THESIS

ON

STEROID METABOLISM
IN NORMAL AND RHEUMATOID ARTHRITIC HUMAN SUBJECTS

SUBMITTED FOR THE DEGREE OF

M.D.

by

IAN FERGUSON SOMMERVILLE

M.B., Ch.B., Ph.D.Edin.

Department of Biochemistry,
University of Edinburgh.

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PREFACE

During the past three decades there has been a remarkable acceleration in the rate of acquisition of new knowledge concerning the fundamental processes of the human body. The young science of biochemistry has gained new dignity year by year, and the benefits to mankind which have resulted and may yet result from its application to the problems underlying the aetiology and prevention of disease have become apparent to all. Few would deny that marked progress has been made in studies of the nature of the secretions of the endocrine glands and that the main impetus to that progress has resulted from an elucidation of the occurrence and nature of the steroid hormones.

The development of techniques for the isolation and characterisation of these substances made possible the identification of hormones secreted by the gonads and the adrenal cortices and of many of their metabolic products. The isolation, in 1929 and 1930, of the main urinary metabolites of the female sex hormones - pregnanediol and oestriol - by Marrian, and of oestrone by Doisy and his co-workers and by Butenandt, was soon followed by the isolation of androsterone, progesterone, oestradiol, testosterone, corticosterone and a large number of related steroids. Thus, /

Thus, for the first time, the biological actions of the pure steroid hormones could be studied, and attempts could be made to elucidate their intermediary metabolism in the body. The complexity of the subject was soon apparent. The rate of secretion of the hormone, the rate of its "inactivation", the response of the target organs, the chemical changes occurring at the site of its action and in other tissues, antagonism and synergism between hormones, the possibility of anti-hormone formation - all these had to be considered in evolving a concept of hormone action. The plurality of actions attributable to one steroid hormone and the importance of the hormones as biological regulators gradually became apparent. Thus it came to be recognised that the sex hormones have many actions not directly related to reproductive function. They influence carbohydrate and protein metabolism, electrolyte and water balance and mitotic activity. It was not difficult to imagine how these substances, acting in abnormal relationship with other hormones or vitamins, influenced to undergo abnormal metabolic changes, or acting upon abnormally "conditioned" tissues might play a part in pathogenesis. Recent work - which will be discussed in this thesis - upon the participation of the steroid hormones of the adrenal cortex in the aetiology of disease has emphasised /

emphasised these possibilities.

In so complex a field of study where so little is known and so much hoped for, attempts to elucidate new facts must be designed in such a way that, if possible, the result is unequivocal and the interpretation reasonably certain. It is here that biochemical research, employing rigid criteria for the purity of the substances studied, and carefully assessed methods for their quantitative determination, has so much to offer.

This thesis is concerned with the intermediary metabolism of steroid hormones in the human body. The female sex hormone - progesterone, has been chosen as the main steroid to be studied for two reasons. First, it was certain that much remained to be learned concerning the chemical mechanism underlying the biological action of this important hormone and especially the part played by the tissues in its metabolic reduction to pregnanediol and closely related steroids. Furthermore, little is known about the extent to which the urinary excretion of pregnanediol reflects endogenous secretion of the parent hormone - a problem with obstetrical and gynaecological implications. Secondly, since pregnanediol has been established as the main end-product of progesterone metabolism /

metabolism (Venning and Browne, 1938) and since a reasonably specific method has recently been described for the quantitative determination of small amounts of this steroid in human urine (Sommerville, Gough and Marrian, 1948) it seemed possible that a study of the proportion of administered progesterone excreted as urinary pregnanediol might constitute a new approach to the study of the possible participation of endogenous steroids in the aetiology of disease and, in particular, might contribute to an understanding of the mechanism whereby certain of the adreno-cortical steroid hormones exert their markedly beneficial action in the treatment of rheumatoid arthritis and related conditions.

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PART I

THE BIOCHEMICAL SIGNIFICANCE OF THE URINARY EXCRETION OF PREGNANEDIOL IN NORMAL HUMAN SUBJECTS WITH SPECIAL REFERENCE TO THE ROLE OF THE UTERUS IN THE METABOLISM OF PROGESTERONE

INTRODUCTION

Prenant (1898) was probably the first to assign an endocrine function to the corpus luteum, in a monograph with the prophetic title - "Sur la valeur morphologique, sur l'action physiologique et thérapeutique possible du corps jaune". In 1902, Fraenkel showed that destruction of the corpora lutea in the rabbit prevented nidation of the ova, and the experiments of Marshall and Jolly, in 1905, indicated that two active principles were produced by the ovary. Bouin (1906) noted the change which occurs in the uterine endometrium after corpus luteum formation and in 1913, Fellner produced this change by the injection of corpus luteum extracts. In 1928, Corner succeeded in maintaining pregnancy in an ovariectomised rabbit by the injection of corpus luteum extracts and in the following year, Corner and Allen described a method for the bioassay of the main active principle of these extracts.

