Urinary Excretion of Pregnanediol in Human Subjects following the Administration of Progesterone and of Pregnane-3α:20α-diol. 2*

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It has been established by the work of many authors that when progesterone is administered for periods of 1–3 days to human subjects in whom endogenous progesterone production is minimal, the amount of pregnanediol subsequently excreted in the urine accounts for only a small proportion (usually < 20 %) of the administered hormone. In the preceding paper (Sommerville & Marrian, 1950) previous work in this field was comprehensively reviewed and it was shown in an extensive series of experiments on normal men, post-menopausal and hysterectomized women that about 9–16 % of intramuscularly injected progesterone is excreted as urinary pregnanediol, and that a slightly higher proportion of the latter is excreted when progesterone is administered orally.

Venning & Browne (1938, 1940) showed that the administration of progesterone during the luteal phase of the menstrual cycle and during pregnancy, at times when 'the process of conversion of progesterone to pregnanediol was already in action', resulted in the excretion of a much higher proportion of the administered hormone as pregnanediol additional to that arising from endogenous sources. More recently, the greater efficiency of conversion of administered progesterone to urinary pregnanediol by the pregnant woman compared to that of women with minimal endogenous progesterone production has been confirmed by Davis & Fugo (1947), who reported that 30–35 % of progesterone injected during early pregnancy was excreted as 'additional' pregnanediol. These authors came to the important conclusion that the 'activity of the corpus luteum must exert some effect on the metabolism of progesterone so that a much greater percentage can be accounted for as the inert metabolite pregnandiol'.

In view of the possibility that the 'activity of the corpus luteum' responsible, according to Davis & Fugo (1947) for this phenomenon, might be the secretion and physiological action of endogenous progesterone, we felt that some light might be thrown on the problem by studying the urinary pregnanediol excretion of human subjects with minimal endogenous progesterone secretion during periods of prolonged daily administration of progesterone. The only earlier workers who appear to have studied urinary pregnanediol excretion during periods of progesterone administration longer than 3 days are Venning & Browne (1940) and Cope (1940). The former workers administered doses of progesterone of about 10 mg./day for 4–8 days, but the pregnanediol recoveries were irregular and showed no definite daily trend. The findings of Cope, on the other hand, were more definite.

Cope injected a case of secondary amenorrhoea with 10, 5, 5, 5 and 5 mg. of progesterone on 5 consecutive days respectively. Only doubtful traces of pregnanediol were recovered from the urine during the first 5 days, but 2·5 mg. was found on the sixth day. In a second experiment 10 mg. of progesterone was injected daily for 5 consecutive days into a case of anovular menstruation. No pregnanediol at all was detected in the urine during the first 3 days, but a total of 4·5 mg. was found in the urine on the fourth and fifth days. Cope suggested that these findings might be explained on the basis of 'a kind of saturation phenomenon comparable to that which is now well known to occur in the excretion of ascorbic acid taken by mouth', and he furthermore predicted that more prolonged daily treatment with progesterone should result in even higher proportions of the administered hormone appearing in the urine as pregnanediol.

In view of the small doses of progesterone injected by Cope and the relative insensitivity of the method of determining urinary pregnanediol which he employed (Venning, 1937, 1938), it seemed doubtful whether very much quantitative significance should be attached to his results. Nevertheless, his work provided an indication that prolonged administration of progesterone might in some way cause a progressive enhancement of the power of the body to convert progesterone into urinary pregnanediol.

* A preliminary account of certain parts of this work was communicated to the Society for Endocrinology on 21 October 1948 and to the first International Congress of Biochemistry, August 1949 (Sommerville & Marrian, 1949).

EXPERIMENTAL METHODS

The selection of subjects for these experiments, the collection of urine specimens and the determination of the pregnanediol in the latter were, except where stated otherwise, as described in the preceding paper (Sommerville & Marrian, 1950).

Progesterone solutions in arachis oil and in ethyl oleate (10–25 mg./ml.) were administered either by intramuscular injection or in gelatin capsules by mouth. Pregnane-3α:20α-diol was administered in solution in arachis oil in capsules by mouth.

