Urinary Excretion of Pregnanediol* in Human Subjects following the Administration of Progesterone and of Pregnane-3 α :20 α -diol. 1

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Since the establishment by Venning & Browne in 1937 of the status of pregnane-3 α :20 α -diol as a metabolic reduction product of progesterone, many figures have been accumulated by different workers on the urinary excretion of pregnanediol in human subjects of both sexes after the injection of the hormone. A critical examination of these figures, however, reveals a somewhat unsatisfactory state of affairs. While there is some measure of agreement that in the majority of human subjects, excluding women during the luteal phase of the menstrual cycle and during pregnancy, the pregnanediol appearing in the urine after the injection of progesterone amounts to approximately 10% of the amount of the hormone administered, wide variations from this rough average have also been reported, and in no one paper are there sufficient figures from any one type of human subject to enable conclusions to be drawn concerning the normal variations which may occur in the conversion of progesterone into urinary pregnanediol. Furthermore, without exception, methods of determining urinary pregnanediol have had of necessity to be employed which are of doubtful accuracy at the low levels of pregnanediol excretion usually encountered in such progesterone metabolism experiments.

These considerations, and the availability of a method for determining urinary pregnanediol, which was believed to be more accurate at pregnanediol excretion levels below about 10 mg./24 hr. than any that had hitherto been described (Sommerville, *et al.* 1948), led the present authors to reinvestigate the conversion of administered progesterone into urinary pregnanediol in human subjects.

In the course of the work described in the present paper the urinary pregnanediol excretion has been studied after the administration of progesterone on 2 successive days to normal men, to normal post-menopausal women with and without pre-treatment with oestrogens, and to hysterectomized women; a comparison has been made in men and in post-

menopausal women between the pregnanediol excreted after intramuscularly and orally administered progesterone; and the pregnanediol excretion following the oral administration of pure pregnane-3 α :20 α -diol to normal men has been determined.

The next paper in this series (Sommerville & Marrian, 1950) describes experiments in which progesterone and pregnane-3 α :20 α -diol were administered to human subjects over long periods.

EXPERIMENTAL METHODS

Progesterone dissolved in arachis oil (10 mg./ml.) was administered on 2 successive days, either by intramuscular injection or in gelatin capsules by mouth. Pregnane-3 α :20 α -diol was administered in solution in arachis oil in capsules by mouth. Subjects for the experiments were selected with great care, particular attention being paid to their willingness to co-operate in the collection of accurate 24 hr. specimens of urine.

Urine specimens (24 hr.) were collected in containers containing 5 ml. of toluene from the subjects in their own homes during a control period of several days prior to the administration of the steroid, during the 2 days of the administration and for the following 5–7 days. Specimens, volumes of which were less than 2500 ml., were made up to that volume with water before withdrawing two 500 ml. samples for the determination in duplicate of the pregnanediol content by the method of Sommerville *et al.* (1948). In the few instances where the 24 hr. volumes were greater than 2500 ml., determinations were carried out on two 500 ml. samples and the appropriate volume correction subsequently applied.

The percentage of administered progesterone, or pregnane-3 α :20 α -diol excreted as urinary pregnanediol, was calculated after correction of the apparent pregnanediol excretion for the control period 'blank' value.

RESULTS

Pregnanediol excretion by post-menopausal women, hysterectomized women and men injected with progesterone

Determinations of urinary pregnanediol excretion in healthy post-menopausal women injected with progesterone do not seem to have been previously reported, but observations have been reported on other types of women with minimal endogenous progesterone production. Thus Venning & Browne

* The term 'pregnanediol' is used to denote the material in urine consisting largely, but not necessarily entirely, of pregnane-3 α :20 α -diol, which is determined by any of the widely used quantitative procedures such as those of Venning (1937, 1938), Astwood & Jones (1941), Guterman (1944, 1945), and Sommerville, Gough & Marrian (1948).

(1938) recovered 12% of the administered dose as pregnanediol in the urine of an ovariectomized woman. Cope (1940) found a 6% recovery in a case of hypoplasia of the uterus and 9% in a case of an-ovulatory menstruation, while Jones & TeLinde (1941) report a recovery of 11.5% in the pre-ovulatory phase of the menstrual cycle.

