where $\varepsilon_{Z^{\pm}}/\varepsilon_{Z^{0}}$ and $\varepsilon_{Z_0^0}/\varepsilon_{Z^{0}}$ are not available by direct spectrophotometric measurement. However, the structural equivalences allow the acceptance of, in principle, the following approximations.

$$(\varepsilon_{Z^{\pm}}/\varepsilon_{Z^{0}}) \simeq (\varepsilon_{Z^{0}}/\varepsilon_{Z^{0}}) = 1 \quad (4)$$

$$(\varepsilon_{Z_0^0}/\varepsilon_{Z^{0}}) \simeq (\varepsilon_{Z_0^{\pm}}/\varepsilon_{Z^{0}}) = y \quad (5)$$

and, then it can be derived

$$\frac{A^{\lambda}}{A^{\infty}}(|H^+| + K_1) - y|H^+| = (k_a + yk_b) + \frac{K_1K_2}{|H^+|}\left(1 - \frac{A^{\lambda}}{A^{\infty}}\right) \quad (6)$$

The values of y may be calculated as a result of dividing the absorbance at pH 7·71 by the absorbance of the solution in KOH 0·16 M.

Thus, taking $pK_1 = 9{\cdot}28$, from equation (6) the values of k_a obtained were $pk_a = 9{\cdot}45 \pm 0{\cdot}02$, $pk_b = 9{\cdot}77 \pm 0{\cdot}04$, pk_c $10{\cdot}14 \pm 0{\cdot}02$, $pk_d = 9{\cdot}81 \pm 0{\cdot}03$.

The useful range of application of equation (6) was from 301 nm (33·2 KK) to 287 nm (34·8 KK), in which an acceptable fit is attained (correlation coefficient = 0·983).

It seems clear that the above mentioned range corresponds to those wavelengths for which the approximations stated in equations (4) and (5) are more closely accomplished.

On the other hand, the results found from the least square method are in good accordance with those obtained from the procedure of deconvolution which it can be considered in supporting the spectral assignments made in this later method.

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A new approach to prostate cancer

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Abstract—Growth of androgen-dependent human prostatic adenocarcinoma implanted in the nude mouse (Honda tumour), is inhibited by 6-methyleneprogesterone. This steroid is a potent inhibitor of both rat and human prostatic 5α-reductase in-vitro. In-vivo, at the studied dose level, it reduces metabolic conversion of testosterone to dihydrotestosterone with minimal effects upon circulating LH and testosterone. These data support the hypothesis that dihydrotestosterone and not testosterone is the main trophic androgen of the human prostatic neoplasm.

Treatment of prostate cancer is based on the hypothesis of Huggins & Hodges (1941) that the tumour depends on testicular androgen for growth so that treatment should be directed towards androgen ablation. To this end castration, either surgical or medical, represents standard therapy (cf. Schultze et al 1987). Although undoubtedly correct in its basic premise of dependence upon androgen secretion of the testis, there is now an impressive body of experimental evidence to show that, in the prostate, testosterone is reduced to dihydrotestosterone (DHT) by the NADPH-dependent enzyme 5α-reductase, and that DHT is the main trophic androgen of the prostate and, by influence, of the tumour (see Petrow 1986).

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In-vitro studies by Petrow & Lack (1981) have shown that 6-methyleneprogestrone (6-MP) is an irreversible inhibitor of rat prostatic 5α-reductase. Its inhibition of the enzyme in human explants of prostatic tissue has been described by Kadohama et al (1983), who additionally demonstrated suppression of metabolic reduction of testosterone to DHT in this model system. MacIndoe et al (1984) have reported 5α-reductase inhibition by 6-MP in homogenates of MCF-7 human breast cancer cells. More recently, Uilenbroek & Woutersen (1988) have found similar inhibition of rat ovarian 5α-reductase activity using both testosterone and progesterone as substrates. 6-MP does not inhibit steroidal aromatase or 3β-hydroxy-5-ene-oxidoreductase (Robertson et al, in manuscript).