RESULTS

Urinary excretion of 'additional' pregnanediol following the administration of progesterone during pregnancy

In the first instance confirmation was sought of the findings of Venning & Browne (1940) and of Davis & Fugo (1947) that the percentage conversion of administered progesterone into urinary pregnanediol is higher in pregnant women than in human subjects with minimal endogenous progesterone production. This was considered to be necessary, since neither of these groups of workers had provided convincing evidence to show that the endogenous pregnanediol excretion during the control periods in their experiments had been sufficiently constant to justify the calculation of the 'additional' urinary pregnanediol formed from the administered progesterone.

The subject was a woman at the twenty-seventh week of her eighth normal pregnancy who was suffering from mitral stenosis. Since the patient was confined in a busy antenatal hospital ward, reliance could not be placed on the completeness of the collection of 24 hr. urine samples. Accordingly, the creatinine content of the urine collected each day was determined by the method of Folin (1914) and the pregnanediol values corrected to the average creatinine value. In calculating the average creatinine excretion, determinations on the few urine specimens which were obviously incomplete were ignored.

The experiment was carried out over five consecutive 5-day periods as detailed below:

Period 1. Control period for the determination of daily endogenous pregnanediol excretion: total prenanediol excretion = 94·2 mg.

Period 2. Experimental period: 60 mg. progesterone injected on first and second days: total pregnanediol excretion (endogenous + 'additional') = 138·2 mg.

Period 3. Control period as period 1: total pregnanediol excretion = 98·4 mg.

Period 4. Experimental period: 60 mg. progesterone injected on first and second days: total pregnanediol excretion (endogenous + 'additional') = 158·2 mg.

Period 5. Control period as periods 1 and 3: total pregnanediol excretion = 112·4 mg.

The results are shown graphically in Fig. 1. It will be seen that the daily endogenous pregnanediol excretions during each of the three control periods were remarkably constant. It was therefore felt to be justifiable to assume for the purpose of calculating the 'additional' pregnanediol excreted during the two experimental periods that the endogenous pregnanediol excretion during period 2 would be fairly

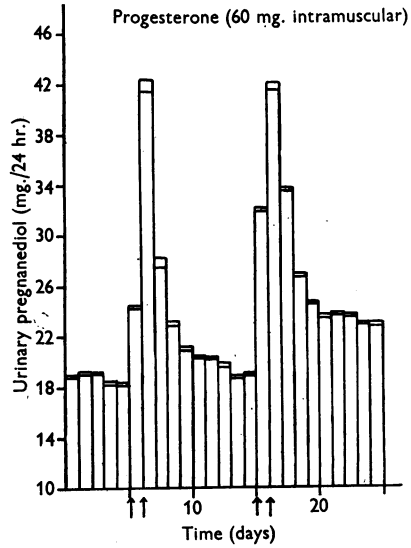

Fig. 1. Effect of progesterone on the excretion of pregnanediol during pregnancy.

represented by the average of those during periods 1 and 3, while that during period 4 would be the average of those during periods 3 and 5. Thus the 'additional' pregnanediol excreted during period 2 is equal to

$$138{\cdot}2 - \left(\frac{94{\cdot}2 + 98{\cdot}4}{2}\right)$$

= 41·9 mg.

= 34·9 % of the administered progresterone,

and the 'additional' pregnanediol excreted during period 4 is equal to

$$158{\cdot}2 - \left(\frac{98{\cdot}4 + 112{\cdot}4}{2}\right)$$

= 52·8 mg.

= 44·0 % of the administered progesterone.

These results clearly confirm the earlier findings of Venning & Browne (1940) and of Davis & Fugo (1947). Provided, therefore, that the administration of progesterone does not stimulate the secretion of endogenous progesterone, it can be concluded that a much higher percentage of administered progesterone is converted into urinary pregnanediol by the pregnant woman than by human subjects in whom the endogenous secretion of progesterone is minimal.

