Short Communication

HORMONAL STATUS AND STEROID METABOLISM IN TWO TRANSPLANTABLE RAT MAMMARY TUMOURS

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Human and rat mammary tumours metabolize steroid hormones and thus have the capacity to modify their local hormonal environment (Adams & Wong, 1968; Jones et al., 1970; King et al., 1964; Miller et al., 1974). In previous studies, we have investigated the metabolism of testosterone by a predominately ovary-dependent DMBA-induced mammary tumour, and have shown it to be sensitive to levels of other hormones present in vitro (Miller, 1976a), or in vivo (Miller, 1976b, c; Buchan et al., 1976). We now report a study of the metabolism of androgens by ovary-independent tumours derived by transplantation. By using multiple tumours transplanted to a single rat, it has been possible to examine tumours derived from the same host animal before and after 2 endocrine manipulations.

All animals were of an inbred strain of Sprague-Dawley rat, obtained from the Animal Diseases Research Association (ADRA), Moredun Institute, Edinburgh. Two lines of transplantable tumours were used: TG3 and TG5. These were histologically carcinomas, originally induced in female "ADRA" rats at 50 days of age by i.v. administration of 5 mg of DMBA. Both tumours were then serially transplanted by dorsal skin implantation of tumour fragments into neonatally-thymectomized hosts. At the time of study TG3 was at its 6th passage and TG5 was at its 7th passage.

Three separate portions of a single donor tumour were implanted at different dorsal sites in each neonatally thymectomized host animal at 50 days of age. Tumours were measured twice weekly throughout the period of study. When the largest tumour reached 1.5 x 1.5 cm in size it was removed through a dorsal skin incision and the animal was immediately bilaterally oophorectomized. Ten days later, a further tumour was excised. The animals were then given daily injections of oestradiol-17β (1 μg in 0.2 ml corn oil) for a further 10 days.

The remaining tumour was excised and the animals were killed, no injection of oestradiol being given on the day of death. Eight rats were so treated (4 with TG3 tumours and 4 with TG5 tumours). Control animals (2 with TG3 tumours and 2 with TG5 tumours) were treated similarly except that a sham oophorectomy was performed in place of an oophorectomy and the injection vehicle replaced oestradiol.

After excision, portions of each tumour (0.5 g) were used for steroid-metabolism studies. Each was finally sliced at 0°C in Krebs–Ringer phosphate buffer pH 7.4 (5 ml). An NADPH-generating system and radioactive precursor (20μCi of either 7α(3H) dehydroepiandrosterone (DHA) or 7α(3H) testosterone) was added. The incubation systems were shaken at 37°C in an atmosphere of O2 for 2 h (TG3 series) or 1 h (TG5 series—on account of the high activity found in the tumour lines). The reaction was stopped by adding methanol to 80% v/v and the incubations stored at
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-10°C until the metabolites were characterized. To measure losses, 500 μg of each non-radioactive carrier steroid to be investigated was added. The metabolites were then extracted and separated into individual metabolites as described previously (Miller et al., 1974). Purification of all steroid fractions except 5α androstenediol was achieved by sequential acetylation and hydrolysis; 5α androstenediol by sequential oxidation and reduction. Methods for derivative formation and characterization of metabolites have been described previously (Miller et al., 1974). Metabolism and conversion of precursors were measured by determining the incorporation of radioactive label into the appropriate metabolites after correction for losses. 5α reduction of testosterone was estimated by combining the production of 5α dihydrotestosterone with that of 5α androstenediol.

The pattern of tumour growth throughout the endocrine procedures investigated is shown in the Figure. Tumour growth was continuous after oophorectomy, but after the administration of oestradiol there was some evidence for accelerated tumour growth, especially in the TG5 line. Sham oophorectomy and administration of corn-oil vehicle had little effect on tumour growth.

**FIGURE.**—Growth patterns of TG3 and TG5 transplantable tumours. Solid dots are values for experimental animals with oophorectomy followed by oestrogen (OE2). Each point represents mean size of the 4 tumours (each from a separate animal) which were subject to both hormone manipulations, with the exception of the final point for TG5 (mean of 3 tumours; one animal killed on Day 18). Open dots are values for control animals with sham oophorectomy followed by corn oil. Each point represents the mean of 2 tumours (each from different animals) which were subject to both manipulations, with exception of the final point for TG5 (value for a single tumour; one animal killed on Day 18). Bars represent s.e. mean. Day 0 represents day of oophorectomy or sham operation.
TABLE I.—Effects of endocrine status on metabolism of 7α(3H) testosterone by TG3 transplanted tumours

| Group         | No. of rats | Endocrine status of tumour donors                                                                 | % Testosterone precursor  
|---------------|-------------|----------------------------------------------------------------------------------------------------|--------------------------
| Control       | 2           | Intact, Sham oophorectomized 10 days earlier, Sham oophorectomized 20 days earlier, + corn oil for last 10 days | Unmetabolized: 2.6, 4.5 | Metabolized by 5α reduction: 65.2, 61.0 |
|               |             |                                                                                                   |                           |                                          |
| Experimental  | 4           | Intact, Oophorectomized 10 days earlier, Oophorectomized 20 days earlier, + 1 μg oestrogen in corn oil for last 10 days | Unmetabolized: 6.5, 4.3, 3.7, 7.8 | Metabolized by 5α reduction: 57.5, 56.2, 59.5, 75.4 |

TABLE II.—Effects of endocrine status on metabolism of 7α(3H) testosterone by TG5 transplanted tumours

| Group         | No. of rats | Endocrine status of tumour donors*                                                                 | % Testosterone precursor  
|---------------|-------------|----------------------------------------------------------------------------------------------------|--------------------------
| Control       | 2           | Intact, Sham oophorectomized, Sham oophorectomized + corn oil, 14.9, —                              | Unmetabolized: 17.8, 32.0 | Metabolized by 5α reduction: 82.4, 66.1 |
|               |             |                                                                                                   |                           |                                          |
| Experimental  | 4           | Intact, Oophorectomized, Oophorectomized + oestrogen, 33.1, 19.4, 25.2, —                            | Unmetabolized: 25.8, 21.2, 27.4, 26.7 | Metabolized by 5α reduction: 69.7, 74.8, 68.9, 67.0 |

* See Table I for details.

