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UPTAKE AND RETENTION OF ANDROGENS BY THE RAT VENTRAL PROSTATE AND CONSIDERATION OF THEIR USE AS SITE DIRECTING AGENTS

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Abstract—Elucidation of the mechanism whereby androgens are accumulated selectively by the prostate may help in the design of drugs for the treatment of prostatic cancer.

The uptake and retention of [^{3}H]testosterone, following intraperitoneal injection, by various tissues in the 24 hr castrate rat has been studied over an extended time course. The selectivity with which prostate, as compared with blood or other tissues, accumulated ^{3}H was shown to be dose-dependent. At a low dose (0.15 μg), selective prostatic accumulation was greater in 24 hr castrate and diethylstilboestrol-treated rats than in normal animals. Testosterone, 5α-dihydrotestosterone and oestradiol, radioactively labelled, were each administered to 24 hr castrate rats by intraperitoneal injection. Specific prostatic accumulation of radioactivity was more dependent on steroid structure at a low dose than at a high dose (0.6 mg) and at the low dose (0.15 μg) followed the order testosterone > 5α-dihydrotestosterone ≥ oestradiol. This order was surprising in view of the androgen receptor binding affinities of these steroids.

It is concluded that small quantities of material could be directed with the greatest specificity to the prostate of castrate or diethylstilboestrol-treated animals if attached to testosterone. Androgens would be more useful for site-directed radiopharmaceuticals than cytotoxic agents.

Hormones may provide the means for specifically directing agents to a target tissue. This idea is of considerable importance with regard to the treatment of cancers of hormone-dependent tissues such as the prostate.

The normal growth and maintenance of the prostate is under the control of androgens, particularly testosterone. Our current understanding of the mechanism of action of testosterone indicates that following passive entry of the steroid into target cells it is converted to 5α-dihydrotestosterone. This latter compound is bound with high affinity by the androgen receptor protein and the resulting complex is translocated to the nucleus [1]. A result of this series of events is the selective retention of androgens in target tissues [2].

Compounds formed from agents chemically coupled to an androgen might also be selectively retained by the prostate. The agents could be either cytotoxic compounds for cancer treatment or suitable isotopes for use as radiopharmaceuticals in emission tomographic scanning procedures [3] of soft tissue deposits (tumours and metastases). With the design of such agents in mind, it is important, in order to maximise accumulation in the prostate relative to other tissues, to recognise conditions that affect the distribution of androgens between tissues.

Current thoughts favour the binding of androgen to the androgen receptor protein as the key step in selective prostatic accumulation. However, the tissue distribution of steroids administered to male animals *in vivo* has not been extensively studied. Furthermore, previous experiments have been conducted using castrated animals and little consideration has been given to the androgen status of the animal [2, 4, 5]. In this study the distribution between rat tissues of administered steroids with different androgen receptor binding affinities has been examined. The effect of animal status and dose on those distributions has also been considered.

Our current knowledge indicates that the mechanism of action of testosterone in the human prostate is similar to that in the rat ventral prostate [6–8]. In particular, no difference in the properties of the androgen receptor protein has been detected in the two species [9]. Our experiments have, therefore, been conducted in the rat. The results are discussed in relation to the design of agents for use in the diagnosis and treatment of cancer of the prostate.

METHODS

Materials. [1α,2α(*n*)-^{3}H]Testosterone, [6,7-^{3}H]-oestradiol and 5α-dihydro[1α,2α(*n*)-^{3}H]testosterone were obtained from the Radiochemical Centre, Amersham, Bucks., U.K. Tritiated steroids were used either at the specific activity received (50 Ci/mmole) or were diluted with unlabelled steroid to yield the required activities of 50 Ci/mmole or 50 mCi/mmole. Diethylstilboestrol was obtained from Koch-Light Laboratories, Colnbrook, Bucks, U.K. All other unlabelled steroids were obtained from Steraloids, Croydon, Surrey, U.K. All other chemicals were of the highest available purity and obtained from B.D.H., Poole, Dorset, U.K.

Animals. Experiments were conducted using male Wistar rats (350–450 g). Animals were used either intact or 24 hr after castration. Castration was per-

formed by the scrotal route, the animals were lightly anaesthetized with Hypnorm [Fluanisone] (5 mg/kg) and Valium (2.5 mg/kg).