Venning & Browne (1938) first reported that pregnanediol (as the glucuronide) could not be recovered from the urine after progesterone administration to two hysterectomized women. Later, however, these workers (Venning & Browne, 1940) recovered 9.6% after administering a larger dose of progesterone to a woman in whom a supravaginal hysterectomy had been performed. Using the same scheme of dosage, 30 mg. on 3 consecutive days, Buxton (1940) recovered 4.1 and 5.4% from two hysterectomized women, but failed to recover any pregnanediol at all from two similar cases. Following the administration of 40 mg. of progesterone on 3 consecutive days, Jones & TeLinde (1941) reported recoveries of 15.6,

2 consecutive days to six healthy post-menopausal women, three young men and four hysterectomized women. The experiment was repeated on four occasions in one subject (P.). The results are shown in Table 1.

In all these experiments the proportion of the administered progesterone recovered in the urine as pregnanediol ranged from 9.1 to 16.0%. There was no clear evidence that the percentage recovery was greater in the normal post-menopausal women than in the hysterectomized women and men. It can therefore be concluded that under these particular experimental conditions the post-menopausal uterus plays no significant role in the conversion of administered progesterone into urinary pregnanediol glucuronide. As will be shown in the following paper (Sommerville & Marrian, 1950), however, the uterus of the post-menopausal woman does seem to take a prominent part in the process when subjects are treated daily with progesterone for periods of longer than about 5-8 days.

Table 1. *Urinary pregnanediol after the administration by intramuscular injection of progesterone to post-menopausal women, hysterectomized women and men*

(Progesterone (60 mg.) injected on each of 2 successive days.)

Subject	Age	Type	Percentage of administered progesterone recovered as urinary pregnanediol
E.	65	Normal post-menopausal woman	15.6
H.	70	Normal post-menopausal woman	12.1
McA.	55	Normal post-menopausal woman	13.7
A.	70	Normal post-menopausal woman	15.6
N.	71	Normal post-menopausal woman	16.0
Mc.	60	Normal post-menopausal woman	15.2
M.	68	Hysterectomized post-menopausal woman	13.3
H.	50	Hysterectomized post-menopausal woman	14.1
R.	52	Hysterectomized ovariectomized woman	14.9
L.	42	Hysterectomized ovariectomized woman	9.1
P.	22	Normal man (four experiments)	<div style="display: flex; align-items: center;"> <div style="font-size: 3em; margin-right: 5px;">{</div> <div> 10.4 10.0 10.8 10.0 </div> </div>
J.P.	23	Normal man	14.7
R.	22	Normal man	9.3

12.9 and 10.9% of urinary pregnanediol from three hysterectomized women.

The excretion of urinary pregnanediol after the administration of progesterone to men was first reported by Buxton & Westphal (1939) who recovered 17% from a normal male and very variable amounts from two men with Addison's disease. Hamblen, Cuyler & Hirst (1940) reported a variation in the proportion excreted as pregnanediol of from 0 to 42.5%, and observed a marked variation in the proportion so excreted when the same subject was investigated on several occasions.

In the present work 60 mg. of progesterone was administered by intramuscular injection on each of

It should be noted that, contrary to the findings of Hamblen *et al.* (1940), repetitions of the experiment in the same subject (P.) gave almost identical results.

Pregnanediol excretion by men and post-menopausal women after oral and intramuscular administration of progesterone

Dorfman, Ross & Shipley (1948) reported the excretion of urinary pregnanediol after the oral administration of progesterone to two men, one with diabetes and the other with Addison's disease. Further observations do not seem to have been reported on oral administration of progesterone, nor

has a comparison been made between the proportion of administered progesterone excreted as urinary pregnanediol after oral and intramuscular administration.

In the present work, progesterone (60 mg. on each of 2 successive days) was administered in capsules by mouth to the three men and to two of the post-menopausal women investigated in the previous

seen that in these cases, which were typical, oral administration resulted in an earlier and more rapid excretion of pregnanediol than that which followed intramuscular injection. This suggests that the absorption of progesterone in oily solution from the gastro-intestinal tract is more rapid than it is from the site of intramuscular injection.