In-vivo, 6-MP shows the characteristic biological properties that result from inhibition of the metabolic conversion of testosterone to DHT. Thus it inhibits growth of the prostate in the castrated rat administered testosterone but *not* DHT (Kendle et al, in manuscript), and enforces marked involution of the prostate in the intact male rate without affecting LH levels

Table 1. Effect of 6-methyleneprogesterone* on Honda tumours in nude mice.

	Weight (mean ± s.e.m.)					
Treatment	Honda tumour (mg)	Ventral prostate (mg)	Seminal vesicle (mg)	Testes (mg)	Liver (mg)	Tumour-free carcase (g)
Control	340·25 ± 21·43	6·28 ± 0·37	73·03 ± 11·00	178·68 ± 8·27	961·16 ± 57·80	19·59 ± 1·44
(n)	(4)	(4)	(4)	(4)	(4)	(4)
6-methyleneprogesterone	196·97 ± 19·57	4·88 ± 0·35	35·22 ± 4·66	178·67 ± 10·29	1147·10 ± 54·85	20·74 ± 0·50
(n)	(6)	(6)	(6)	(6)	(6)	(6)
P	< 0·01	< 0·05	< 0·05	n.s.	n.s.	n.s.
% Inhibition	42·1	22·3	51·8			

* 20 mg kg^{-1}, administered as a 0·96% solution in ethanol–sesame oil (1:18).

(Petrow et al 1984; Marts et al 1987; Kendle et al, in manuscript), or, at the 20 mg kg^{-1} dose level, circulating testosterone levels (Kadohama et al 1985; Marts et al 1987). Significantly, these growth-inhibitory effects upon the prostate are carried over to the Dunning R-3327-H prostatic adenocarcinoma implanted in the Copenhagen-Fisher rat (Petrow et al 1984) and in the Noble rat (Kadohama et al 1985). These tumours are generally regarded as appropriate models of human androgen-dependent prostate cancer. These data led Petrow (1986) to develop the hypothesis that human prostate cancer in its androgen-dependent phase depends mainly upon DHT and not testosterone for growth, and that therapy should be directed towards elimination of prostatic DHT with a 5α-reductase inhibitor leaving circulating testosterone levels largely intact. We now report data supporting this hypothesis.

Ito et al (1985) have described a serially transplantable human prostate cancer (Honda tumour) implanted in the nude mouse which *retains* its human biological profile and endocrine dependence. This tumour has been employed in the present study.

Materials and methods

Transplantation of the Honda tumour into the nude mouse was performed as described earlier (Ito et al 1985). Tumour fragments of ca 1 mm diameter were implanted into the flanks of nude mice using a trocar. The mice were treated with 50 μL of 6-MP solution (20 mg kg^{-1}) immediately following transplantation of the tumour and thereafter daily for 30 days. The ethanol-propylene glycol (1:9) medium used previously for dissolving 6-MP (Petrow et al 1984) proved to be extremely irritant to the nude mouse causing deep erosion of skin and muscle. The steroid was consequently administered in ethanol-sesame oil (1:18), but even with this modification, the solution caused redness of the skin and accumulation of sesame oil at the site of injection forcing termination of the experiment after 30 days.

Results and discussion

Table 1 shows that there was a 42% inhibition of growth of the tumour with inhibitory effects upon growth of ventral prostate (22%) and seminal vesicles (52%). There was some reduction in the retention of secretion in the lumen of the seminal vesicles in treated mice. No significant differences were observed between treated and control mice in the histology of the prostates and of the tumours, which retained the histological features of poorly differentiated adenocarcinomas. Prostate specific antigen stained by the peroxidase-antiperoxidase method were essentially the same in treated and control tumours. Tumour-free body weights, and weights of the testes and livers did not differ between treated animals and controls.

These data provide experimental support for the hypothesis that androgen-responsive human prostate cancer depends mainly upon DHT and not testosterone for growth. It follows that 5α-reductase inhibitors may represent a new treatment of androgen-dependent prostate cancer which by-passes the trauma of surgical or medical castration. Finally, as developed elsewhere (Petrow 1986), there is a strong likelihood that 5α-reductase inhibitors can be successfully employed for prophylaxis of the disease in cancer families.

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