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Studies of Antiprostatic Agents in the Baboon[1] (38204)

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Studies on diseases of the human prostate, both benign and cancerous, have been greatly hampered by the relative unavailability of a suitable animal model system. However, recent studies in the baboon indicate that the prostate of this subhuman primate resembles that of man in some respects (1, 2) and can be investigated as a possible surrogate for human studies, particularly those involving chemotherapeutic agents for cancer of the prostate.

The present study consists of a number of investigations concerned with several prostatic parameters, following the administration of 3 antiprostatic agents to baboons, and include the effects on the following: (1) several enzymatic systems of the baboon prostate, (2) the localization of labeled zinc in the gland, and (3) prostatic weight, histology and blood flow. All of these prostatic facets have unique features which can be affected by a number of hormonal agents, e.g., androgens and estrogens, influencing prostatic function and anatomy. The experiments were designed to ascertain the manner in which these parameters would be altered by the several drugs administered. These drugs have been shown to have antiprostatic actions in rodents and dogs, but, as far as we know, they have not been studied extensively in baboons.

Methods and Materials. Adult male baboons (Papio anubis) weighing 20 kg or more were used in the studies. The prostate removal technique has been previously described (3, 4). The total gland was removed from the animals and the 2 parts of the baboon prostate (caudal and cranial) separated with ease (1–4). These lobes resemble, respectively, the central and peripheral zones of the human prostate. A slow iv infusion of Ringer's lactate solution was maintained through the duration of the experiments.

The techniques for determining prostatic blood flow utilizing ^{133}Xe and for measuring the ^{65}Zn in the prostate, blood and other tissues have also been described in detail elsewhere (2, 4–7).

Arginase was determined by the method of Yamanaka *et al.* (8) and 5α-reductase by the procedure of Kirdani *et al.* (9).

Estramustine phosphate (Estracyt, supplied by the A. B. Leo Company, Helsingborg, Sweden) dissolved in 5% glucose and diethylstilbestrol diphosphate (Stilphostrol) in the commercially available solution were given intravenously 3 times weekly for 4 weeks; and Flutamide (SCH 13521, supplied by Schering Corporation, Bloomfield, New Jersey) was injected intramuscularly, also 3 times weekly for 4 weeks. The latter compound was suspended in 0.9% saline containing carboxymethylcellulose (0.5%) before injection. The control animals were injected with saline only. All drugs were given at a dose of 5 mg/kg/wt. Phencyclidine hydrochloride (Sernylan) was given intramuscularly (1 mg/kg) for anesthesia. The control and experimental groups consisted of 3 baboons each.

Results and Discussion. 1. *Effects on weight and histology of the baboon prostate.* All drugs produced a definite decrease in the weight of the prostate (Table I), which usually appeared to involve the caudal pros-

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TABLE I. Effects of Estracyt, Flutamide and Stilbestrol on Prostatic Weights in the Baboon.

	(Mean ± Standard error)	
	Prostatic weight (gm)	Body weight (kg)
Control[a]	10.18 ± 1.01	25.3 ± 1.46
Estracyt	6 91 ± 0.31	23.8 ± 1.18
Stilbestrol	5.90 ± 0.28	24.4 ± 1.21
Flutamide	3.44 ± 0.11	20.1 ± 1.38

[a] Published values for normal baboon: prostate weight 10.07 ± 1.00 gm, body weight 25.93 ± 1.46 kg (7).

tate more than the cranial one. The decrease in weight was at least as large as that observed following castration in baboons (7).

The histology of the prostates was evaluated, particularly in regard to the ratio of glandular tissue to that of the stroma, and the morphology of the acinar nuclei. The most pronounced changes following the administration of all the drugs were observed in the acinar nuclei, which became flattened, developed columnar shapes and contained variable amounts of chromatin. Many large nuclei were particularly evident following the administration of Estracyt. The ratio of glandular tissue to that of stroma decreased following the administration of all three drugs.

