TABLE II. Influence of Population Size of Type d *H. influenzae* on Presence of Cells Initially Susceptible of Transformation to Type c.

Transforming principle	Approximate population sizes 1.0×10^6	1.0×10^5	1.0×10^4
TPC	— (1) + (4)	— (1) + (4)	— (5)
TPC	+ (3) — (2)	+ (3) — (2)	— (5)

TPC = Transforming principle isolated from type c.
+ = Type c present.
— = No type c found.
No. in parentheses of either + or — symbols indicates No. of samples examined in each experiment.

occurs during a 48-hour growth period. They do not differ significantly from the results obtained with R cells derived from type d under comparable circumstances.

In Table II are listed data which determine the population sizes of type d which must be used in order to ensure the initial presence of susceptible cells. Here again, no significant difference is demonstrated between R and S cell population sizes which contain susceptible cells prior to growth.

Discussion. The slightly greater degree of irregularity seen in these experiments on S cells, as compared with previously published observations on R cells, may be explained by the fact that the identification of presence of new types is much less efficient because of the large number of recipient cells; the specific antibody concentration in the transforming environment is too low to separate completely the recipient cells from the induced new type by agglutination and sedimentation. A stronger concentration can be expected to increase greatly the efficiency of detection of induced new type specific cells.

Since the type d populations used for induction of type c were derived directly from a single clone (colony), the populations can be viewed as homogeneous with respect to the type specific trait. Moreover it was very difficult to demonstrate the presence of an R colony before exposure to the transforming environment. Therefore, if susceptible cells arise by mutation, they are unlikely to be derived solely as mutants of R cells. The factor responsible for susceptibility must arise independently of the R and S genetic traits, for it has not been possible to demonstrate a significant difference in the frequency of susceptible cells in R and S populations.

Summary. 1. Normal strains of types b and d *H. influenzae* have been directly transformed to new types of *H. influenzae;* type b was changed to types a, c and d, and type d into types a, b and c. 2. The rate of occurrence in type d populations of cells susceptible of transformation has been shown to be not significantly different from that found earlier in R cell populations derived from type d. Comparisons in the presence of more potent anti d antibody have been made subsequently between populations of Rd and type d for frequency of cells susceptible (initially or during growth for 24 hours) to transformation to type c. No real differences could be demonstrated when this more efficient selective agent was used.

Received October 23, 1951. P.S.E.B.M., 1951, v78

Hemodilution as a Result of Estrogen Therapy. Estrogenic Effects in the Human Female. (19162)

CARROLL L. WITTEN AND JAMES T. BRADBURY.
From the Institute of Medical Research and Department of Obstetrics and Gynecology, University of Louisville, Ky.

Many articles concerning the effects of estrogenic substances on the so-called "blood picture" may be found in the literature. There are numerous publications concerning the effects of estrogens on the blood composition of laboratory animals. Vollmer and Gordon(1), Tyslowitz *et al.*(2,3), Castrodale(4), and