In 1929, pregnanediol, a saturated dihydroxy alcohol, was isolated from human pregnancy urine by Marrian. This substance was identified by Butenandt in 1931 and was later shown to have the steric configuration of pregnane-3α,20α-diol. As progesterone had not yet been isolated, the biochemical

significance /

significance of this steroid could not be appreciated at that time. In 1933, however, Allen and Meyer described a method for the separation of the oestrogenic and progestogenic fractions in corpus luteum extracts, and in 1934, the isolation of progesterone (Δ^4 pregnene-3,20-dione) in pure crystalline state was announced by four groups of workers (Butenandt, Westphal and Hohlweg; Slotta Ruschig and Fels; Allen and Wintersteiner; and Hartmann and Wettstein). Later in the same year Butenandt et al, and Fernholz prepared progesterone by partial synthesis from stigmasterol. It was now obvious that pregnanediol could be a metabolic product of progesterone formed by reduction of the C_4 to C_5 double bond (Δ^4) and the C_3 and C_{20} ketone groups. The isolation by Hartmann and Locher in 1935 of the C_5 stereoisomer of pregnane-3α,20α-diol,- allopregnane-3α,20α-diol, from pregnancy urine, supported this idea. Allopregnane-3α,20α-diol was isolated from human pregnancy urine by Marker and Rohrmann in 1939.

Pregnanediol was isolated from pregnant mare's urine by Marker et al. in 1937, from bovine pregnancy urine by Marker in 1938, from the urine of the pregnant chimpanzee by Fish, Dorfman and Young in 1942 and from rabbit pregnancy urine by Verly, Sommerville and Marrian in 1950.

Pregnanediol /

Pregnanediol was isolated in small amounts from the urine of ovariectomised women by Hirschmann (1940) and of adult men (1944). Marker et al. (1938) claim to have isolated large amounts of this steroid from bull's urine.

In 1936, Odell and Marrian reported the presence of an acid-hydrolysable combined form of pregnanediol in human pregnancy urine and, later in the same year, Venning and Browne showed that this was a water-soluble complex - the sodium salt of pregnanediol glucuronide. In the following year, Venning successfully developed a method for the rough quantitative determination of this complex in human pregnancy urine. This work opened up a new field of research and in a series of important experiments, Venning and Browne (1938, 1940) established beyond doubt that pregnanediol is a metabolic product of progesterone. Thus they recovered pregnanediol glucuronide from the urine after the administration of progesterone to women in whom endogenous progesterone production could be assumed to be minimal and they studied for the first time the excretion of this steroid during the luteal phase of the menstrual cycle and during the course of normal pregnancy.

In 1939, the recovery of pregnanediol glucuronide, after the administration of progesterone to men, was reported /

reported by Buxton and Westphal. In the following year, Hamblen, Cuyler and Hirst confirmed this observation and reported that the proportion of administered progesterone excreted in this way varied considerably from individual to individual - from 0.0 per cent. to 42.5 per cent in their series, and also in the same individual when the procedure was repeated. In 1941 Seegar Jones and Telinde reported a recovery of 10.0 per cent. in the preovulatory phase of the menstrual cycle.

Conflicting reports appeared in the literature concerning the effect of pretreatment with oestrogen upon the proportion of administered progesterone excreted as pregnanediol glucuronide. Venning and Browne (1938) obtained results which suggested that pretreatment with oestradiol benzoate increased the recovery of pregnanediol glucuronide after progesterone administration to women with secondary amenorrhoea and hypoplastic endometria. Cope (1941) did not confirm this observation nor was it confirmed in a series of post-menopausal women investigated more recently (Sommerville and Marrian, 1950a). Smith and Smith (1940), on the other hand, compared the effect of progesterone alone and progesterone plus oestradiol benzoate on the excretion of pregnanediol during pregnancy, and suggested - although with little experimental /

experimental evidence - that oestrogen favoured the conversion of progesterone to pregnanediol. These workers also observed an increase in the urinary excretion of pregnanediol glucuronide of endogenous origin during the administration of diethylstilboestrol to pregnant diabetic women (Smith, Smith and Hurwitz, 1946). It appears, however, that this apparent increase may be due to the determination of the glucuronides of substances other than pregnanediol in the final product of the Venning method, since it has been shown that pregnanediol excretion actually falls during the administration of diethylstilboestrol to normal or diabetic pregnant women (Sommerville, Marrian and Clayton, 1948).