2. *^{65}Zn uptake by the baboon prostate.* Zinc has been postulated to have a unique relationship to the prostate, in which organ it is thought to play an essential role in the function and integrity of the gland (7, 10–12). Furthermore, high concentrations of zinc have been demonstrated not only in the prostate of rodents and dogs (12–17), but also in the gland of the baboon and human (11, 12, 15, 16). It is possible that those agents which decrease the zinc concentration in and/or uptake of labeled zinc by the prostate may affect the vital functions of the gland. Thus, the uptake of radioactive zinc could be used as an index for the antiprostatic actions of administered drugs.

Not only is the concentration of zinc very high in the prostate of the baboon, but its uptake is largely under hormonal control (10, 18–21). Thus, the administration of testosterone causes an increased uptake of radioactive zinc by the baboon prostate. The effects of estrogens on the uptake by the baboon gland have not been reported, though conflicting results have been published for the rat, i.e., high doses in mature animals cause a moderately decreased uptake of ^{65}zinc by the dorsolateral prostate, whereas low doses in adult castrated or in immature animals led to a marked increase (7, 22).

Tissues were removed 4 hr following intravenous administration of 100 μCi of ^{65}Zn. The results are expressed as dpm/gm of wet tissue and are shown in Table II. It is evident that all drugs inhibited the deposition of ^{65}Zn in the caudal prostate, with the most profound effects being shown by Estracyt and stilbestrol. The effects on the cranial prostate were less significant and the total amount of ^{65}Zn taken up by this part of the gland was 1/5 of that in the caudal prostate. Only Flutamide appeared to significantly inhibit the ^{65}Zn uptake. Parenthetically, the levels of radioactivity found in the prostates of the control animals are very similar to those previously reported for

TABLE II. Effects of Estracyt, Flutamide and Stilbestrol on ^{65}Zinc Uptake by Baboon Tissues.

	(Results expressed as mean dpm/gm of wet tissue ± SEM)					
	Caudal prostate	Cranial prostate	Sem. vesicles	Pancreas	Blood (dpm/ml)	Muscle
Control	14490 ± 5920	2760 ± 840	2820 ± 760	26840 ± 3330	620 ± 160	520 ± 230
Estracyt	2370 ± 660	2440 ± 630	1570 ± 160	34880 ± 11720	570 ± 90	690 ± 320
Flutamide	6330 ± 3080	1850 ± 590	2340 ± 210	21450 ± 2620	400 ± 10	670 ± 30
Stilbestrol	2910 ± 720	3140 ± 790	2020 ± 80	24140 ± 2720	440 ± 60	930 ± 230

normal baboons in a number of other studies (2, 3, 20).

The pancreas of the baboon is known to take up large amounts of ^{65}Zn, actually more than the prostate (23). In order to test the specificity of the drug effects on pancreatic zinc, the ^{65}Zn levels in the head of the pancreas were determined and are shown in Table II. No significant alteration of ^{65}Zn uptake was evident in the pancreas following the administration of any of the drugs.

The effects on the seminal vesicles are also shown in Table II, with only Estracyt appearing to have a significant influence on the deposition of ^{65}Zn. Interestingly, stilbestrol had only a minor effect.

Since the levels in the various organs have to be considered in light of the ^{65}Zn concentration in the blood, the dpm/ml of blood are shown for comparison in Table II. It is apparent that the levels of ^{65}Zn in the blood 4 hr after injection are much lower than those observed in the tissues. This applies to muscle tissue also (Table II).

3. *Effects on prostatic blood flow.* Since the deposition of ^{65}Zn may be influenced by the blood flow to the prostate, such flow was determined in the caudal prostate. The results are shown in Table III. No significant changes in the amount of blood flowing to the prostate were produced by the administered drugs; and all the values fall within the range reported for normal baboons (2, 3).

4. *Arginase activity.* This prostatic enzyme has been shown to be under androgenic control, i.e., its activity is increased by the administration of testosterone and decreased by estrogens (8). It has also been established that the presence of Mn^{2+} is necessary in the incubation medium, if the full activity of arginase in prostatic tissue is to be realized (24). This applies to human and rat prostates. Activation of arginase by Mn^{2+} in the homogenate of prostates of normal baboons was tested (Table IV) and shown to be much higher than that exhibited by normal human prostate or the ventral prostate of the rat (Fig. 1). The activation rate resembled that shown by human benign prostatic hypertrophy tissue (24). Arginase activity was much higher in the caudal than in the cranial prostate of the baboon.

