TABLE I. Cultural Characteristics and PSH Agglutinability of Non-Fermenting Gram-Negative Rods.

| Organism | No. of strains | Gelatin | Citrate | Pigment | Fluorescence (Sellers) | $N_2$ agar (Sellers) | Motility (broth) | Agglutination (PSH) |
|---|---|---|---|---|---|---|---|---|
| *A. fecalis* | 8 | — | + | — | — | + | + | + |
| | 4 | — | + | — | — | — | — | + |
| | 4 | — | + | — | — | — | + | + |
| *Ps. aeruginosa* | 5 | + | + | + green | + | + | + | — |
| | 1 | + | + | + lemon yellow | + | + | + | — |
| | 1 | + | + | — | + | + | + | — |
| | 1 | + | + | + green | + | — | + | — |
| | 1 | — | — | + green | + | — | + | — |
| | 2 | — | + | — | + | — | + | — |
| Unidentified | 3 | — | + | — | — | — | — | — |
| | 1 | + | + | — | — | — | + | + |
| | 1 | — | — | + brown | — | — | — | — |
| | 1 | + | — | — | — | — | + | — |
| | 1 | — | — | — | — | — | — | ± |

*Ps. aeruginosa*. The possibility of using the agglutinating properties of PSH as a tool in the differentiation of *A. fecalis* from other Gram-negative rods, especially *Ps. aeruginosa*, is being further investigated.

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## Hepatic Metabolism and the Anti-Androgenic Activity of Cyproterone Acetate.* (31341)

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Recent reports suggested that cyproterone acetate (Cyp A) might exert anti-androgenic effects directly on receptors within target organs. Junkmann and Neumann(1,2) showed that this compound inhibited fetal endogenous androgens without affecting the secretion of ICSH. These authors and others (3) demonstrated that Cyp A also inhibited the action of exogenous androgens in castrate rats. It is not certain, however, whether Cyp A exerted an anti-androgenic effect by increasing the destruction of androgens in the liver (4,5) or by interfering with the effect of androgens upon target organs. The present study was designed to investigate the first of these possibilities, comparing the systemic effect of this compound when administered subcutaneously with that produced by a splenic depot which drains directly to the liver(6).

*Materials and methods.* Charles River male rats were castrated at birth. When 9-12

* Cyproterone acetate (1,2α-methylene-6-chloro-$\Delta^6$17α-hydroxyprogesterone acetate) was synthesized by Dr. Wiechert, Schering AG, Berlin, Germany. The authors are indebted to Dr. Karl H. Kimbel of Berlin Laboratories, Inc., for the supply of Cyp A, to Mrs. Maxine Wollman for technical and clerical assistance, and to Gordon E. Mestler for drawing the graph. The present work was supported in part by the Harris McLaughlin Foundation and an unrestricted research grant to this medical school from USPHS.

TABLE I. Effect of Location of Cyp A on Its Absorption Rate and Weight of the Seminal Vesicles of Androgen-Stimulated, Castrated Rats.

| Groups listed in Fig. 1 | # of rats | Avg wt (g) | Absorption* # of mg | Absorption* % of original wt of pellets | P value† |
|---|---|---|---|---|---|
| 1 | 9 | 389 | — | — | .01; 1-7 |
| 2 | 5 | 377 | .27 | .9 | .01; (2, 3)-(4, 5)-(6, 7) |
| 3 | 5 | 348 | .25 | .8 | .05; (3, 5, 7)-(2, 4, 6) |
| 4 | 5 | 360 | .51 | 1.1 | .01; (4, 6)-(5, 7) |
| 5 | 5 | 389 | .54 | 1.2 | .05; 5-1 |
| 6 | 5 | 410 | .59 | 1.0 | N.S.; 6-1 |
| 7 | 5 | 479 | .65 | 1.1 | .01; 7-6 |
| 8 | 9 | 370 | — | — | .001; (8-9-10) |
| 9 | 9 | 338 | 1.04 | 1.7 | .001; (9-10) |
| 10 | 10 | 349 | .88 | 1.5 | .001; 10-8 |

* Absorption = mean daily loss in wt of pellets of Cyp A.

† The P value of the differences between the mean weights of the seminal vesicles of the groups listed, using "F" and "t" statistics and Dunnett's multiple comparison test(8) where applicable.

N.S. = not significant ($P > .05$).

months old, the experimental animals received pellets of Cyp A either in the spleen or subcutaneously; pellets of cholesterol were implanted in the other of these two sites. Control animals received cholesterol in both sites. Each pellet was cylindrical, 2 mm in diameter and weighed 5 mg. Pellets were desiccated and weighed before implantation and again after sacrifice of the animals. Reduction in weight of the pellets while in the animals was calculated as the average daily loss in mg and in percent of total pellet weight.