An investigation of the effect of hysterectomy on the conversion of progesterone to pregnanediol glucuronide also led to contradictory results and differing interpretations. This problem has been re-investigated in the present work and relevant contributions to the subject will be discussed in section 2.

The introduction of the Venning method (1937,1938) permitted a new approach to the problems of the placental origin of progesterone. Thus, in 1938, Jones and Weil reported removal of the corpus luteum at the eighth week of pregnancy followed by continuation of the pregnancy which terminated in spontaneous delivery /

delivery of a healthy infant at term. Pregnanediol glucuronide disappeared from the urine for several days after the excision (it was probably present in amounts too small to be detected by the Venning method) but soon reappeared in increasing amounts. This and other similar experiments indicate extra-ovarian sources of progesterone, and whereas it is reasonably certain that the placenta is the main site of progesterone production during pregnancy, the possibility that the adrenal cortices may play a subsidiary role cannot be overlooked. Progesterone and allopregnanolone were isolated from adrenal extracts by Beall and Reichstein in 1938 and pregnanediol was isolated from the urine of women with adrenal virilism by Butler and Marrian (1937). Furthermore, Cuyler, Ashley and Hamblen (1940) and Horwitt, Dorfman, Shipley and Fish (1944) observed the excretion of urinary pregnanediol after the administration of desoxycorticosterone to human subjects.

In 1940, Venning and Browne reported that when progesterone was injected during the luteal phase of the menstrual cycle, or during early pregnancy, a greater increase in pregnanediol excretion resulted than when the hormone was injected into women in whom endogenous progesterone secretion was minimal. This result, which is of considerable interest, was confirmed /

confirmed by Davis and Fugo (1947) using a different method for the determination of the steroid.

The method of Venning also led to preliminary observations on the rôle of the liver in the metabolism of progesterone. Thus Masson and Hoffman (1945) found that when progesterone was administered orally to rabbits the biological effect was eight times greater in partially hepatectomised than in intact animals, but the proportion of pregnanediol glucuronide excreted was similar in amount in both groups of animals.

In 1937, two 3-hydroxy-20-ketosteroids - pregnan-3α-ol-20-one and allopregnan-3α-ol-20-one were isolated from human pregnancy urine by Marker and his co-workers, and a third stereoisomer - allopregnan-3β-ol-20-one was isolated from the same source by Pearlman, Pincus and Werthessen in 1942. In 1943, Strickler et al., applying the method of Venning to the urine of a girl with hirsutism, obtained evidence for the presence of the glucuronide of a 20-ketosteroid in the final product. In 1945, Mason and Kepler identified pregnane-3α,17,20-triol as a glucuronide in the "pregnanediol glucuronide" fraction of the urine of women with adrenal hyperplasia. (This acid-labile 17-hydroxy steroid was first isolated from such urine by Butler and Marrian in 1937).

In /

In 1946, Marrian and Gough made the important observation that the "sodium pregnanediol glucuronidate" obtained from human pregnancy urine by the Venning method is contaminated by ketonic glucuronides notably a water-soluble derivative of pregnan-3α-ol-20-one. This substance constituted about 20.0 per cent. of the "sodium pregnanediol glucuronidate". In the following year, Sutherland and Marrian separated the non-ketonic and ketonic sodium glucuronidate fractions by a modification of the procedure of Girard and Sandulesco (1936), isolated pure sodium pregnane-3α,20α-diol glucuronidate and showed that the ketonic fraction consisted mainly of sodium pregnan-3α-ol-20-one glucuronidate. (Heard, Hoffman and Mack had shown in 1944 that the glucuronic acid in sodium pregnanediol glucuronidate was conjugated with the C_3 hydroxyl group of the pregnanediol. The glucuronide isolated by Sutherland and Marrian must be conjugated in the same way but the form of conjugation of the allopregnanediols is not yet known).

These observations implied serious disadvantages in the use of the Venning method for the determination of pregnanediol, although that procedure still finds favour with those wishing to determine "progesterone metabolites" as one enigmatic complex composed of the glucuronides of several substances including pregnanediol /

pregnanediol and the pregnanolones. In this connection it should be added that it cannot be assumed that all the glucuronides determined by the method of Venning are, in fact, metabolites of progesterone. Methods for the determination of pregnanediol as its glucuronide have the further disadvantage that fictitiously low results may be due to bacterial hydrolysis of the conjugate in inadequately preserved urine.

Thus, although the method of Venning had contributed so much to our knowledge of the parallelism between the excretion of progesterone metabolites in the urine and the secretion of progesterone by the ovary or placenta, some other method was required for a more detailed investigation of the excretion of pregnanediol and of the metabolism of progesterone.