Urinary pregnanediol excretion during continued daily administration of progesterone to normal post-menopausal women

Preliminary experiments on pregnanediol excretion during continued daily administration of progesterone were carried out upon two post-menopausal women. One (F.) received 60 mg. progesterone per day intramuscularly for 10 days, while the other (N.) received the same daily dose administered orally for 15 days. The results are shown

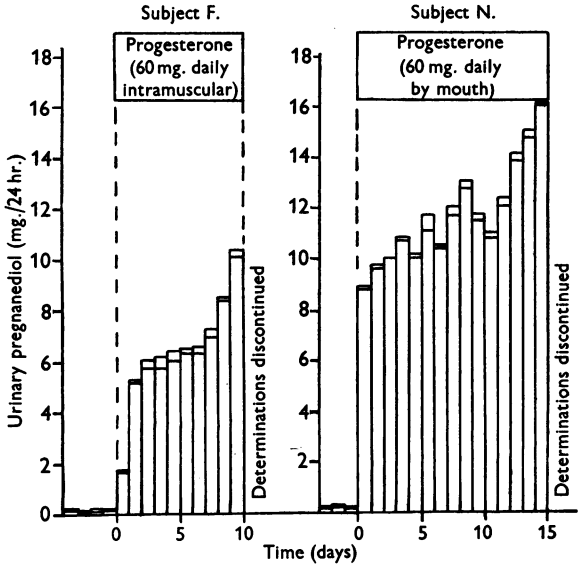

Fig. 2. Pregnanediol excretion during prolonged administration of progesterone to normal post-menopausal women.

graphically in Fig. 2. It will be seen that in subject F. the daily pregnanediol excretion was maintained at an almost constant 'plateau' level, corresponding to about 10 % of the daily dose of progesterone, between the third and eighth days. The pregnanediol excretion then rose sharply, and by the tenth day, when the experiment was discontinued, it had reached a value corresponding to 17 % of the daily dose of progesterone. It will also be seen that a similar phenomenon occurred in subject N. who received the progesterone orally. In this experiment the pregnanediol excretion was somewhat irregular, but it will be clear that after reaching a rather ill-defined plateau level corresponding to about 17 % of the daily dose of progesterone (second to sixth day), the pregnanediol excretion rose to 25 % of the daily dose of progesterone by the fifteenth day. These preliminary results indicated that in post-menopausal women treated with progesterone, either by injection or orally, the power to convert the administered hormone into urinary pregnanediol becomes considerably enhanced after about 6–8 days of the treatment.

In order to confirm this interesting finding and to see whether a second 'plateau' of pregnanediol excretion would ultimately be obtained four further experiments of longer duration were carried out upon three more post-menopausal women. The details of these experiments and the results obtained are shown in Table 1 and Fig. 3.

It will be seen from Fig. 3 that in every experiment the daily pregnanediol excretion rose considerably after reaching a temporary initial 'plateau' level and ultimately attained a second 'plateau'. The results of the two preliminary experiments were thus amply confirmed and extended. This rise in pregnanediol excretion from the initial 'plateau' level will hereafter be referred to as a 'priming' effect.

It will be seen from Table 1 that the 'priming' effect, expressed as the percentage increase of the final 'plateau' level above the initial 'plateau' level, was greatest in the experiment in which the progesterone was administered intramuscularly. It may also be pointed out that in the three experiments in which oral administration was employed, a dose of 60 mg./day produced a greater 'priming' than was obtained with 40 mg./day. As will be seen later, these observations may be of some significance.

Urinary pregnanediol excretion during continued daily administration of progesterone to normal men

The fact that marked 'priming' was observed in post-menopausal women receiving progesterone orally suggested at first that the effect was probably not associated in any way with the physiological action upon the uterus, since it is generally recognized that orally administered progesterone shows only a small fraction of the physiological activity, as judged by the rabbit uterus test, of that shown by the injected hormone. Furthermore, previously reported experiments (Sommerville & Marrian, 1950) in which progesterone was administered by injection on 2 successive days to post-menopausal and to hysterectomized women had suggested that the post-menopausal uterus might have no significant role in the conversion of administered progesterone into urinary pregnanediol. It was confidently expected, therefore, that a 'priming' effect, quantitatively similar to that observed in post-menopausal women, would be obtained in men.

Experiments were therefore carried out on three normal men as follows:

Subject M., aged 30; 40 mg. progesterone per day orally for 18 days.

Subject R., aged 62; 60 mg. progesterone per day orally for 18 days.