Fig. 1. Urinary pregnanediol excretion after the administration of progesterone to a normal man (subject P.) by intramuscular and oral routes. Arrows indicate administration of 60 mg. progesterone. The values obtained in the duplicate determinations are indicated by the double lines at the tops of the columns.

Fig. 2. Urinary pregnanediol excretion after the administration of progesterone to a post-menopausal woman (subject H.) by intramuscular and oral routes. Arrows indicate administration of 60 mg. progesterone. The values obtained in the duplicate determinations are indicated by the double lines at the top of the columns.

series. The recovery experiment was repeated on three occasions in one of the male subjects.

In Figs. 1 and 2 the rate of excretion of pregnanediol following the oral administration of progesterone is compared with that following intramuscular injection of the hormone in one male and in one post-menopausal female subject. It will be

In Table 2 the pregnanediol recoveries following the oral administration of progesterone to the three male and two female subjects are summarized and compared with those obtained from the same subjects after intramuscular injection. Particular attention is drawn to the following two points: (i) the pregnanediol recoveries following oral administration of

Table 2. Urinary pregnanediol after the administration of progesterone to men and to post-menopausal women by oral and intramuscular routes

(Progesterone (60 mg.) administered on each of 2 successive days.)

Subject	Age	Type	Percentage of administered progesterone recovered as urinary pregnanediol	
			Oral	Intramuscular
P.	22	Normal man	12.8	10.4
R.	22	Normal man	11.5	9.3
J.P.	23	Normal man (three experiments)	18.6	14.7
			17.4	
			19.6	
N.	71	Post-menopausal woman	18.0	16.0
H.	70	Post-menopausal woman	14.5	12.1

progesterone on three separate occasions to the same subject (J.P.) were virtually the same; (ii) the recoveries following oral administration were in each case slightly higher than the recoveries in the same subjects following intramuscular injection. This latter finding is of some importance in connexion with the use of urinary pregnanediol determinations for clinical diagnostic and prognostic purposes. In view of the well-established fact that the physiological activity of orally administered progesterone, as judged by the rabbit-uterus test, is very small in comparison with its activity by intramuscular injection, this new finding suggests that the amount of pregnanediol in the urine may have little or no quantitative relationship to the amount of endogenous progesterone acting on the target organs.

Pregnanediol excretion after the administration of progesterone to post-menopausal women pre-treated with oestrogen

The evidence in the literature concerning the effect of oestrogen treatment on the urinary excretion of pregnanediol following the administration of progesterone to women is conflicting. Venning & Browne (1940) reported that, in two cases of amenorrhoea with hypoplastic endometria, virtually no pregnanediol could be recovered from the urine. However, after pre-treatment with oestradiol benzoate for several days the same subjects were found to excrete 12 and 18% of the administered hormone as pregnanediol. Venning & Browne (1940) suggested that this effect upon the pregnanediol excretion was due to the 'building up' of the endometrium by the oestrogen. Contrary results were reported shortly afterwards by Cope (1940). In experiments on a similar case he found that pre-treatment with oestradiol benzoate resulted in a slight diminution (6-3%) of the proportion of administered progesterone excreted in the urine as pregnanediol. Evidence of a different character has been advanced by Smith & Smith (1940) to suggest that oestrogen increases the proportion of administered progesterone converted into urinary pregnanediol. They claimed that in toxæmic and diabetic pregnant women progesterone administered alone resulted in a smaller excretion of pregnanediol additional to that arising from endogenous sources than occurred when progesterone was injected together with oestradiol benzoate.

In the present work the problem has been studied in three post-menopausal women with prolapse of the uterus. In two of these (H. and B.) 60 mg. of progesterone was injected intramuscularly on each of 2 successive days, while in the third (K.) the dose was 120 mg./day. The progesterone administration was preceded by the injection of 30 mg. of oestradiol benzoate/day for 6 successive days with subjects H. and B., and for 20 successive days with subject K. The experiment was followed by total hysterectomy

in subjects H. and B. and by endometrial biopsy in subject K. Histological section revealed an atrophic endometrium in H., a proliferative endometrium with foci of secretory glands in B. and a proliferative endometrium in K.