Treatment. All radioactive steroids were administered by intraperitoneal injection. Before injection the steroids were dissolved in ethanol which was made 1% with respect to normal saline. At various times following injection animals were killed by cervical dislocation. Tissue and blood samples were removed and frozen at −70° until processed.

In some experiments intact animals were pretreated with diethylstilboestrol. Diethylstilboestrol was dissolved in arachis oil (1 mg/0.5 ml) and injected subcutaneously in the neck. Diethylstilboestrol (1.0 mg) was administered on days 1 and 2 at 10.00 a.m. At 1.00 p.m. on day 2 these animals received [^{3}H]testosterone in saline by intraperitoneal injection.

Determination of total tissue/blood radioactivity. Aliquots of tissue or blood (0.25 g/0.25 ml) were completely combusted in a Packard 306 tritium/carbon oxidiser. The resulting separated fraction of ^{3}H as water was collected in scintillation fluid I and taken for radioactive counting.

Radioactive counting. Samples were counted in a Packard Model 3375 scintillation counter. Dpm were determined using the automatic external standard.

Scintillation fluid. I. Monophase (Packard Instrument Co. Inc.).

RESULTS

The distribution of a number of administered steroids between different tissues (liver, kidney, blood, brain, heart, lung, spleen and prostate) of the rat has been studied. The effect of dose, animal status and steroid structure has been considered. For brevity and clarity the results reported do not include those for lung and spleen because in each experiment the results fell between those of brain and heart.

Effect of dose

[^{3}H]Testosterone (100 μCi, sp. act. 50 mCi/mmole), at a dose of 0.6 mg, was administered to 24 hr castrate rats. The ^{3}H concentration in various tissues over a 4 hr time period was determined. The ^{3}H concentration in liver and kidney was higher than that in prostate at all time points. The ^{3}H concentration in blood differed little from that in prostate whereas the ^{3}H concentration in prostate was 2–4 times greater than that in brain or heart. The mean values determined from a minimum of 4 experiments are shown in Fig. 1.

If the dose of [^{3}H]testosterone (25 μCi, sp. act 50 Ci/mmole) administered was reduced to 0.15 μg, the distribution of radioactivity between the tissues changed markedly. The concentration of ^{3}H determined in liver and kidney fell to values similar or lower than those measured in prostate. At all time points the ^{3}H concentration in prostate was 5–10 times that in blood, brain or heart (see Fig. 2a).

Animal status

The experiments described so far were conducted in 24 hr castrate rats. It was of interest, bearing in mind the treatment of patients, to study the distribution of testosterone between the tissues of the intact rat. A low dose of [^{3}H]testosterone (25 μCi, sp. act. 50 Ci/mmole) was given to normal rats. The concentration of radioactivity in prostates of normal animals was lower than that in prostates of 24 hr castrate rats given this low dose. Furthermore, the relative distribution of radioactivity between the tissues of normal animals also differed from those measured in castrates at this low dose, being nearer to that observed after higher dose (100 μCi, sp. act. 50 mCi/mmole) administration to castrates. That is, the ^{3}H concentration in the prostate rose relative to the ^{3}H concentration in all other tissues.