TABLE III. Effects of Estracyt, Flutamide and Stilbestrol on Blood Flow to Caudal Prostate.

(Expressed as ml/min/gm ± SEM)	
Control[a]	0.163 ± 0.28
Estracyt	0.203 ± 0.54
Flutamide	0.143 ± 0.19
Stilbestrol	0.177 ± 0.10

[a] Published values for normal baboons: 0.205 ± 0.11 (4), 0.186 ± 0.031 (3), 0.155 ± 0.070 (3).

The effects of the various drugs on arginase activity in the caudal and cranial prostates of the baboon are shown in Table IV. All the drugs produced a significant reduction of the arginase activity in the caudal prostate, with stilbestrol and Estracyt leading to the lowest levels of activity. Consequently, the level of arginase after administration of Estracyt could be measured in the cranial prostate only.

5. *5α-reductase activity.* This prostatic enzyme is responsible for the conversion of testosterone to dihydrotestosterone. The latter is thought to be the active form of the hormone in the prostate (25). Interestingly, the level of 5α-reductase activity is under the control of androgens, i.e., the activity increases when testosterone or dihydrotestosterone are administered and decreases when estrogens are given (25).

In Table IV are shown the values for 5α-reductase activity in the baboon prostate following administration of the drugs. It is evident that profound depression of the enzyme activity was induced by each drug, with the enzyme system (17β-dehydrogenase) responsible for the conversion of testosterone to androstenedione being less affected than 5α-reductase.

Comments. Each of the drugs given to the baboon produced a definite effect on one or another parameter of prostatic function measured, though the effect differed with the drug administered. Thus, the most profound effect on prostatic weight was induced

