washed away. The lipids are then extracted by stirring up the wet precipitate with alcohol and ether.

To one volume of plasma in a centrifuge tube one adds in succession, with stirring, 15 volumes of water, 1.25 volumes of colloidal iron solution (Merck's "Dialyzed Iron" with 5% Fe_2O_3), and 0.65 volume of a 1:1 aqueous solution of $MgSO_4.4H_2O$. The precipitate is centrifuged for 5 minutes, and is then washed by centrifugation with 15 volumes of water plus 0.65 volume of the 1:1 $MgSO_4.4H_2O$ solution. The washing can be repeated as many times as necessary for desired completeness. The last washing can be done without adding the $MgSO_4$. For routine analyses 2 washings suffice.

The washed precipitate is transferred to a volumetric flask marked to contain 10 times the volume of the plasma sample. For the transfer 4 volumes of absolute alcohol and 4 volumes of ether are used as follows. The precipitate is suspended in 2 volumes of the alcohol, and transferred as completely as possible to the flask. To finish the transfer one then uses in succession 1 volume of alcohol, 1 volume of alcohol, 2 volumes of ether, and 2 volumes of ether, finally filling to the mark with ether. The mixture is filtered.

For complete extraction of the lipids the presence of water is necessary in about the ratio of 1 volume to 6 of alcohol-ether, which is approximated by the above conditions. If a larger proportion of alcohol-ether per volume of plasma were taken, water would have to be added also in order to insure quantitative extraction of the lipids.

The clear filtrate contains all the plasma lipids, and we have not found in it any evidence of non-lipid extractives.

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Desoxycorticosterone Acetate Is Estrogenic in the Human Female.

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It has previously been shown that women, after surgical castration, excrete estrogens.[1] Furthermore, it was noted that in some

[1] Frank, R. T., Goldberger, M. A., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 615.

women, many months after removal of both ovaries, the vaginal smears[2] and vaginal mucosa[3] do not reveal the usual regressive changes associated with estrogen deprivation. Both these observations suggested that there is some extra-ovarian source for estrogen formation in the body. The question arose as to whether the adrenal cortex may not be the source of these estrogens. To determine this point, a number of post-menopause women (with vaginal smear signs of estrogen deprivation) were given synthetic adrenal cortex hormone, in order to see if the hormone would have an estrogenic effect. The latter was evaluated on the basis of changes in the vaginal smears as previously reported with estrone and estradiol benzoate by Papanicolaou and Shorr[4] and confirmed by Salmon and Frank.[3]

Kendall and his co-workers[5] and Reichstein and Steiger[6, 7] have isolated a number of active crystalline compounds from cortical hormone extracts. Synthesis of desoxycorticosterone acetate (21-hydroxyprogesterone), apparently the most active of the synthetic adrenal cortex steroids, was performed by Steiger and Reichstein.[8] In the present study desoxycorticosterone* was used, since it was shown by Miescher, Fischer and Tschopp[9] that the physiologic action of the synthetic adrenal cortex hormone was prolonged by esterification.

A group of 10 women with typical estrogen deficiency vaginal smears were injected with desoxycorticosterone acetate, 3 times weekly, in individual doses of 5 and 10 mg. The hormone was administered, intramuscularly, in concentrations of 5 mg per cc of sesame oil. Vaginal smears were taken 3 times weekly. The total doses administered varied from 50 to 230 mg over periods of 14 to 56 days.

The vaginal smears revealed signs of an estrogenic effect as early as 96 hours after the first injection of the desoxycorticosterone

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

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4 Papanicolaou, G. N., and Shorr, E., *Am. J. Obs. and Gyn.*, 1936, **31**, 806.

5 Kendall, E. C., Mason, H. L., Hoehn, W. M., McKenzie, B. F., *Proc. Staff Meeting of Mayo Clinic*, 1937, **12**, 136.

6 Reichstein, T., *Helv. Chem. Acta*, 1937, **20**, 953.

7 Steiger, M., and Reichstein, T., *Nature*, 1938, **141**, 202.

8 Steiger, M., and Reichstein, T., *Nature*, 1937, **139**, 925.

9 Miescher, K., Fischer, W. H., and Tschopp, E., *Nature*, 1938, **142**, 435.

* For the desoxycorticosterone acetate used in this investigation, I am indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J., and Mr. R. Mautner of the Ciba Company, Summit, N. J.

acetate. This was manifested by the appearance of large, squamous epithelial cells and a decrease in the number of leucocytes and "atrophy" cells. All cases showed a full estrogen effect at the end of 8 days, with doses varying from 40 to 60 mg. At this time, the smears showed complete absence of leucocytes and "atrophy" cells and consisted entirely of well shaped, squamous, epithelial cells with small, deeply-staining nuclei. The changes in the smears during the period of corticosterone administration were strikingly similar to those observed after the administration of estradiol benzoate.

It is interesting to note that the patients experienced some relief of the menopause symptoms. The clinical improvement, however, was not as marked as one would expect from the cytologic changes in the smears. Comparable smear changes induced with estradiol benzoate are usually associated with much more striking relief of the symptoms. It is also worthy of note that the corticosterone injections had no appreciable effect on the blood pressure and, in the doses given, induced no signs or symptoms of virilism. This is particularly significant in view of the virilism and hypertension associated with adrenal cortex tumors and would seem to indicate that the adreno-genital syndrome is not caused by desoxycorticosterone.

A fact of considerable significance is that desoxycorticosterone, in doses of 0.1 to 0.5 mg, had no estrogenic effect in rats. Does this signify that desoxycorticosterone is converted in the human into an estrogen and excreted as such?

Summary and Conclusions. Typical estrogenic effects were produced with synthetic adrenal cortex hormone (desoxycorticosterone acetate) in the vaginal smears of post-menopause women. On the basis of these observations, it is suggested that the estrogens excreted after the menopause or after surgical castration probably have their origin in the adrenal cortex. This may explain the persistence of estrogen effects in the vaginal mucosa and vaginal smears after surgical castration and may, possibly, also account for individual variations in the severity of symptoms experienced at the menopause or after surgical castration.