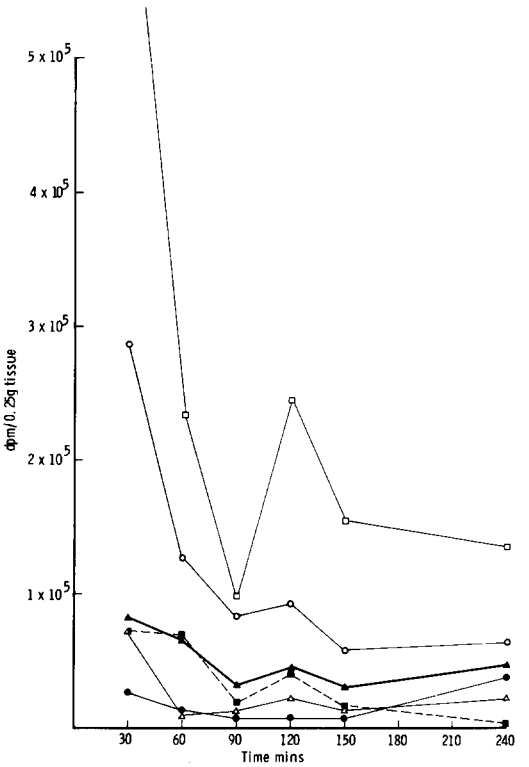

Fig. 1. 24 hr following castration each animal received 100 μCi [^{3}H]testosterone (50 mCi/mmole) i.p. in 0.5 ml of 1% ethanol in 0.15 M saline. Animals were killed by cervical dislocation 60, 90, 120, 150, and 240 min post injection. Tissues were removed and radioactive content was determined. Results are expressed per 0.25 g tissue and are the means of at least four experiments per time point. □——□ Liver; ○——○ kidney; ▲——▲ prostate; ■- - -■ blood; △——△ heart; ●——● brain.

24 hr castration results in a fall in plasma testosterone. A fall in plasma testosterone can also be brought about by treatment with diethylstilboestrol. A group of normal rats were therefore pretreated for 26 hr with diethylstilboestrol, after which time [^{3}H]testosterone (25 μCi, 50 Ci/mmole) was administered by intraperitonal injection. The ^{3}H concentration of various tissues was determined and found to be more similar to that observed in 24 hr castrate rats after [^{3}H]testosterone treatment at the same low dose.

Plasma testosterone concentration was determined in groups of 3 normal, 24 hr castrate and 26 hr