Subject P., aged 22; 60 mg. progesterone per day orally for 18 days.

Table 1. *Urinary pregnanediol in post-menopausal women during prolonged treatment with progesterone*

Subject	Daily dose of progesterone and route of administration (mg.)	Duration of progesterone treatment (days)	Pregnanediol excretion: Initial plateau (as % of daily dose of progesterone)	Pregnanediol excretion: Final plateau (as % of daily dose of progesterone)	Pregnanediol excretion: Increase (final plateau—initial plateau, as % of initial plateau)
H.	50 (intramuscular)	22	10·0	22·5	125
H.	60 (oral)	21	14·0	24·0	71
D.	40 (oral)	22	25·5	36·0	41
G.	40 (oral)	27	15·0	20·0	33

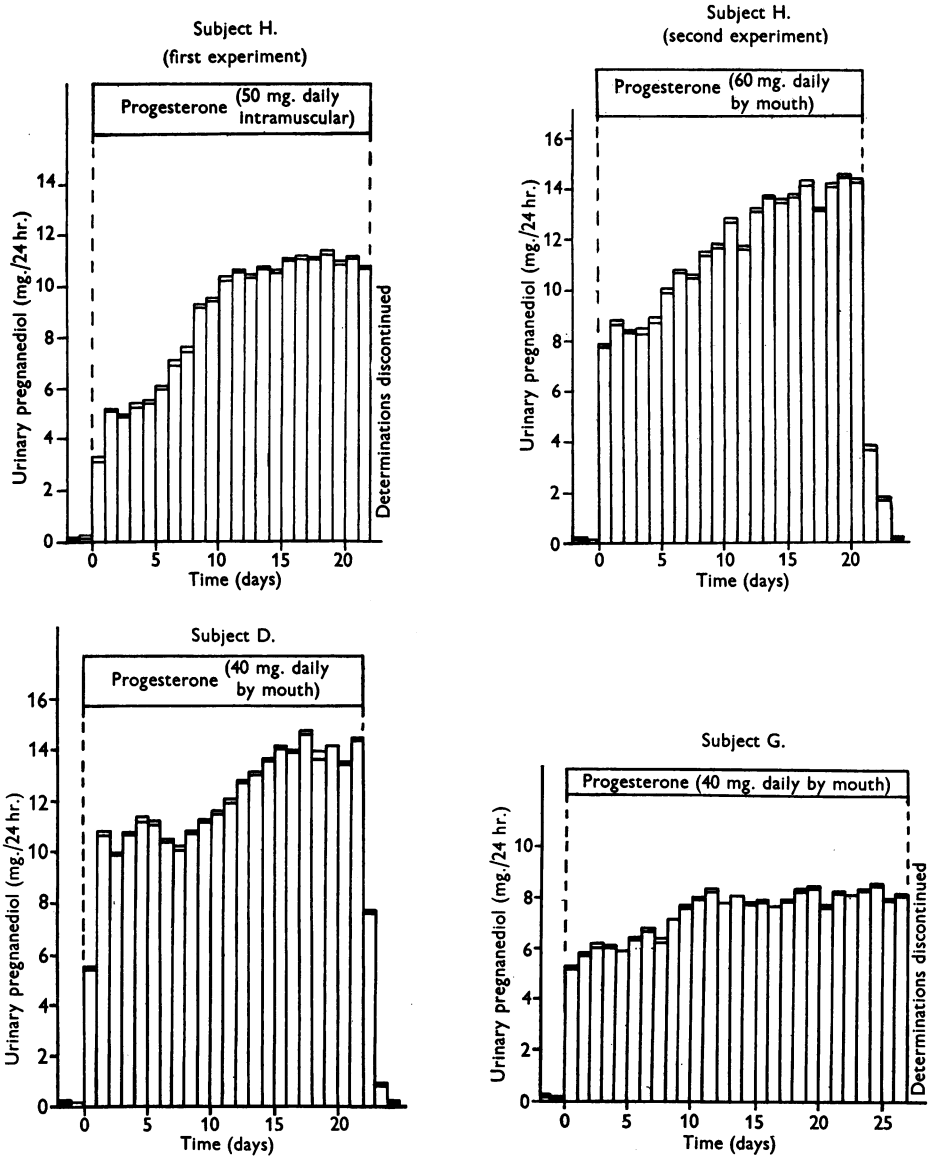

Fig. 3. Pregnanediol excretion during prolonged administration of progesterone to normal post-menopausal women.

