawal bleeding from previously stimulated uteri of postmenopausal women as an assay end point. Alpha estradiol and estrone were equally effective in causing withdrawal bleeding, and each was about two-thirds as potent as estradiol benzoate. Beta estradiol was found to be altogether ineffective in a total dosage of 45 mg given over a 3 week period.

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Effect of Antrum Transplantation on Gastric Secretion in Experimental Animals.* (17784)

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In previous studies from this laboratory, the profound reduction in gastric secretion of Pavlov pouch dogs produced by removal of the antrum of the stomach has been recorded(1). In the present experiments, quantitative collections of gastric juice from Pavlov and Heidenhain pouch dogs and from animals with totally isolated stomach preparations have been made for control periods lasting several weeks. The antrum of the stomach has then been separated from the fundus, using great care not to interfere with its blood supply, and subsequently transplanted as a diverticulum into the duodenum and into the colon. The results have demonstrated quite conclusively that when the antrum is excluded from the gastro-intestinal tract so that it does not come in contact with food, it exerts little or no effect on gastric secretion. When it is transplanted into the duodenum, a very profound augmentation in gastric secretion results, and this is even more marked when the antrum is transplanted into the colon.

(1) *The Effect of Exteriorization of the Antrum of the Stomach on Gastric Secretion in Pavlov Pouch Dogs.* Pavlov pouches were prepared in 3 normal dogs and quantitative collections of the gastric secretion from the isolated pouches were made for control periods of several weeks by means of the tight-fitting nylon plastic cannulas previously described (2). The antrum of the stomach was then carefully separated from the fundus and the pylorus transected. The fundus was anastomosed to the side of the second portion of the duodenum. The proximal end of the antrum was infolded and closed and the pyloric portion brought to the surface as a fistula. Care

* This work was aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

1. Woodward, E. R., Bigelow, R. R., and Dragstedt, L. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 473.

2. Dragstedt, L. R., Haymond, H. E., and Ellis, J. C. *Surg., Gynec., and Obst.*, 1933, v56, 799.