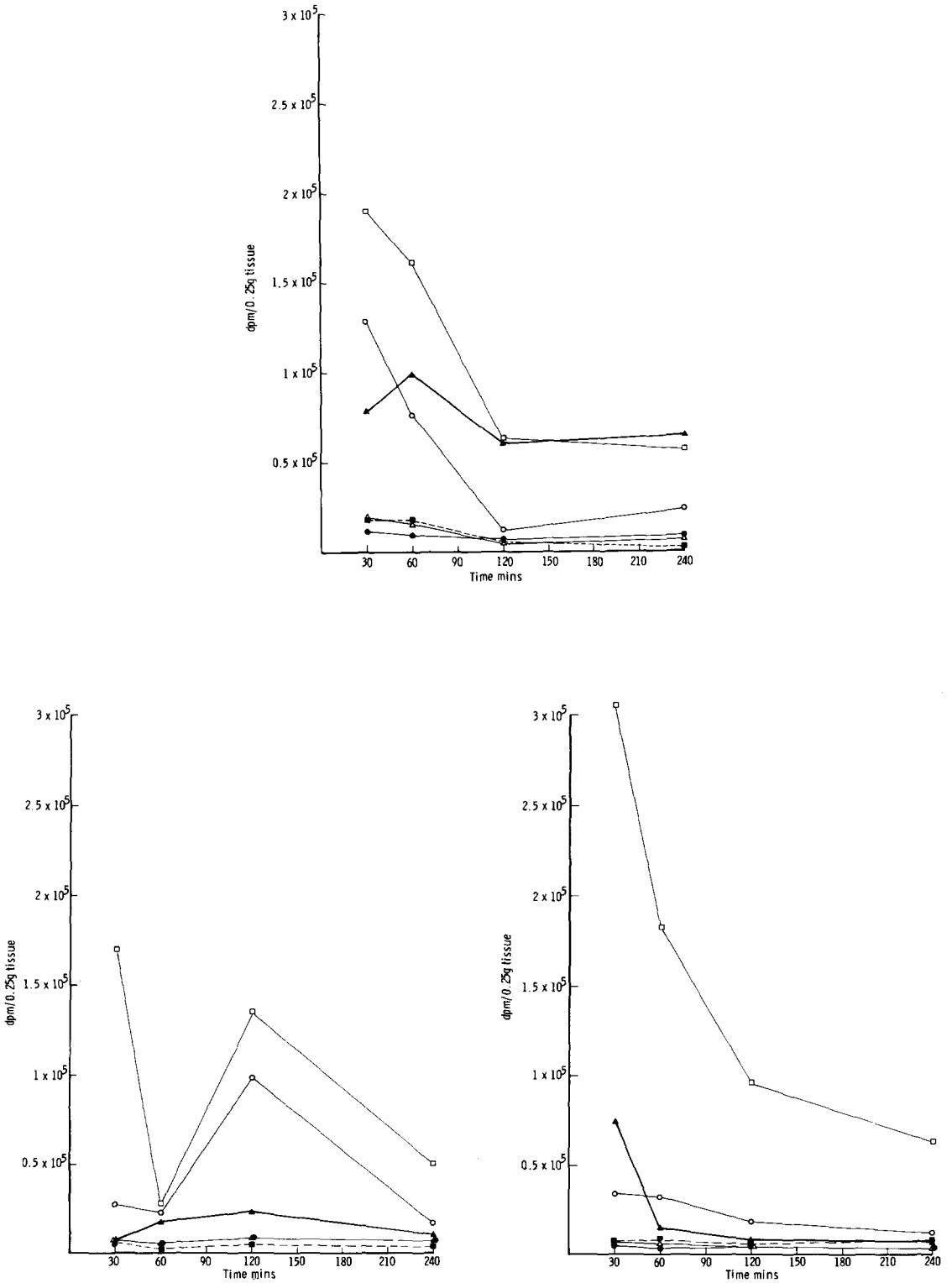

Fig. 2. 24 hr following castration each animal received 25 μCi (50 Ci/mmole) of (a) [^{3}H]testosterone; (b) [^{3}H]5α-dihydrotestosterone; (c) [^{3}H]oestradiol i.p. in 0.25 ml of 1% ethanol in 0.15 M saline. Animals were killed by cervical dislocation 30, 60, 120 and 240 min post injection. Tissues were removed and the radioactive content was determined. Results are expressed per 0.25 g tissue or per 0.25 ml blood and are the means of two experiments. □——□ Liver; ○——○ kidney; ▲——▲ prostate; ■- - -■ blood; △——△ heart; ●——● brain.

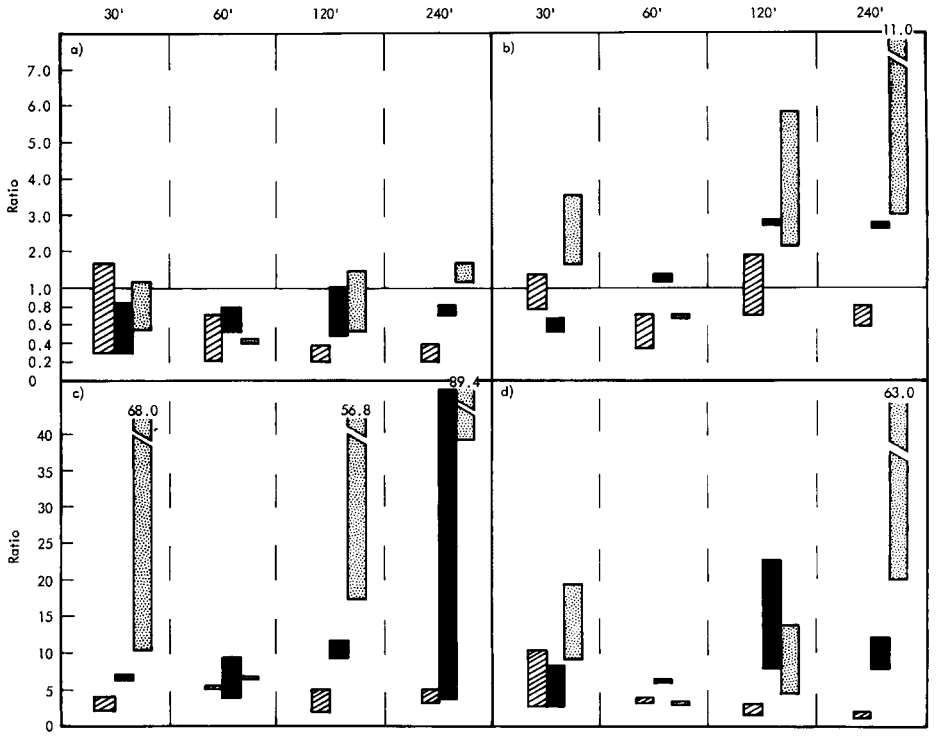

Fig. 3. The ratio of the ^3H concentration in the prostate to the ^3H concentration in the liver (a), kidney (b), blood (c) and heart (d) have been plotted for the intact ▨, 24 hr castrate ■ and diethystilboestrol-treated ⊡ rats. The bars represent the range over two experiments of results obtained at 30, 60, 120 and 240 min following the i.p. injection of 25 μCi of [^3H]testosterone (50 Ci/mmole).