In 1941, Astwood and Jones described a method for the determination of the free form of pregnanediol liberated by acid hydrolysis. The determination was gravimetric and it is certain that substances other than pregnanediol, e.g. certain 20-ketosteroids were present in the final product when this method was applied to human pregnancy urine. In the same year, however, Talbot et al. utilised the sulphuric acid reaction as a colorimetric method of determination. Pregnanolone and allopregnanolone have almost no chromogenic /

chromogenic power in this reaction and this modification, therefore, increased the specificity of the method. The combined Astwood-Talbot procedure was less time-consuming and technically more simple than the Venning method, but unsatisfactory results were obtained when it was applied to urines containing less than about 10 mg. per twenty-four-hour urine specimen and it was, therefore, unsuitable for studies of pregnanediol excretion during the menstrual cycle or early pregnancy, or for the determination of pregnanediol excretion after progesterone administration.

An investigation of the procedure by Professor Marrian and Dr. Nancy Gough revealed deficiences in the technique of extraction and purification of the pregnanediol, many of which could be corrected and it was found that a serious source of error lay in the conditions of the repeated precipitation whereby the extracted steroid was purified (Sommerville, Gough and Marrian, 1947). After various modifications had been tested in a series of recovery experiments in which pure sodium pregnane-3α,20α-diol glucuronidate or pure pregnane-3α,20α-diol were added to male urine, a method was finally evolved which permitted of the determination of more than about 0.4 mg. of pregnanediol in one-fifth of a twenty-hour urine specimen (Sommerville, Gough and Marrian, 1948).

The /

The method was simpler and less time-consuming than the above-mentioned procedures and appeared to be more accurate than any which had hitherto been described. The procedure takes thirty-six hours (including overnight incubation of the first precipitation mixture) but six determinations (three in duplicate) can be carried through daily by one person.

Many substances other than pregnanediol yield colours with concentrated sulphuric acid and it was necessary to test the specificity of the purification process by the addition of large amounts of other steroids prior to the first precipitation. Allopregnanediol, pregnanolone, allopregnanolone, pregnane-3,17,20-triol, androsterone, isoandrosterone, dehydroisoandrosterone, cholesterol, Δ^2 or Δ^3 androstene-17-one and Δ^5 androstadiene-17-one were investigated. It was not surprising to find that allopregnane-3α,20α-diol is determined along with pregnane-3α,20α-diol, although, since the former steroid is about 20.0 per cent. less chromogenic in the sulphuric acid reaction than the latter, its recovery is not quantitative. The other steroids investigated would not interfere unless present in very large amounts as in the urine of certain cases of adreno-cortical tumour.

Also based upon the combined Astwood-Talbot procedure, /

procedure, methods have been described for the qualitative or semi-quantitative determination of pregnanediol for clinical diagnostic or prognostic purposes. Such abreviations of the original procedure have been described by Guterman (1946) and Mack and Parks (1946, 1947). A method which is as rapid as these and more accurate was described in 1948 (Sommerville, Marrian and Kellar). This is a shortened version of the method of Sommerville, Gough and Marrian, permits of the reasonably accurate determination in three hours of more than about 0.4 mg. per 100 ml. of urine and is suitable for the determination of urinary pregnanediol during pregnancy but is unsuitable for a study of progesterone metabolism.

In order to investigate whether, as suggested by the results of Hamblen et al. and others using the method of Venning, there is considerable variation in the proportion of administered progesterone excreted as urinary pregnanediol, and in order to obtain a series of "control" figures as a preliminary to work such as that described in this thesis, a reinvestigation of this problem was undertaken under the supervision of Professor Marrian in 1947, When progesterone was administered to different groups of human subjects, and the pregnanediol excretion determined by the method of Sommerville, Gough and Marrian, the range of proportion of the hormone so excreted was as follows: /

follows: normal post-menopausal women:- 12.1 per cent. to 16.0 per cent., hysterectomised post-menopausal women:- 13.3 per cent. to 14.9 per cent., oestrogen pre-treated post-menopausal women:- 15.1 per cent. to 16.1 per cent., men:- 9.3 per cent. to 14.7 per cent. Furthermore, it was found that when the administration was repeated in the same individual, the results were remarkably constant. Thus, when 120 mg. of progesterone was administered to a normal man on four occasions:- 10.4 per cent., 10.0 per cent., 10.8 per cent. and 10.0 per cent. of the hormone was excreted as urinary pregnanediol. In a comparison of the proportion of pregnanediol excreted after oral and intra-muscular administration, it was found that excretion was more rapid after oral administration, and that a slightly higher proportion of the metabolite appeared in the urine. This proportion was also remarkably constant in a given individual - e.g. when 120 mg. of progesterone was administered orally to a normal man on three occasions, 18.6 per cent, 17.4 per cent, and 19.6 per cent. was excreted as urinary pregnanediol, (Sommerville and Marrian, 1950a). The observation of Venning and Browne (1938) and Davis and Fugo (1947) that a much higher proportion of the progesterone administered to a pregnant woman is excreted as urinary pregnanediol than after administration /