1. Vollmer, E. P. and Gordon, A. S., *Endocrinology,* 1941, v29, 828.

Crafts(5) reported various experiments in the rat, dog, and monkey. All recorded a drop in erythrocyte count following the injection of estrogens and some reported a lowered level of hemoglobin. Friedlander, Laskey, and Silbert(6), in a study on bilaterally oophorectomized women, recorded a reduction of about 25% in blood volume from the pre-operational level. This loss in blood volume was readily restored by small doses of estrogen. Kretzschmar and Barnes(7) in the early clinical testing of diethylstilbestrol reported a slight lowering of red cell count, white cell count, and hemoglobin, and postulated that "while such a drop was within the limits of technical accuracy, it might be a reflection of the increase in blood volume which is said to occur with the administration of estrogenic substances." Karnaky(8), in a study of 65 patients being treated with stilbestrol, concluded that "the blood picture is not altered even with a dosage of 181,960 mg of stilbestrol over a period of slightly less than a year." His figures, however, reveal a lower erythrocyte count and hemoglobin in the stilbestrol-treated women. He explained this apparent difference by the statement that some of those receiving stilbestrol were menometrorrhagic cases, so a lowered red cell count and hemoglobin might be expected. He compared the average blood counts of the estrogen-treated group with those of an untreated group, but did not record the changes in any patient before and during treatment. In contrast to Karnaky's claims are those of Bateman(9) who reported a moderate anemia in 7 out of 10 women treated with stilbestrol for carcinoma. Bateman also noted that the blood volume was increased as much as 40% above the normal and that it decreased after discontinuing the stilbestrol therapy. Hamblen(10) states that "blood volume is increased due to alterations in electrolyte and water metabolism resulting from administration of estrogens," but on another page makes the contrary statement that, "although estrogens favor the storage of water and electrolytes, the water retention is not intense enough to produce hemodilution."

It was deemed of value to determine, under controlled conditions, the effects of estrogens on the blood of the human female—with the thought in mind that some correlations with the vasomotor symptoms of post-menopausal syndrome might be forthcoming. Also, with the present widespread use of estrogenic substances, it is of interest to understand some of the effects of this ovarian hormone on extragenital tissues.

Methods. This paper summarizes the observations on 16 female outpatients of the Louisville General Hospital, to whom estrogens were administered. These women were suffering from minor menstrual irregularities and varied in age from 15 to 52 years. In the younger women, the estrogen treatment and the blood studies were completed in the inter-menstrual interval so none of the blood changes could be attributed to menstrual blood loss. Venous blood samples were drawn on each visit and the red cell count, hemoglobin, and hematocrit were determined. At least 2 control determinations were obtained before estrogens were administered on alternate days for a total of 2 to 4 intramuscular injections. The estrogen dosage consisted of

TABLE I. Changes in Blood Composition During Estrogen Therapy.

	Erythrocyte count	Hemoglobin, g	Hematocrit, mm
Max decrease	1110000	1.9	11
Min ,,	210000	.4	2
Avg ,,	665000	1.1	6
Avg % decrease	14.8%	8.5%	15%

2. Tyslowitz, R., and Dingemanse, E., *Endocrinology,* 1941, v29, 817.

3. Tyslowitz, R., and Hartman, C., *Endocrinology,* 1941, v29, 349.

4. Castrodale, D. *et al., Endocrinology,* 1941, v29, 363.

5. Crafts, R. C., *Endocrinology,* 1941, v29, 606.

6. Friedlander, M., Laskey, N., and Silbert, *Endocrinology,* 1936, v20, 329.

7. Kretzschmar, N. R., and Barnes, A. C., *Am. J. Obst. and Gynec.,* 1942, v43, 668.

8. Karnaky, K. J., *Am. J. Obst. and Gynec.,* 1949, v58, 404.

9. Bateman, J. C., *Blood,* 1951, v6, 639.

10. Hamblen, E. C., *Endocrinology of Woman,* 1945, Charles C. Thomas, p46.

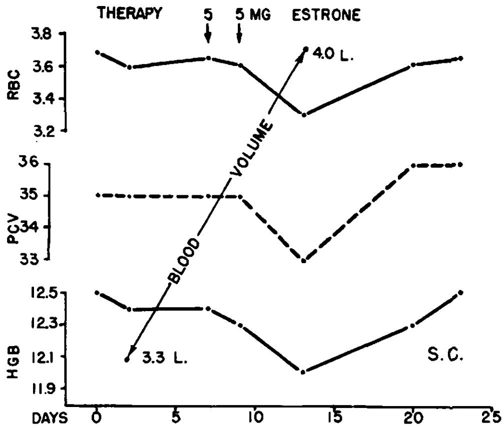

FIG. 1. Changes in blood composition and blood volume as result of 2 injections of estrone on alternate days.

either 5 mg of Theelin (Estrone) or 0.4 mg of α-estradiol dipropionate per injection. On 3 of the 16 patients, blood volumes were determined by the intravenous injection of Evans Blue T-1824 dye before and during estrogen treatment.

