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URINARY EXCRETION OF PREGNANEDIOL IN POST-MENOPAUSAL WOMEN AND MEN DURING PROLONGED DAILY ADMINISTRATION OF PROGESTERONE*

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SUMMARY

Progesterone (50 mg/day) was administered by intramuscular injection to six intact post-menopausal women and to one man for periods of 13–20 days, and by mouth to one intact post-menopausal woman for 15 days. Determinations of the daily urinary excretion of pregnanediol by these subjects gave results in marked contrast to those of Sommerville & Marrian [1950].

It was reported by Sommerville & Marrian [1950] (see also Sommerville [1950]) that on administering progesterone daily, either by intramuscular injection or by mouth, to intact post-menopausal women for periods up to 27 days, the daily urinary excretion of pregnanediol, as determined by the method of Sommerville, Gough & Marrian [1948], reached a plateau level by the 2nd or 3rd day and then at about the 5th to 8th day rose in a regular step-wise manner to a second plateau level which was maintained for the duration of the experiment. This phenomenon was termed a 'progestosterone "priming" effect'. It was also reported that on administering progesterone daily to men, hysterectomized-ovariectomized women and hysterectomized post-menopausal women no such effect was observed, the pregnanediol excretion being maintained at a virtually constant level during the whole of the experimental period.

There was apparently no doubt about the results, since clear-cut 'priming' was reported in six experiments on intact post-menopausal women, while the complete absence of 'priming' was reported in three experiments on men and in three on hysterectomized women. On the basis of these findings Sommerville & Marrian [1950] stated that 'it has been clearly demonstrated that the enhanced power of the normal post-menopausal woman to excrete urinary pregnanediol after prolonged daily administration of progesterone depends upon the uterus', and that 'the conclusion can hardly be avoided that the post-menopausal uterus after prolonged exposure to the action of progesterone or one of its metabolic products is able to effect the conversion of progesterone into urinary pregnanediol, or at least some part of that conversion'.

The apparent finding of a clear-cut difference in the pattern of the urinary excretion of pregnanediol between intact post-menopausal women on the one hand, and men and hysterectomized women on the other, was unexpected at the time by the senior

* A preliminary account of this work has been given by Marrian, Russell & Atherden [1953].
of the two authors concerned. However, considerable confidence was placed in the validity of the experimental findings in view of the excellent agreement between duplicate determinations and the consistent regularity of the pregnanediol excretion patterns, and it was felt that the experimental results obtained seemed to justify the conclusion that the uterus must be concerned in the conversion of progesterone to pregnanediol.

Slight uncertainty concerning the reliability of the published results first arose when Engel, Patterson, Wilson & Schinkel [1950] obtained no ‘priming’ effect and no plateau level of excretion of progesterone metabolites in an experiment in which urinary ‘steroid alcohols’ were determined daily in a woman with cancer of the breast who was being treated with 100 mg progesterone per day for 10 days. These doubts were increased when Russell [1952] was unable to show any difference in the patterns of urinary pregnanediol excretion between ovariectomized, ovariectomized-hysterectomized, and male rabbits during prolonged daily administration of progesterone by intramuscular injection.

These doubts concerning the validity of the results of Sommerville & Marrian [1950] were still further increased when other results obtained in this laboratory at about the same time could not be confirmed. Sommerville, Marrian, Duthie & Sinclair [1950] (see also Sommerville [1950]) had claimed to have shown conclusively that a considerably greater percentage of intramuscularly administered progesterone was excreted in the urine as pregnanediol by rheumatoid arthritic post-menopausal women and men than by non-arthritic post-menopausal women and men. In further investigations carried out with the cooperation of Drs Duthie and Sinclair of the Northern General Hospital, Edinburgh, Marrian & Atherden [1953] were unable to confirm the findings of Sommerville et al. [1950]. In view of this the desirability of reinvestigating the findings of Sommerville & Marrian [1950] was apparent.

EXPERIMENTAL METHODS

Subjects

The first experimental subjects were four post-menopausal women (L, B, O and D) and one man (H) who were hospitalized for various causes. They were not suffering from renal or hepatic disease.

Although it seemed unlikely that the metabolic fate of the administered progesterone could have been significantly influenced by the clinical states of these particular subjects, this possibility could not be entirely excluded. Accordingly, three further experiments were carried out on post-menopausal women who were leading normal active lives in their own homes (G, V and N). It should be noted that the last of these was one of the experimental subjects who was reported to have shown marked ‘priming’ by Sommerville & Marrian [1950].

Progesterone administration

Since Sommerville & Marrian [1950] reported that the ‘priming’ effect in post-menopausal women was more pronounced when the progesterone was administered by intramuscular injection than when it was given by mouth, intramuscular administration was used in all but one of the experiments in the present work. In the case of subject N oral administration was adopted out of deference to the wishes of the
subject herself, and also because this mode of administration had been employed for this subject in the experiments of Sommerville & Marrian [1950].

In all cases 50 mg progesterone per day were administered, the experimental periods ranging from 13 to 20 days. It should be noted that in each of the six experiments on post-menopausal women reported on by Sommerville & Marrian [1950] there was pronounced 'priming' for several days before the 13th day of progesterone administration.

In the experiments on subjects L, B, O, D, H and N solutions of progesterone in olive oil (20 mg/1-0 ml.) were employed. In those on subjects G and V a commercial preparation of progesterone in an unknown oily vehicle (British Drug Houses, Ltd.) was used.

Collection of urine specimens and determinations of pregnanediol

Complete 24 hr urine specimens were collected with 5 ml. toluene as preservative from each subject for 1 day before the commencement of the progesterone treatment and daily for the whole duration of the latter.