administration to a subject with minimal progesterone secretion, was confirmed, and the steady excretion of the steroid during the control periods enabled a fairly accurate estimate to be made of the amount of pregnanediol of exogenous origin. Thus, after the administration of 120 mg. of progesterone on two occasions to a woman at the twenty-seventh and twenty-ninth weeks of a normal pregnancy, 35.0 per cent. and 45.0 per cent. of the administered hormone was excreted as pregnanediol.

In the course of this work, the urinary excretion of pregnanediol following the administration of pure pregnane-3α,20α-diol was studied for the first time. Venning and Browne (1938) had shown that when "sodium pregnanediol glucuronidate" was administered by intramuscular injection, a large proportion was excreted as pregnanediol glucuronide in the urine (58.0 per cent. and 43.0 per cent. in their experiments) but they were unable to recover pregnanediol after injection of the steroid into two hysterectomised post-menopausal women. When pregnane-3α,20α-diol was administered to normal men in gelatin capsules by mouth (Sommerville and Marrian, 1950a) it was found that the recovery of pregnanediol from the urine was very similar to that obtained after the oral administration of progesterone to the same subjects. This result suggests the interesting possibility that administered progesterone may /

may be largely reduced to pregnanediol in the body and that the low recoveries of urinary pregnanediol following administration of either the hormone or its metabolite may be due to excretion of the reduction product by other routes and/or further metabolism into as yet unidentified products.

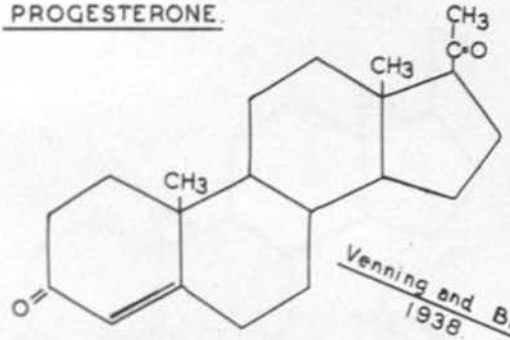
The experiments leading to the evolution of methods for the determination of urinary pregnanediol (Sommerville, Gough and Marrian, 1948; and Sommerville, Marrian and Kellar, 1948); the experiments in which pregnanediol excretion was studied after the administration of progesterone to men, post-menopausal women, hysterectomised women, oestrogen pre-treated post-menopausal women and during normal pregnancy; the excretion of pregnanediol after the excision of the corpus luteum of pregnancy; and the investigation of the effect of diethylstilboestrol upon the excretion of pregnanediol during normal and diabetic pregnancy were described in a thesis submitted for the degree of Doctor of Philosophy in 1948.

Part I of the present thesis is divided into five sections. In the first section, a series of experiments is described in which an attempt is made to elucidate some aspects of the biochemical significance of pregnanediol excretion during pregnancy, by a study of the urinary excretion of the steroid during prolonged administration of progesterone to human subjects.

The /

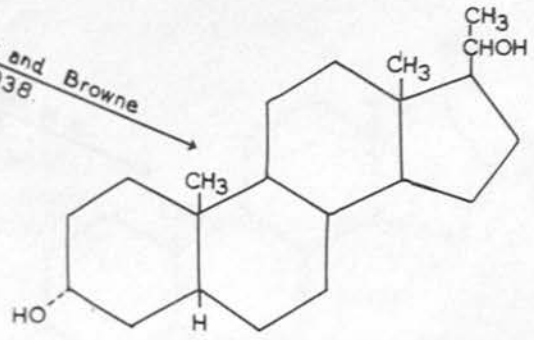
The second section constitutes an investigation of the possibility that the administration of vitamin E (d,l-alpha-tocopherol acetate) influences the metabolism of progesterone. In the third section, preliminary observations on the urinary excretion of pregnanediol during normal pregnancy are described and the results discussed in relationship to the urinary excretion of the glucuronides determined by the method of Venning (1937, 1938). In the fourth section a substance with progesterone-like activity (ethinyltestosterone) is investigated in relation to urinary pregnanediol excretion. In the fifth section, an investigation is made of the possibility that the more sensitive method employed in the present work could be applied to the determination of urinary pregnanediol in an experimental animal.