diethylstilboestrol-treated rats. Values of 6.67 ± 0.94, 1.05 ± 0.39 and 1.1 ± 0.51 nmole/l, respectively, were obtained.

A comparison of the relative distribution of ^3H between the tissues of normal, 24 hr castrate and diethylstilboestrol-treated rats is shown in Fig. 3. The ratio of the ^3H concentration in prostate to the ^3H concentration in liver, kidney, blood and brain was determined respectively for each treatment and at a number of time points over a 4 hr period. The data presented represents the range over two experiments per point.

The experiments described above were repeated using a high dose of injected [^3H]testosterone (100 μCI, 50 mCi/mmole). No significant difference in ^3H concentration between the corresponding tissues of the normal, 24 hr castrate and diethylstilboestrol-treated rats could be detected (unpublished data).

Steroid specificity

Receptor binding may play a key role in retention of steroids by the prostate. 5α-Dihydrotestosterone has a higher binding affinity than testosterone for the androgen receptor protein [10]. There have been reports of the presence of the oestradiol receptor protein in rat and human prostate [11–13]. It was therefore of interest to compare the distribution of administered 5α-dihydrotestosterone or oestradiol with that of testosterone between the tissues of the 24 hr castrate rat.

The ^3H concentration was determined in tissues of 24 hr castrate rats after the injection of [^3H]5α-dihydrotestosterone or [^3H]oestradiol, respectively (25 μCi, 50 Ci/mmole). The mean results are plotted in Figs 2b and 2c.

In comparison with ^3H concentrations determined after the injection of [^3H]testosterone at the same dose (Fig. 2a), it can be seen that at all time points the radioactive concentration in the prostate was higher after the administration of [^3H]testosterone than after injection of [^3H]5α-dihydrotestosterone or [^3H]oestradiol. The concentration of ^3H in the prostate relative to other tissues was also greater with [^3H]testosterone than with the other steroids.

There were small differences in ^3H concentration between corresponding tissues after the injection of [^3H]5α-dihydrotestosterone or [^3H]oestradiol. ^3H concentrations were higher in liver after injection of [^3H]oestradiol while the ^3H concentrations in kidney were higher after the injection of [^3H]5α-dihydrotestosterone. Although the total concentration of ^3H in the prostate was similar in both cases, the time courses differed. [^3H]Oestradiol concentrations were higher initially, but fell rapidly, whereas [^3H]5α-dihydrotestosterone concentrations reached a maximum at 2 hr.

The ^3H distribution between various tissues of the 24 hr castrate rat were determined over a 4 hr time course following the injection of a high dose (100 μCi, sp. act, 50 mCi/mmole) of either [^3H]5α-dihydrotestosterone or [^3H]oestradiol. There were

no significant differences between these results and those obtained after administration of [^{3}H]testosterone at a high dose (unpublished data).

DISCUSSION

The results clearly show (Fig. 1 and Fig. 2a) that the selective concentration of ^{3}H in the prostate as compared with other tissues, after administration of [^{3}H]testosterone, is dose-dependent. These results are in general agreement with previous attempts to show target tissue accumulation of androgens. No selective accumulation of radioactivity could be demonstrated following administration of [^{14}C]testosterone, i.e. low specific activity [4, 5], but could be demonstrated after administration of [^{3}H]testosterone, i.e. of higher specific activity [2], and also in agreement with the conclusions of Eakins *et al.* [14]. Fang *et al.* [2], however, obtained lower radioactive concentrations in liver after administration of [^{3}H]testosterone of high specific activity than were obtained here. Although they administered a similar dose of [^{3}H]testosterone, their animals had been castrated for 4 days.