Each urine specimen was made up to a volume of 2500 ml., and pregnanediol determinations in duplicate were carried out on 500 ml. portions by the method of Sommerville et al. [1948].

RESULTS

The results are shown in Figs. 1–4. The double bars at the tops of the columns indicate the results of duplicate determinations. In those instances where there was exact agreement between duplicate determinations, this is indicated by a single bar cut with a vertical line.

In deciding whether any 'priming' effect was demonstrated in these experiments, it is essential to be quite clear concerning the meaning of the term 'priming' effect', as it was used by Sommerville & Marrian [1950]. This term was used to describe a regular step-wise increase in the daily urinary pregnanediol excretion after a preliminary plateau level of excretion which was maintained from about the 2nd to the 3rd day until the 5th to the 8th. Sommerville & Marrian [1950] reported that this regular step-wise increase in the urinary pregnanediol excretion after a preliminary plateau occurred in all six of the post-menopausal women who were studied, while in the four cases in which the administration of progesterone was continued for more than 15 days, the excretion reached a second, higher plateau level, which was maintained.

It will be seen that in the present work no indication whatsoever of 'priming' was observed with the post-menopausal subjects L, B, O, V, G and N (Figs. 1, 3 and 4). While the results from the first three of these must be considered with some reserve, since the women were undergoing hospital treatment for various ailments, no such reserve is necessary in considering the results from subjects V, G and N who were entirely comparable with the post-menopausal subjects studied by Sommerville & Marrian [1950].

The pattern of pregnanediol excretion shown by subject D (Fig. 1) somewhat resembled those described by Sommerville & Marrian (1950). There was a suggestion of a preliminary plateau level of pregnanediol excretion (days 4 and 5), followed by
a brief step-wise increase (days 6 and 7) to a higher plateau level (up to day 11). However, it will be observed that this second plateau level was not maintained. Furthermore it is important to draw attention to the fact that a somewhat similar pattern of pregnanediol excretion was observed in the case of the single man studied (subject H, Fig. 2). In this case there was a brief preliminary plateau level of excretion (days 2–4), followed by a step-wise increase (days 5–7).
Fig. 2. Urinary pregnanediol excretion during prolonged daily administration of progesterone by intramuscular injection to a man (hospitalized).

Fig. 3. Urinary pregnanediol excretion during prolonged daily administration of progesterone by intramuscular injection to intact post-menopausal women (not hospitalized).
Fig. 4. Urinary pregnanediol excretion during prolonged daily administration of progesterone by mouth to an intact post-menopausal woman (not hospitalized).

DISCUSSION

If it is assumed that there were no gross errors in the determination of the daily pregnanediol excretion, the evidence presented here must be accepted as conclusive proof that the 'progesterone “priming” effect', as described by Sommerville & Marrian [1950], is not usually seen in intact post-menopausal women treated daily for long periods with progesterone. The finding that in one case there was a pattern of pregnanediol excretion rather dubiously resembling those described by Sommerville & Marrian [1950] provides no confirmation of the results and conclusions of the latter, since a similar pattern of pregnanediol excretion was also observed in the one male subject studied.

Since the completion of the present work Rothchild [1953] has reported results which are also at variance with those of Sommerville & Marrian [1950]. In this work progesterone was administered intravenously to nine women with cervical carcinoma, who, with one doubtful exception, had had amenorrhoea for some time previously. Only three of these subjects showed patterns of pregnanediol excretion which in any way resembled the 'priming' curves of Sommerville & Marrian [1950], and of these three one had been hysterectomized some years earlier. Rothchild's work is open to the criticism that his experimental subjects were not normal. However, in these women the carcinoma was not in the uterus itself, while the occurrence of withdrawal bleeding after cessation of the progesterone treatment showed that in all cases the functional activity of the uterus was not affected. Furthermore, it was shown that
the pregnanediol excretion in these subjects following a single dose of progesterone was not significantly different from that which was found in comparable experiments on normal women during the preovulatory phase of the menstrual cycle.

It is difficult to find any satisfactory explanation for the discrepancy between the results of the present work and those of Rothchild [1953] on the one hand, and those of Sommerville & Marrian [1950] on the other. Two possible explanations must, however, be considered.

It might be supposed that in the present work and in that of Rothchild [1953] the determinations of urinary pregnanediol were at fault, either because of incomplete collection of urine samples or because of technical incompetence on the part of the analysts concerned. This explanation seems to be a somewhat improbable one for the following reasons: in the present work care was taken to impress upon all experimental subjects the importance of providing complete 24 hr urine specimens, and there was no more reason to suppose that incomplete samples were obtained than there was in the work of Sommerville & Marrian [1950]. Before the commencement of the present work both the accuracy of the method of Sommerville et al. [1948] and the competence of the analysts were rigorously tested by recovery experiments in which known amounts of pure pregnane-3α:20α-diol were added to acid-hydrolysed urine of men and male rabbits. The outcome of these tests was completely satisfactory. Rothchild [1953] also carried out preliminary recovery experiments to test the accuracy of the method, and obtained satisfactory results.

It seems justifiable to conclude that the results of the present work and also those reported by Rothchild [1953] are true ones, and accordingly the possibility must be considered that the consistent finding of 'priming' in intact post-menopausal women and the consistent lack of 'priming' in hysterectomized women and men, which was reported by Sommerville & Marrian [1950], was due to chance in the selection of experimental subjects. While this is not outside the bounds of possibility, it may not be a sufficient explanation of the difference between the earlier and the present investigation.

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REFERENCES
Engel, L. L., Patterson, H. R., Wilson, H. & Schinkel, M. [1950]. J. Biol. Chem. 183, 47.