On the day of implantation of pellets, and daily thereafter for a total of 10 days, each animal was injected subcutaneously with approximately .1 ml of sesame oil containing .1 mg of testosterone propionate per 100 g of body weight. On the eleventh day, the rats were sacrificed and weights recorded for the body, ventral prostate, and the seminal vesicles after removal of the coagulating glands. Three dosages of Cyp A were used in Exp 1. The dosage which showed the greatest effect was employed in Exp 2 to ascertain that the previous results could be confirmed.

*Results and discussion.* It was first established that comparable amounts of Cyp A were absorbed from subcutaneous and splenic depots. The average daily loss of weight, calculated as a percentage of total weight of pellets when implanted, was 1.2% for those in the spleen, and 1.1% for those placed subcutaneously (Table I).

It was reasoned, that, if the anti-androgenic action of Cyp A were due chiefly to increased inactivation of androgen by the liver, this effect would be enhanced if Cyp A were supplied directly to the liver. If the liver were not the major site of action, however, Cyp A, like other steroids(7), would be expected to undergo degradation in the liver before exerting its anti-androgenic action.

In castrated rats receiving only exogenous androgen, the weight of the seminal vesicles was significantly greater than that in rats given either 45 or 60 mg of Cyp A in subcutaneous depots ($P < .05$, $P < .01$, Exp 1; $P < .001$, Exp. 2; Fig 1, Table I). The reduction in weight of the seminal vesicles was significantly greater with subcutaneous than with splenic pellets of the anti-androgen ($P < .05$, Exp 1; $P < .001$, Exp 2). In fact, splenic depots of Cyp A exerted no significant anti-androgenic effect, since the weight of the seminal vesicles was comparable to that in rats receiving no Cyp A (Fig. 1, Table I).

Exp 1 and 2, which were conducted at different seasons (June and February) and in different years, gave results that were in essential agreement. The weight of the seminal vesicles was less in Exp 1 than in Exp 2, because in the latter case the organ was kept moist during the weighing.

Weight of the prostate reflected the same tendencies as observed with weight of the

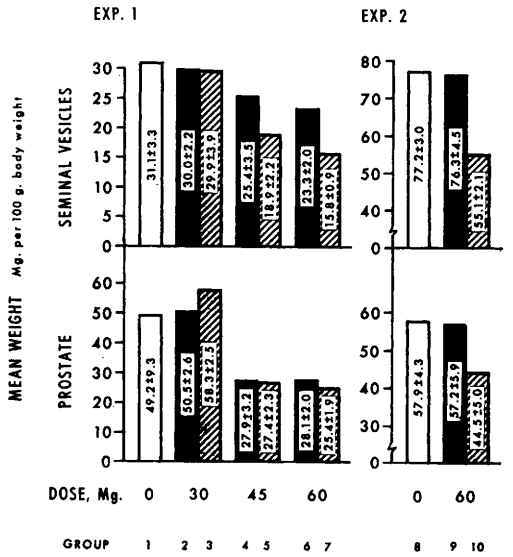


FIG. 1. Comparative inhibition of androgenic stimulation by splenic and subcutaneous depots of cyproterone acetate. □—splenic cholesterol and subcutaneous cholesterol; ■—splenic Cyp A and subcutaneous cholesterol; crosshatched area—splenic cholesterol and subcutaneous Cyp A. (Mean ± standard error)

seminal vesicles, although fewer of these tendencies were statistically significant.

Cyp A was markedly anti-androgenic. Absorption of .6 to .8 mg per day from subcutaneous pellets reduced by 30 to 50% the weight of seminal vesicles in rats receiving daily injections of .4 mg of testosterone propionate.

*Summary and conclusions.* The effects of splenic *vs.* subcutaneous depots of pellets of an anti-androgen, cyproterone acetate (Cyp A), were studied in castrate rats receiving daily injections of .1 mg of testosterone propionate per 100 g body weight. Mean loss of weight of pellets was similar in both sites, indicating comparable absorption. Inhibition of growth of seminal vesicles was significantly less with splenic than with subcutaneous depots of Cyp A ($P<.05$, Exp 1; $P<.001$, Exp 2). These data provide evidence that (a) this compound does not exert its anti-androgenic effects by increasing the inactivation of testosterone propionate in the liver, and that (b) Cyp A can be destroyed in the liver before producing systemic effects as an anti-androgen. The effectiveness of Cyp A as an anti-androgen was shown by a 40% reduction in androgenic response in studies employing a ratio of 2 parts of Cyp A (supplied by pellets) to 1 part of testosterone propionate (supplied in oil).

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## Tissue Culture Characteristics and Oncogenicity of Three SV40-Induced Hamster Tumors. (31342)

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*In vitro* transformation by simian virus 40 (SV40) of fibroblast cultures of hamster lung or liver has resulted in altered growth characteristics of these cells and their production of undifferentiated sarcomas or fibrosarcomas on injection into hamsters(1). Serially cultured human fibroblasts, infected with SV40, have changed morphologically into epithelial-like cells(2,3), and hamster renal cultures have been transformed by SV40