Red cell counts were made using standardized pipettes, Hayem's solution and a Spencer Bright Line counting chamber. *Hemoglobin* was determined as acid hematin by a standardized procedure utilizing the electrophotometer. *Hematocrit comparisons* were obtained from oxalated blood in Wintrobe tubes centrifuged for 30 minutes at 3000 r.p.m. *Blood volume* was determined by (a) preparing a standard by the addition of 0.1 cc of a 1:100 dilution of 1% T-1824 Evans Blue dye to an accurately pipetted 5 cc portion of heparinized whole blood. (b) injecting 1 cc of 1% T-1824 Evans Blue dye into the antecubital vein and 5 minutes later withdrawing 5 cc of blood from the other arm and placing the venous blood into a heparinized tube. (c) centrifuging the 2 blood specimens (a) and (b) to obtain the plasma. (d) comparing the relative concentration of Evans Blue dye in the plasma of the standard and the unknown at 620 mμ in the electrophotometer, and computing therefrom the total blood volume.

Results. In the 16 patients there occurred a consistent lowering of the erythrocyte count, hemoglobin and hematocrit during the 4 to 10 days in which estrogen was administered. On cessation of the estrogen therapy there resulted a subsequent return of initial levels within a period of 5 to 9 days. The magnitude of these changes is summarized in Table I.

The trends presented may best be illustrated graphically. The first 3 figures (Fig. 1-3) are those of 3 patients of this series and illustrate the lowering of the erythrocyte count, hemoglobin, and hematocrit correlated with the computed blood volume changes during estrogen administration. The increases in blood volumes in these 3 patients (21%, 19%, and 15%, respectively) demonstrate

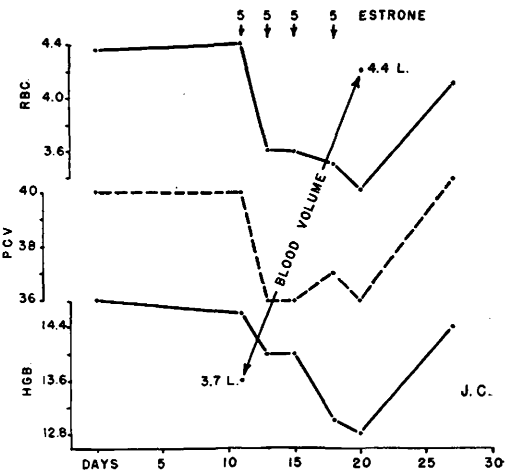

FIG. 2. Changes in blood composition and blood volume as result of 3 injections of estrone on alternate days.

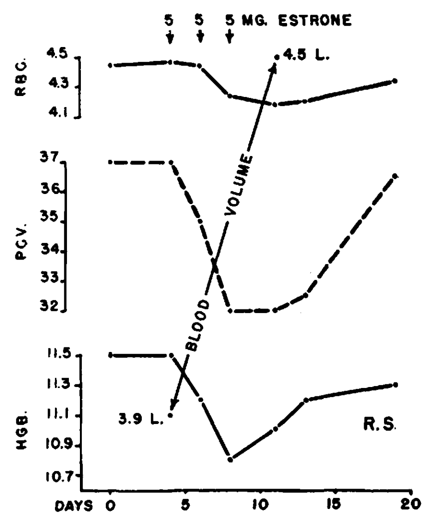

FIG. 3. Changes in blood composition and blood volume as result of 4 injections of estrone in 1 wk.

TABLE II. Total Circulating Red Cell Volumes in Three Patients Before and During Estrogen Treatment. Calculated from the hematocrits and the blood volume estimations based on injections of Evans Blue dye.