The general histological appearance of both tumour lines was not changed by either oophorectomy or oestrogen administration, in terms of cell number, cell type, necrosis or polyploidy.

The % metabolism of testosterone and its conversion to 5αreduced products by TG3 tumours are shown in Table I. All TG3 tumours, irrespective of the animal from which they were derived or its endocrine status, metabolized large amounts (86–99%) of the testosterone precursor. Despite this high metabolic activity, oophorectomy caused an increase in tumour testosterone metabolism in each animal. This effect was consistently reversed by the administration of oestrogen to the oophorectomized animals. In each animal, oophorectomy was also associated with an increase in tumour 5αreduction of testosterone. Subsequent oestrogen administration to oophorectomized animals then led to decreased tumour production of 5αreduced metabolites, although tumour 5αreduction still remained higher than that in corresponding tumours from the intact individuals. Of the individual 5αreduced metabolites investigated, the effects were most marked on 5αandrostenediol, perhaps because its production was at least twice that of 5αdihydrotestosterone in all TG3 tumours. The rise in 5αreduced metabolites produced by oophorectomy was significantly different from the small change caused by sham ablation in the control group (P<0.05 as based on t test of the logarithms of the ratio between the groups.) Similarly, the fall in tumour 5α-reduction apparent after administration of oestradiol was significantly different.
from the effect of injection vehicle in control animals ($P<0.01$).

The results obtained from incubations of TG5 tumours are presented in Table II. As with the TG3 series, oophorectomy was associated in each animal with an increase in tumour metabolism and 5α-reduction of testosterone; these effects were reversed after administration of oestradiol to the animals. Both 5α dihydrotestosterone and 5α androstenediol were equally affected by treatment. Transformation to both these 5α-reduced metabolites was alone sufficient to account for the changes in testosterone metabolism produced by hormonal manipulation.

The metabolism of 7α[3H]DHA was investigated in duplicate portions of the same TG3 tumours used to study the metabolism of testosterone. The distribution of radioactivity from 7α[3H]DHA after incubation is shown in Table III. The metabolism of this steroid (16–43%) was less extensive than that of testosterone. Oophorectomy and oestrogen treatment failed to influence the overall metabolism of DHA or the production of its major metabolite, Δ5 androstenediol. Metabolism of 7α[3H]DHA was also similar in tumours from control animals subjected to sham oophorectomy followed by injection of vehicle.

As both TG3 and TG5 tumours maintain their histological appearance during serial and multiple transplantations, they provide useful models for studying the effects of sequential operations within individual animals, without the need to take biopsy samples from individual tumours, a procedure which alone may change tumour behaviour. In the present communication we have utilized these tumour lines to study the effects of endocrine manipulation on steroid metabolism.

Compared with ovary-dependent DMBA-induced tumours (Miller, 1976b), both the ovary-independent transplantable tumour lines had a high capacity to metabolize 3H-testosterone, especially by 5α-reduction. In both TG3 and TG5 tumours, oophorectomy was associated with increased metabolism of testosterone, whereas administration of oestradiol reversed the effect. These changes could be accounted for largely by parallel changes in the production of 5α-reduced metabolites. However, it is unlikely that the changes were nonspecific since the overall metabolism of dehydroepiandrosterone and conversion to its major metabolite Δ5 androstenediol were, by contrast, uninfluenced by endocrine manipulation. Furthermore, there were no obvious histological changes in tumour appearance, such as increase in cell number after oophorectomy or cell death after oestradiol treatment, which might directly account for the changes in testosterone metabolism resulting from endocrine manipulation.

The pattern of changes in testosterone metabolism after endocrine manipulation does not differ from that observed previously with DMBA-induced ovary-dependent tumours (Miller, 1976b). Thus the sensitivity of steroid metabolism to endocrine manipulation does not differenti-
ate between tumours dependent on the ovary for their growth and those which are not. However, the transplantable tumours were derived originally from DMBA-induced tumours, and at least one of the lines (TG5) was ovary-dependent in its first and second transplant generations. Since (1) oestrogen receptors are present in the tumours (Hawkins et al., 1978) and (2) tumour growth, whilst not ovary-dependent, may be enhanced by oestradiol-17β administration (Figure), it seems likely that these tumours have retained some hormonal sensitivity during transplantation.

Similar progressive changes in hormone dependency with succeeding transplant generations have been shown for other transplantable tumours (Sluyser & Van Nie, 1974; DeSombre et al., 1976; Horn et al., 1976). The transplantable TG3 and TG5 tumours may thus resemble the transplantable R3230AC tumour, which has been described by Hilf (1972) as ovary-independent but hormone-sensitive.

It is thus possible that the changes in steroid metabolism observed in these transplantable ovary-independent TG3 and TG5 tumours following endocrine manipulation are related, at least in part, to some residual hormonal sensitivity.

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