The results, which are shown graphically in Fig. 4, clearly show that no 'priming' whatsoever occurred in these three men.

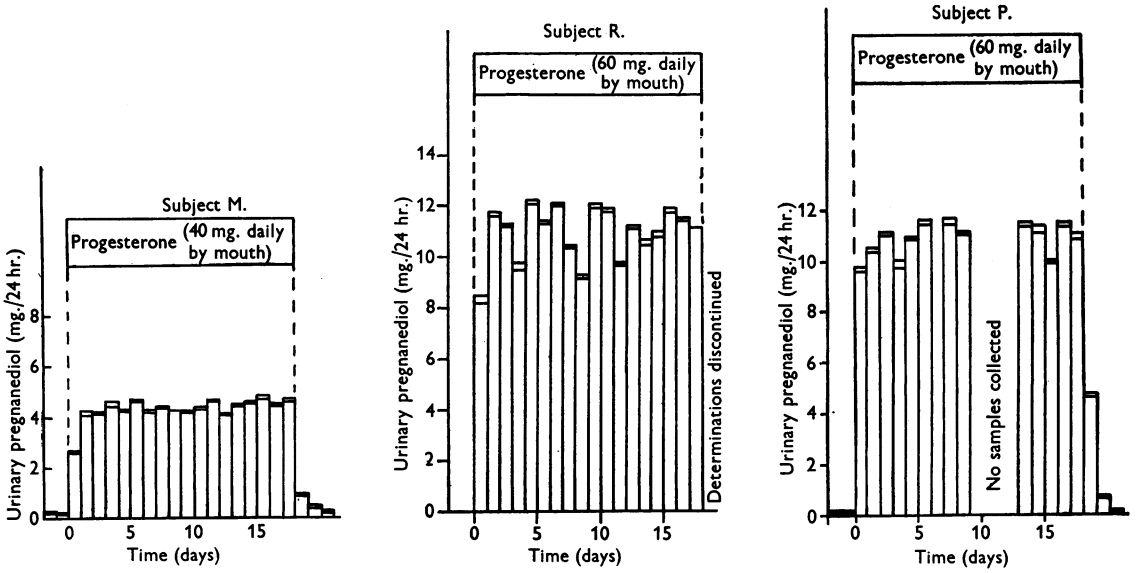

Fig. 4. Pregnanediol excretion during prolonged administration of progesterone to normal men.

