TABLE II.
Concentration of Sulphanilamide in Aliquot Portions, Before and After Production of Violet Substance by Irradiation.

Before radiation, mg per 100 cc	After radiation, mg per 100 cc	% decrease
6.3	**2.7**	**53**
6.3	**3.4**	**46**
7.0	3.0	57
7.0	3.0	57
9.0	4.5	50
9.8	**7.0**	**29**
11.0	**6.4**	**42**
12.2	6.1	50

chemotherapeutic effect (insufficiently accounted for by the very feeble bactericidal power of the drug itself) remains to be ascertained.

In offering this phenomenon as the explanation of the cyanosis during sulphanilamide administration we do not suggest that light is the causative factor *in vivo*. It would seem that a transformation so easily produced by light *in vitro* might well be brought about by some of the rapid and powerful metabolic changes constantly occurring in the tissues.

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Skin Absorption of Dihydroxyestrin in Humans.

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The present investigation was undertaken to determine whether estrogenic hormone can be absorbed through the human skin. Absorption was estimated on the basis of the application to the human female of the Allen-Doisy test. Papanicolaou and Shorr[1] have demonstrated that the human vaginal secretions after the menopause or castration exhibit certain striking cytological features, characterized chiefly by the presence of small, round or oval epithelial cells ("deep cells") with rather large, well-staining nuclei and associated with a varying number of leucocytes. These investigators have shown that following administration of adequate amounts of estrogenic hormone the leucocytes and "deep cells" disappear and are

[1] Papanicolaou, G. N., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 585.

supplanted by large, squamous epithelial cells, characteristic of the follicular phase of normally menstruating women. In the present study an attempt was made to determine whether similar vaginal smear changes could be produced in human females by means of skin inunctions with an estrogenic hormone.

A group of women were chosen who were considered, on clinical grounds, to have either marked ovarian deficiency (post-menopause or X-ray castration) or no ovarian function at all (bilateral oöphorectomy). Of these, 14 were selected whose vaginal smears during a period of observation of 2 weeks were found to be constantly "negative" (Reaction I or O),[2] indicating absence of estrogenic activity. The vaginal mucous membrane changes of this group of women, as revealed in the smears, constitute sensitive test objects for estrogenic substances.

Seven groups of 2 cases each were put on a lanolin ointment containing concentrations of dihydroxyestrin* varying from 500 to 25,000 R.U. per ounce. The ointment was applied to the face, neck, and chest nightly, one hour before retiring. By varying the concentration of the hormone in the ointment, as well as the quantity of ointment used with each inunction, the amount of dihydroxyestrin applied to the skin was varied from 500 R.U. to 100,000 R.U. per week. It is obvious that, since it would be impossible to prevent loss of some of the ointment on bed clothes, all calculations would have to be only approximate. Vaginal smears were taken at weekly intervals, prepared and classified as previously described.[2]

At the end of 1 week, the smears of the 2 patients who had used 100,000 R.U. of dihydroxyestrin began to show early signs of cell changes, consisting of a diminution in the number of leucocytes and an increase in the size and number of the epithelial cells (Reaction II). During the following week, with continued inunctions, the leucocytes and "deep cells" grew steadily less in number and were supplanted by increasingly larger numbers of squamous epithelial cells (Reaction III). At the end of 3 weeks, the smears consisted entirely of large squamous epithelial cells with small, deeply-staining nuclei (Reaction IV). A total of 300,000 R.U. of the estrogen was applied before this effect was obtained.

The group using 40,000 R.U. per week began to show early estrogen effects in the smear (Reaction II) at the end of 2 weeks and a full estrogen effect (Reaction IV) at the end of 4 weeks when

[2] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 612.

* For the dihydroxyestrin ointment used in this study, I am indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

160,000 R.U. had been used. The smears of the 2 cases using 10,000 R.U. per week showed only an incomplete estrogen effect at the end of 8 weeks, when 80,000 R.U. had been used. Although the squamous epithelial cell was the predominating type of cell in the smears of these cases a few "deep cells" and leucocytes were still present at the end of 8 weeks.

The 6 cases using 1,000 to 4,000 R.U. per week did not show any significant effect until after the 6th week. The changes in the smears were slight, consisting of a relative diminution in the number of leucocytes and "deep cells" and the appearance of scattered, larger epithelial cells. Although these patients kept on using the same amount of ointment for 6 more weeks, there was comparatively little further progress in the smears, leucocytes and "deep cells" being constantly present, though somewhat reduced in number. The smears of the 2 women using 500 R.U. per week showed no significant changes at the end of 12 weeks. Apparently these patients were not absorbing enough of the dihydroxyestrin to produce an estrogenic effect on the vaginal mucosa.

Of the 14 cases treated, a full estrogen effect (Reaction IV) was produced in 4; an incomplete effect (Reaction III) in 2; a slight effect (Reaction II) in 6, and no reaction in 2 cases. A full estrogen effect (Reaction IV) in these cases was produced with total doses of dihydroxyestrin varying from 160,000 to 300,000 R.U. The time elapsed before the full estrogenic effect appeared in the smear varied from 3 to 4 weeks. The quickest response was obtained in 3 weeks in a woman who used 12 ounces of an ointment containing 25,000 R.U. per ounce, representing a total skin application of 300,000 R.U. in 3 weeks. Beginning estrogen effects were noted as early as 1 week after the institution of inunctions when the concentrated preparation was used (25,000 R.U. per ounce) and as late as the 6th week with the weaker ointments (1,000 to 4,000 R.U. per ounce). The vaginal smears began to show signs of regression (manifested by the appearance of "deep cells" and leucocytes) within 2 weeks after the discontinuation of the inunctions.

It seems from these experiments that in order to produce a full estrogen effect more than 10,000 R.U. of dihydroxyestrin must be applied to the skin per week; when less than this amount is applied only a partial effect is produced in the smears, even though the inunctions are continued for 12 weeks.

It is interesting to note that 6 of the patients volunteered the information that they experienced considerable relief of the pruritus vulvae and vaginal discomfort of which they had formerly com-

plained. This symptomatic improvement coincided with the appearance of an estrogenic effect in the vaginal smear. The skin at the site of inunctions, even in the cases using the most concentrated ointments, exhibited no signs of irritation.

Since the completion of this investigation, the attention of the writer was drawn to a publication by Loeser[3] in which he reports the use of dihydroxyestrin benzoate inunctions in women, using changes in the endometrium and clinical symptoms as an index of absorption. In 5 cases in which endometrial studies were done, he reported no significant changes in the endometrium following inunctions with a total of more than one gram (approximately 6,500,000 R.U.) of the hormone in each case. However, on the basis of clinical symptoms (swelling of the breasts, abdominal cramps and vaginal discharge) which appeared after the inunctions, Loeser concludes that the dihydroxyestrin benzoate is absorbed through the skin. It is worthy of note that in none of the cases reported in the present communication were any of the symptoms described by Loeser observed. The systemic symptoms noted by Loeser are probably attributable to the much larger doses of hormone which were used in his cases.

It appears from this study that dihydroxyestrin is absorbed through the skin of the human female and, when applied in sufficient concentration, exerts an estrogenic effect upon the epithelium of the vaginal mucous membrane. This is shown by the transformation of the vaginal smear, following inunctions with adequate amounts of an ointment containing dihydroxyestrin, from the "negative" phase, in cases of advanced estrogen deficiency, to a "positive" phase characteristic of normal ovarian activity.

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3 Loeser, A. A., *J. Obst. and Gynec., Br. Emp.*, 1937, **44**, 710.