PROGESTERONE.



Venning and Browne 1938

Δ4-PREGNENE-3,20-DIONE



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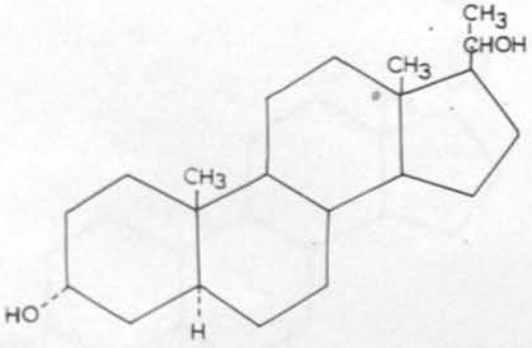

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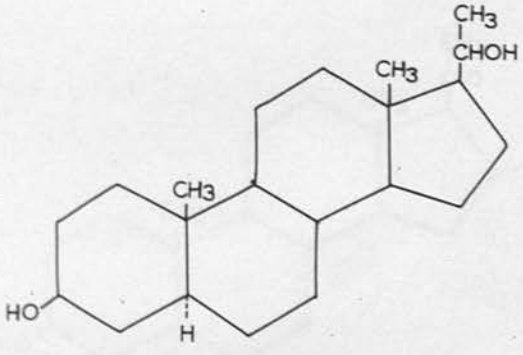

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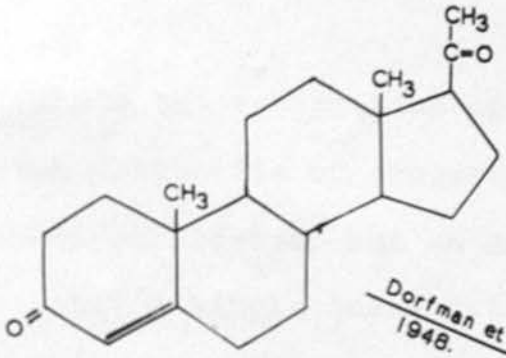
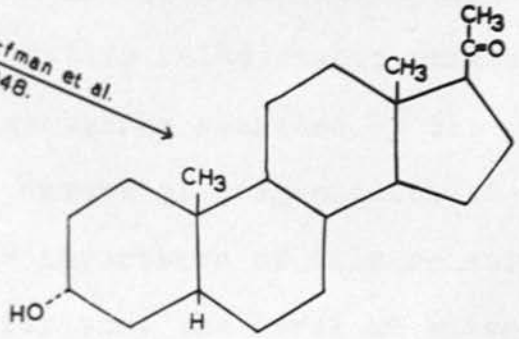
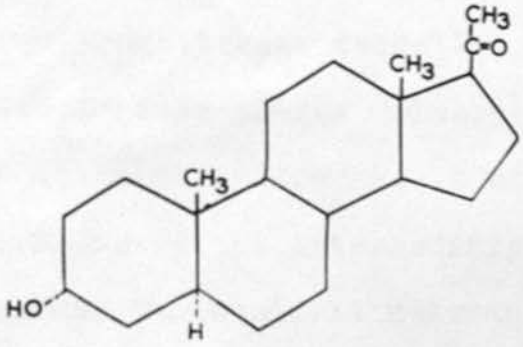


Δ^4-PREGNENE-3,20-DIONE.

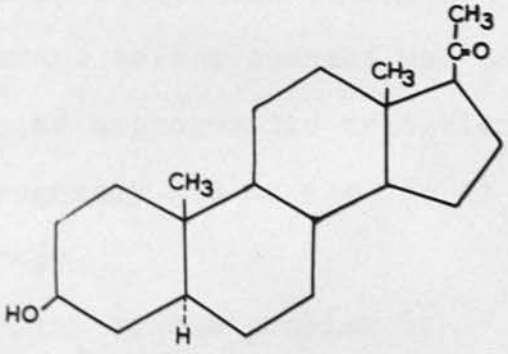
Dorfman et al. 1948.



PREGNAN-3α-OL-20-ONE.



allo PREGNAN-3α-OL-20-ONE.



allo PREGNAN-3β-OL-20-ONE.

SECTION I

URINARY EXCRETION OF PREGNANEDIOL AFTER PROLONGED ADMINISTRATION OF PROGESTERONE AND OF PREGNANEDIOL TO HUMAN SUBJECTS

Since the recognition of pregnanediol as an important metabolite of progesterone, the possibility has been considered, and on occasion the assumption made, that a simple quantitative relationship exists between the amount of progesterone secreted by the ovary or placenta and the amount of pregnanediol excreted in the urine. The importance of this relationship lies in the possibility that the level of endogenous progesterone secretion could thus be assessed by determination of the urinary pregnanediol excretion, and its elucidation would not only contribute to our knowledge of the intermediary metabolism of the hormone, but would be relevant to the current use of urinary pregnanediol assay as a prognostic criterion in the complications of pregnancy and as a guide to rational progesterone therapy.