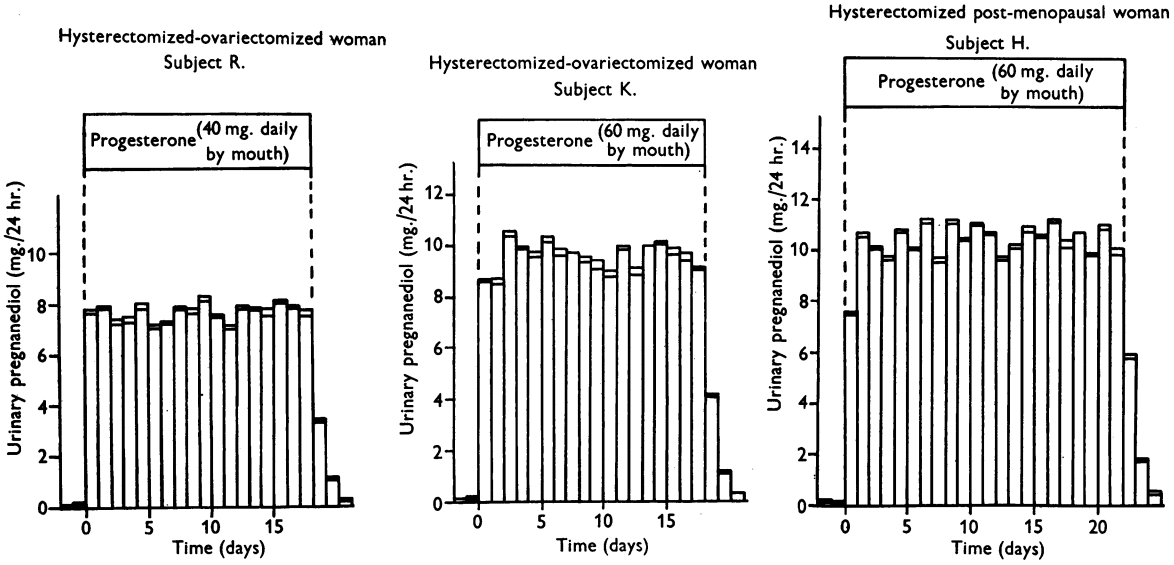

Fig. 5. Pregnanediol excretion during prolonged administration of progesterone to hysterectomized-ovariectomized and hysterectomized post-menopausal women.

Urinary pregnanediol excretion during continued daily administration of progesterone to hysterectomized (ovariectomized or post-menopausal) women

The unexpected lack of any 'priming' effect in men suggested the possibility that although the post-menopausal uterus may have no role in the conversion of administered progesterone to urinary pregnanediol in experiments of only a few days' duration, it might nevertheless be responsible for the 'priming' observed in post-menopausal women after administration of progesterone for longer periods.

Accordingly, experiments as detailed below were carried out upon three women who had been hysterectomized not less than 2 months previously.

Subject R., aged 42; hysterectomized, ovariectomized; 40 mg. progesterone per day orally for 18 days.

Subject K., aged 45; hysterectomized, ovariectomized; 60 mg. progesterone per day orally for 18 days.

Subject H., aged 50; hysterectomized, post-menopausal; 60 mg. progesterone per day orally for 22 days.

The results shown in Fig. 5 indicate quite definitely the complete lack of any 'priming' in these three cases. There can be little doubt therefore that the uterus is necessary for the progesterone 'priming' effect observed in post-menopausal women.

The results, shown in Fig. 6, indicate clearly that no 'priming' occurred as the result of continued daily administration of pregnane-3α:20α-diol.

DISCUSSION

It is clear from the previous work of others and of the present authors that the uterus is not essential for the conversion of administered progesterone into urinary pregnanediol; nor does it seem that the normal post-menopausal uterus plays any significant role in the conversion process when progesterone is administered over periods of only a few days (cf. Sommerville & Marrian, 1950). In the present

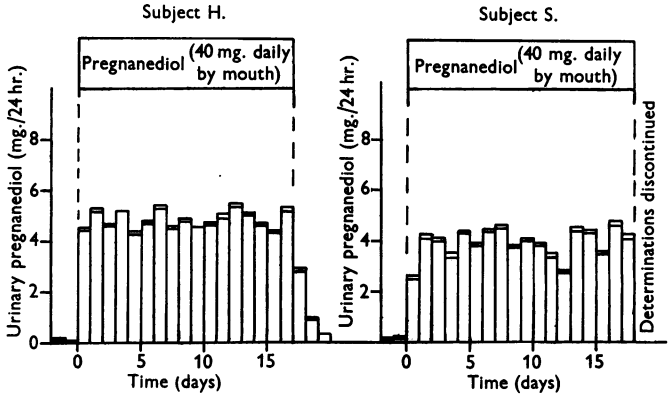

Fig. 6. Pregnanediol excretion during prolonged administration of pregnane-3α:20α-diol to normal post-menopausal women.

Urinary pregnanediol excretion during continued daily administration of pregnane-3α:20α-diol to normal post-menopausal women

In view of the possibility, which is discussed later in this paper, that the 'priming' phenomenon in post-menopausal women may be associated with the action of progesterone in causing structural changes in the uterus, it seemed of importance to test for 'priming' activity related steroids which are known or suspected to be progesterone metabolites, but which lack the physiological activity of the hormone. Unfortunately, owing to the lack of sufficient quantities of other possible progesterone metabolites, pregnane-3α:20α-diol is the only one of these steroids which could be tested.

Experiments, as detailed below, were carried out on two normal post-menopausal women, one of whom (H.) had previously shown definite 'priming' with progesterone.