In keeping with these results, it was also found that the selective concentration of [^{3}H]testosterone in the prostate after low dose administration is also dependent on animal status. The concentration of [^{3}H]testosterone was higher in the prostates of 24 hr castrate or diethylstilboestrol-treated rats than in the prostates of normal animals; that is, higher in animals with low circulating testosterone levels. After high dose administration no effect of animal status could be demonstrated. At this large dose the diluting effect of endogenous testosterone was presumably insignificant.

Using radioactive steroids of high specific activity, i.e. low dose, the radioactive concentration determined in the prostate was also dependent on the nature of the administered steroid. The prostatic ^{3}H concentration was higher after administration of [^{3}H]testosterone than after injection of [^{3}H]5α-dihydrotestosterone or [^{3}H]oestradiol. Contrary to the commonly held view, it would thus appear that androgen receptor binding is not a key factor in prostatic steroid accumulation. The androgen receptor binding affinity of testosterone is only one-tenth that of 5α-dihydrotestosterone [10]. Alternative factors that may be involved include binding to plasma proteins, plasma membrane transport, metabolic pools and metabolism. The plasma protein, sex hormone binding globulin, has a greater affinity for 5α-dihydrotestosterone than testosterone. This protein is present in the plasma of a number of animals; however, available evidence [15] suggests that it is not present in the rat. The transport of testosterone across the plasma membrane may be facilitated. There have been reports [16, 17] to suggest that steroids may be actively transported across the plasma membrane of target cells. Once in the target cell testosterone is rapidly metabolised to 5α-dihydrotestosterone [2], which may be more available for androgen receptor binding than administered 5α-dihydrostestosterone. 5α-dihydrotestosterone is reduced in prostate to compounds of lower androgen receptor binding affinity [18]. This data also indicates that the selection of putative androgen-linked compounds (for cancer treatment), based only the criterion of androgen receptor binding affinity, would be inappropriate.

After administration of low specific activity testosterone, i.e. a high dose, the radioactive concentration in the prostate was much lower than that of liver or kidney and only slightly greater than that of other tissues. At this dose the concentration difference between tissues was not critically dependent on steroid structure, a similar distribution was obtained after injection of radioactively labelled testosterone, 5α-dihydrotestosterone, or oestradiol. Similar data using labelled steroids of low specific activity were obtained by Sturman *et al.* [19] in dogs and by Kirdani *et al.* [20] in dogs and baboons.

Steroid linked cytotoxic agents for cancer treatment are likely to be administered in high doses. The recommended dose of a commercial preparation Estracyt (A.B Leo, Helsingborg, Sweden), a nitrogen mustard derivative of oestradiol, is 150 mg/day. This is an oral preparation but 75% absorption has been shown to occur [21]. This would approximate to a 112 mg injected dose to an average man of 70 kg body wt, or 0.80 mg/0.5 kg body wt. This is a dose very similar to that used in the high dose experiments here reported. Furthermore, the recommended dose of other cytotoxic drugs (e.g. adriamycin, vinblastine) is in the range of 0.1–2.0 mg/kg. At these doses testosterone is not accumulated specifically by the prostate; thus it seems unlikely that androgens would be suitable agents to specifically direct cytotoxic agents to the prostate. It is possible, however, that if a cytotoxic compound could be attached to testosterone, so that the accumulation of this steroid in prostatic cells was not altered, an adequate concentration of the cytotoxic might be achieved selectively at this site on administration of smaller doses.

A major requirement of a suitable radiopharmaceutical for scanning emission tomographic procedures is good discrimination between tissue and background. It has been estimated that a ratio of five to one is required [22]. The total amount of material accumulated in the tissue is less critical provided a suitably energetic, short half-life isotope can be introduced. Metastatic deposits of prostatic adenocarcinoma commonly occur in lymph nodes. On the basis of the data presented, where [^{3}H]testosterone concentrations in the prostate of five to ten times that in blood can be achieved, it would seem highly probable that a derivative of testosterone would form a suitable radiopharmaceutical. Furthermore, since castration and diethylstilboestrol treatment are commonly used for carcinoma of the prostate, effects of endogenous testosterone could be eliminated [23].

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