The experimental approach to the problem is fraught with difficulty. A direct comparison cannot be made between the blood level of progesterone and the urinary level of the metabolite since it is not yet possible to determine the amount of chemically identifiable progesterone in the serum of pregnant women. /

women. Furthermore, even if such a method were available, it would by no means follow that the blood level of the hormone so determined would be a reliable index to the amount secreted. The results of the bioassay of the progestational activity of pregnancy serum indicate that the hormone is present in very small amounts (Hoffmann and von Lám, 1942; Hooker and Forbes, 1948) and this finding, and the relatively high excretion of pregnanediol in the urine of pregnant women suggest very rapid utilisation and metabolism of the hormone. It is even possible that the blood is a site for the metabolic reduction of progesterone to pregnanediol. On the other hand, the possibility may be considered that progesterone is present in the blood - not only protein-bound (Hooker and Forbes,1948) but in a chemically conjugated form, and that the low results obtained by bioassay are due to the low biological activity of this hypothetical conjugate.

Observation of the biological effects of the endogenous hormone may throw some light on the level of endogenous secretion. Thus the endometrium reflects the action of progesterone on one tissue, but this action is modified by other hormones and a correlation of endometrial histology and urinary pregnanediol excretion gives no reliable indication of the quantitative relationship between hormone and metabolite.

As it is not possible, therefore, to estimate the level of secretion of endogenous progesterone, attention has been turned to experiments in which this variable is replaced by the administration of weighed amounts of the pure hormone, in the hope that the fate of administered progesterone closely resembles that of the endogenous hormone.

When it was found that the administration of progesterone to subjects in whom endogenous progesterone secretion was thought to be negligible, resulted in the excretion of about 10.0 per cent. of the administered hormone as urinary pregnanediol, some observers were tempted to calculate that a daily urinary excretion of 100 mg. of pregnanediol during pregnancy must represent a daily endogenous secretion of 1,000 mg. of progesterone. On the other hand, as has been mentioned in the introduction, when progesterone is administered during pregnancy, about 50.0 per cent. of the hormone is excreted as urinary pregnanediol additional to that of endogenous origin. This experiment suggests that when the body is already under the influence of progesterone the metabolism of the hormone is in some way altered. Discussing this problem, Davis and Fugo (1947) state "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 pregnanediol". /

pregnanediol". This is not, however, the only possible interpretation which can be derived from this observation, since it may be related to any of the factors known and unknown which are associated with the presence of an active corpus luteum or placenta - for example, gonadotrophic, oestrogenic or adrenocortical activity. Furthermore, it may be doubted whether it is entirely justifiable to calculate the proportion of the administered hormone which is excreted as pregnanediol over and above that of endogenous origin. In this calculation it is assumed that the excretion level of endogenous origin is not affected by the administered hormone and although this assumption gains support from the levels of the adjacent control periods of excretion of endogenous pregnanediol it is not open to direct investigation.

In order to investigate whether this apparent change in the metabolism of progesterone is, in fact, due to the secretion and physiological activity of that hormone, it was 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 administration of progesterone.

The only earlier workers who appear to have studied urinary pregnanediol excretion during periods of /

of progesterone administration longer than three days are Venning and Browne (1940) and Cope (1941). The former workers administered doses of progesterone of about 10 mg. per day for four to eight days but the pregnanediol recoveries were irregular and showed no definite daily trend. Cope injected a case of secondary amenorrhoea with 10, 5, 5, 5 and 5 mg. of progesterone on five consecutive days. It is doubtful whether much quantitative significance can be attached to the results in view of the small doses injected but it is interesting to note that whereas only doubtful traces of pregnanediol were recovered from the urine during the first five days, 2.5 mg. was found on the sixth day. A similar effect was observed in a second experiment on a case of anovular menstruation. Cope suggested that these findings might be explained on the basis of a saturation phenomenon comparable to that associated with the excretion of orally administered ascorbic acid, and his results provided a suggestion that prolonged administration of progesterone might, in some way, cause an enhancement of the power of the body to convert progesterone into urinary pregnanediol.