Subject H., 40 mg. pregnane-3α:20α-diol per day orally for 17 days.

Subject S., 40 mg. pregnane-3α:20α-diol per day orally for 18 days.

work, however, 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. Accordingly, the conclusion can hardly be avoided that the post-menopausal uterus after prolonged exposure to the action of progesterone or of one of its metabolic products is able to effect the conversion of progesterone into urinary pregnanediol, or at least some part of that conversion.

At the present time there is no definite evidence to show whether this 'priming' effect of progesterone is, or is not, associated with its physiological action in inducing structural changes in the uterine endometrium and myometrium. The observation that the 'priming' was greater with intramuscularly than with orally administered progesterone, and the observation that in the three oral administration experiments the higher dose gave the greatest 'priming', suggest that perhaps this may be the case. However, the figures at present available are obviously too few to constitute clear-cut evidence in favour of this view. Furthermore, it may be

doubted whether 40 mg. of progesterone per day administered orally without oestrogen would induce any structural changes in the uterine endometrium or myometrium.

The observation that the oral administration of pregnane-3α:20α-diol gave no 'priming' in post-menopausal women is compatible with the theory that the effect may be due to progesterone *per se*. The possibility must be borne in mind, however, that 'priming' might result from other progesterone metabolites which have not yet been tested.

It is possible, but by no means certain, that the increased excretion of pregnanediol observed by Cope (1940) after 5–6 days administration of progesterone may have been due to the same 'priming' phenomenon which is reported here. The possibility that the 'priming' effect might be due to a 'saturation phenomenon', such as was suggested by Cope (1940), must therefore be briefly considered.

The fact that in men and in hysterectomized women a steady 'plateau' of pregnanediol excretion is established within 2–3 days after the commencement of progesterone administration and is maintained for periods up to 22 days indicates that there can certainly be no 'saturation phenomenon' in the absence of the uterus. It is, furthermore, unlikely for two reasons that the 'priming' in normal post-menopausal women could be due to a saturation of the uterine tissue with progesterone and/or its metabolites. First, if such a saturation were to occur, then in experiments of short duration the pregnanediol excretion should be relatively lower in normal post-menopausal women than in hysterectomized women. As has been previously shown, however, the presence or absence of the uterus seems to have little effect on the excretion of pregnanediol in experiments in which progesterone is administered for 2 days only (Sommerville & Marrian, 1950). Secondly, if the 'priming' effect were due to saturation of the uterine tissue with progesterone and/or its metabolites, one would expect that the clearance of pregnanediol, as judged by its rate of disappearance from the urine after stopping the administration of progesterone, would be delayed in the experiments of long duration in comparison with those of short duration. No such delay in pregnanediol clearance in experiments of long duration was in fact observed (see Fig. 3, Subject H., second experiment).

It is not unlikely that the relatively high percentage conversion of administered progesterone into 'additional' urinary pregnanediol which occurs during pregnancy and also possibly during the luteal phase of the menstrual cycle may be due to a 'priming' of the uterus with endogenous progesterone. At the present time, however, it would be premature to assume that the two phenomena are necessarily due to the same cause.

SUMMARY

1. Previous findings by Venning & Browne (1940) and Davis & Fugo (1947) that the pregnant woman can convert a higher proportion of administered progesterone into urinary pregnanediol than can human subjects in whom endogenous progesterone production is minimal have been confirmed.

2. It has been shown that when progesterone is administered daily (intramuscularly or orally) to normal post-menopausal women for periods up to 27 days, the daily urinary pregnanediol excretion reaches on the second or third day a preliminary plateau level which is maintained until the fifth to eighth day. The daily pregnanediol excretion then rises and at about the twelfth to sixteenth day reaches a second plateau which is then maintained. This phenomenon has been termed a 'progesterone "priming" effect'.

3. No 'priming' effect was observed when progesterone was administered daily to normal men, hysterectomized-ovariectomized and hysterectomized post-menopausal women. It has been concluded, therefore, that the uterus is necessary for the manifestation of progesterone 'priming'.

4. No 'priming' effect was observed when pregnane-3α:20α-diol was administered daily (orally) to normal post-menopausal women.

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