The proportions of administered progesterone excreted as pregnanediol were 16.1, 16.2, 15.1% respectively in the three cases. These proportions do not appear to be significantly different from those obtained with post-menopausal women receiving no oestrogen pre-treatment (see Table 1). It may be concluded from these results that under the conditions of these experiments the administration of oestradiol benzoate did not significantly affect the conversion of the administered progesterone into urinary pregnanediol; furthermore, the results suggest that the state of the uterine endometrium had little influence on the conversion. The present authors do not, however, regard the matter as being in any way settled, since it is not improbable that under different experimental conditions oestrogen may influence the conversion of administered progesterone into urinary pregnanediol. It is clear that a final decision on this problem must await the results of further work.

Urinary pregnanediol excretion in men following the oral administration of pregnane-3 α :20 α -diol

Venning & Browne (1938) were unable to recover pregnanediol glucuronide from the urine of two hysterectomized women after the injection either of progesterone or of pregnane-3 α :20 α -diol. However, when sodium pregnanediol glucuronide was injected into the same subjects, 58 and 43% was recovered in the urine. In view of these findings it is surprising that further studies on the urinary excretion of pregnanediol following the administration of pregnane-3 α :20 α -diol have not been attempted. To the present authors it seemed that such a study might be informative.

Because of the low solubility of pregnane-3 α :20 α -diol in oil it was not practicable to administer sufficient by injection to give an accurately measurable urinary excretion. Accordingly, administration in oil solution by mouth was adopted. Solutions containing 2.4-3.0 mg./ml. were obtained by dissolving weighed amounts in minimal volumes of boiling ethanol, adding arachis oil, boiling off the excess of ethanol in a water bath and then making up to volume with more arachis oil. Such solutions were administered in measured amounts by mouth in capsules on 2 successive days to three young male subjects whose pregnanediol excretions following the oral administration of progesterone had previously been determined. The results are shown in Table 3. For comparison, the pregnanediol recoveries following the oral administration of progesterone to these subjects are also included in this table.

Table 3. *Urinary excretion of pregnanediol following the oral administration of pregnane-3 α :20 α -diol to men on 2 successive days*

Subject	Recovery of pregnanediol following the oral administration of progesterone (%)	Pregnane-3 α :20 α -diol administered orally (mg.)	Recovery of pregnanediol following the administration of pregnane-3 α :20 α -diol (%)
R.	12.8	2 $\times$ 48	12.3
P.	18.6	2 $\times$ 56	16.3
J. P.	11.5	2 $\times$ 59	13.4

It is seen that the recoveries of pregnanediol in the urine following the oral administration of pregnane-3 α :20 α -diol are very similar to those obtained in the same subjects following the oral administration of progesterone. So similar are the recoveries in the two sets of experiments that it is tempting to speculate that administered progesterone may be largely reduced to pregnanediol in the body, and that the low recoveries of urinary pregnanediol, following administration of the hormone, may be due to excretion of the reduction product by other routes or further metabolism into products so far unidentified or to a combination of both processes.

SUMMARY

1. Following the intramuscular injection on 2 successive days of progesterone into normal men,

normal post-menopausal women and hysterectomized women, 9–16 % of the amount injected is subsequently excreted in the urine as pregnanediol. There is no evidence from these experiments that the post-menopausal uterus plays any significant role in the conversion of progesterone into urinary pregnanediol glucuronide.

2. The urinary excretions of pregnanediol following oral administration of progesterone to normal men and normal post-menopausal women were slightly higher than those observed in the same subjects following intramuscular administration of progesterone.

3. No evidence was obtained to support the view that oestrogen pre-treatment of women with atrophic endometria increases the proportion of administered progesterone excreted in the urine as pregnanediol.

4. Following the oral administration of pregnane-3 α :20 α -diol to normal men the urinary excretions of pregnanediol were very similar to those observed in the same subjects following oral administration of progesterone.

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