Patient	Total circulating red cell volume	
	Before estrogen, l	During estrogen, l
S. C.	1.15	1.32
J. C.	1.48	1.58
R. S.	1.44	1.49

that the decreases in red cell count, hemoglobin and hematocrit were due to a hemodilution produced by a marked increase in plasma volume. When the hematocrit values obtained on the day of blood volume determinations were used to determine the total volume of circulating red blood cells, it was obvious that there was no appreciable increase in the volume of red cells during the experiment (Table II). Similar changes in the composition of the blood were noted after another estrogen (estradiol dipropionate), Fig. 4 and 5.

Discussion. The studies confirm the earlier observations of other authors who have reported changes in blood composition which may have been due to an increased blood volume in laboratory animals treated with estrogen. The loss of blood volume following oophorectomy as noted by Friedlander *et al.*, and its correction by estrogen therapy suggests that the vasomotor symptoms of the surgical or natural menopause may be, in part, a reflection of blood volume changes. The hemodilution observed in our studies also suggests the possibility that the increased estrogen level during pregnancy may be a causative factor of the increased blood volume and the "physiologic anemia" that occurs in pregnant women. Studies in women who experience a definite premenstrual edema would be of interest to determine whether an intravascular fluid increase parallels the extravascular fluid increase. Hematological studies during therapy with estrogen and progesterone or testosterone would show whether these hormones modify or augment the action of estrogen.

Summary. (1) In all 16 patients in this study there was a lowering of the erythrocyte count, hemoglobin and hematocrit during the 4 to 10 days that estrogen was administered. On cessation of estrogen therapy there was a subsequent return to the initial blood constituent levels within a period of 5 to 8 days. (2) Evans Blue dye studies made in 3 of the 16 women demonstrated that the blood volume increased sufficiently to account for the lowering of the red cell count, hemoglobin and hematocrit. (3) During short periods of estrogen therapy there may be a significant hemodilution. Further studies are necessary to determine whether this effect is transitory or whether it may be maintained indefinitely.

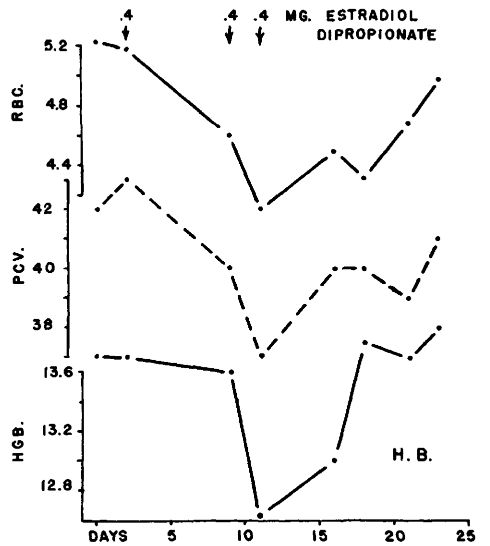

FIG. 4. Changes in blood composition as result of 3 injections of estradiol dipropionate in 8 days.

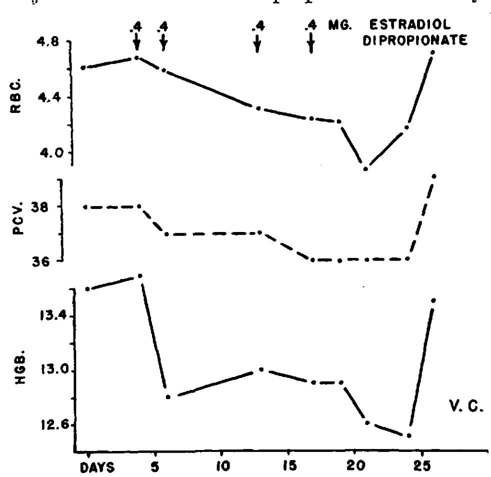

FIG. 5. Changes in blood composition as result of 4 injections of estradiol dipropionate in 10 days.

Received October 11, 1951. P.S.E.B.M., 1951, v78.