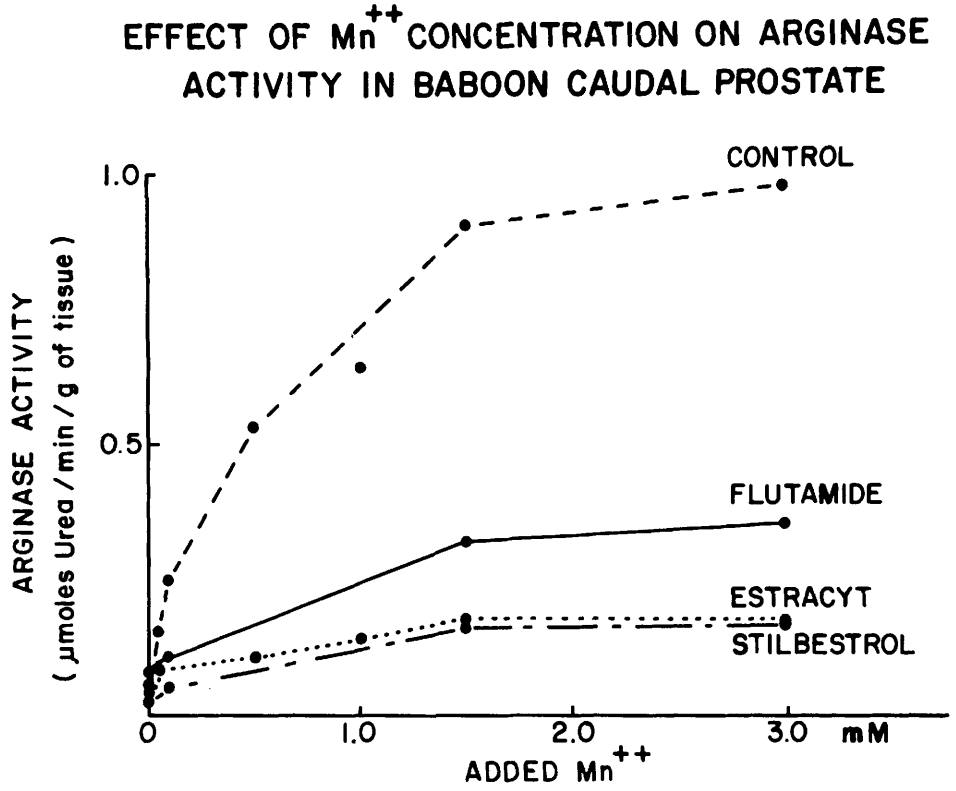

FIG. 1. Effect of Mn^{++} concentrations on arginase activity of baboon prostates. The results indicate that Mn^{++} is needed for optimal enzyme activity, as has been shown for human and rat prostates (24); and that the decreased levels of arginase activity caused by the administration of the 3 drugs to baboons are real and not due to Mn^{++} deficiency in the assay.

by Flutamide, an action very similar to that produced by the drug in dogs (26). Furthermore, the reduction in the weight of the baboon prostate was more intense than that produced by castration alone (7), and indicates a probable direct effect of Flutamide on the prostate rather than through a pituitary-testicular action.

On the other hand, Estracyt and stilbestrol produced their most intense effects on ^{65}Zn uptake and reduction in arginase and 5α-reductase activities. It is possible that the

TABLE IV. Effect of Estracyt, Stilbestrol and Flutamide on Arginase, 5α-Reductase (5α-R) and 17β-Dehydrogenase (17β-D) Activity.[a]

	Arginase Activity (μmoles urea/min/gm)		5α-R Activity (expressed as conversion of T to DHT in μg/gm)	17β-D Activity (expressed as conversion of T to A in μg/gm)
	Caudal prostate	Cranial prostate	Caudal prostate	Caudal prostate
Control	0.74 ± 0.11	0.39 ± 0.08	0.233 ± 0.055	0.323 ± 0.091
Estracyt	0.19 ± 0.04*	0.26 ± 0.04	0.075 ± 0.012**	0.174 ± 0.042
Stilbestrol	0.15 ± 0.02**		0.090 ± 0.014**	0.138 ± 0.035
Flutamide	0.44 ± 0.05		0.082 ± 0.013**	0.280 ± 0.074

[a] Values were obtained in 3 animals and are expressed as mean ± S.E.M. Analysis by the t-test revealed: *$P < 0.05$, **$P < 0.02$, T = testosterone, DHT = dihydrotestosterone, A = androstenedione.

effects of Estracyt and stilbestrol may be, at least partially, mediated through decreased pituitary secretion of gonadotrophic hormones and/or testosterone production by the testes, though a direct prostatic effect cannot be ruled out, particularly in the case of Estracyt.

Since no significant changes in prostatic blood flow were induced by any of the drugs administered, their effects on the prostate cannot be ascribed to alterations in supply of substances in the blood and point to a more direct action of the drugs on the gland.

The 3 agents used in the present study apparently have different modes of antiprostatic action. Thus, diethylstilbestrol apparently affects the prostate by inhibiting pituitary release of LH which leads to reduced testosterone secretion by the testes. The decreased levels of testosterone are then responsible for the changes in prostatic size and histology, the reduced arginase and 5α-reductase activities, and lowered uptake of labeled zinc. A direct action of stilbestrol on the prostate has not been ruled out.

Estracyt is a nitrogen mustard of estradiol-17β(E_2), E_2-3[bis-(2-chlorethyl)]-carbamate-17-dihydrogen phosphate, which probably affects the prostate either through the alkylating effects of the mustard moiety and/or through the action of the released estrogen moiety (9). However, the exact mechanism of Estracyt action in the baboon, or for that matter in the human, remains to be established.

Flutamide (SCH 13521, 4′-nitro-3′-trifluoromethylisobutyranilide) has been shown to produce atrophy of the prostate in rats and dogs apparently through an antiandrogenic action (26), though the exact mechanisms of its antiprostatic effects have to be fully elucidated.

The results of the present study appear to indicate that data obtained with the baboon prostate should be given considerable weight in their extrapolation to possible effects on the human prostate.

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