In the course of previous experiments in which an attempt was made to study the effect of oestrogen administration on the proportion of administered progesterone /

progesterone excreted as urinary pregnanediol, the present worker had obtained two interesting results which appeared to substantiate some of these speculations. During the daily administration of 60 mg. of progesterone by intramuscular injection to a postmenopausal woman for ten days and by mouth to a similar subject for fifteen days, the pregnanediol excretion showed the following trends. In the first case, the daily pregnanediol excretion was maintained between the third and eighth days at an almost constant plateau level corresponding to about 10.0 per cent. of the daily dose of progesterone. The pregnanediol excretion then rose sharply on the eighth, ninth and tenth days and by the tenth day, when the experiment was discontinued, it had reached a value corresponding to 17.0 per cent of the daily dose of progesterone. In the second case, after reaching a preliminary plateau level corresponding to from 16.0 per cent. to 18.0 per cent. of the daily dose of progesterone, the pregnanediol excretion rose to 27.0 per cent. of the daily dose of progesterone by the fifteenth day. In a third experiment in which 40 mg. of progesterone was administered daily by mouth to a young man for sixteen days, a plateau corresponding to about 10.0 per cent. of the daily dose was maintained throughout the experment. No definite conclusion could be drawn from these /

these experiments in view of the number of variables involved - dose, route of administration, age and sex of subjects but in view of the theoretical considerations outlined above, it seemed probable that a more extensive investigation along these lines would yield interesting results.

Subjects for these experiments were selected with great care, particular attention being paid to their willingness to co-operate in the collection of accurate twenty-four hour specimens. The results bear witness to their felicity in that respect. The normal post-menopausal women were on the waiting lists of the gynaecological units of the Royal Infirmary, Western General Hospital and Deaconess Hospital. They were all suffering from a mild degree of prolapse of the uterus (without disturbance of bladder function) and the investigations were made possible through the courtesy of Professor R.J. Kellar, Dr. W.I.C. Morris and Dr. T.N. MacGregor. The twenty-four hour specimens were collected from all the subjects in their own homes during a control period of several days prior to the administration of the steroid and throughout the experiments. As in the other experiments described in this thesis specimens of urine of volumes less than 2,500 ml. were made up to that volume with water before withdrawing two 500 ml. samples /

samples for the determination in duplicate of the pregnanediol content by the method of Sommerville, Gough and Marrian (1948). In the few instances where the twenty-four hour volumes were greater than 2,500 ml. determinations were carried out on two 500 ml. samples and the appropriate volume correction subsequently applied.

Progesterone, dissolved in arachis oil (10 mg. per ml.) was administered either by intra-muscular injection or in gelatin capsules by mouth. As mentioned in the introduction, it has been shown that when progesterone is administered by mouth to human subjects, the proportion excreted as urinary pregnanediol is approximately 3.0 per cent. higher than when the hormone is injected, (Sommerville and Marrian, 1950a).

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

Four experiments were carried out in an attempt to elucidate the following points suggested by the two preliminary experiments.

(i) to determine the height to which the pregnanediol excretion will rise in such experiments (since the level was still rising when the preliminary experiments were terminated).

(ii) /

(ii) to determine whether a second plateau of pregnanediol excretion is attained and whether it is maintained.

(iii) to determine whether the phenomenon is related to the physiological activity of the administered hormone.

1. Subject 'H':

Post-menopausal woman, aged 70 years.

Progesterone, 50 mg. administered daily for 22 days by intra-muscular injection.

The results are shown in Fig. 2 and Table 1.

After attaining a short plateau corresponding to about 10.0 per cent. of the administered progesterone, the excretion of pregnanediol rose rapidly and a second plateau corresponding to about 22.0 per cent. of the administered hormone was reached at about the seventeenth day.

2. Subject 'H':

Progesterone, 60 mg. administered to the same subject daily for 22 days in gelatin capsules by mouth.

The results are shown in Fig. 2 and Table 2.

The urinary excretion of pregnanediol rose from a level corresponding to about 14.0 per cent. of the administered progesterone at the fourth day to about 24.0 per cent. at the eighteenth day. The rise was more /

more gradual than in the previous experiment and the second plateau had just been established at the end of the experiment.

3. Subject 'D':

Post-menopausal woman, aged 60 years.

Progesterone, 40 mg. administered daily for 22 days in gelatin capsules by mouth.

The results are shown in Fig. 3 and Table 3.

The first plateau of pregnanediol excretion was unusually high in this case - about 25.0 per cent. of the administered hormone. It was maintained until about the nineth day when the level began to rise and attained about 36.0 per cent. on the nineteenth day. The excretion had returned to the control period "blank" value forty-eight hours after the last dose of progesterone.

4. Subject 'G':

Post-menopausal woman aged 58 years.

Progesterone, 40 mg. administered daily for 27 days in gelatin capsules by mouth.

The results are shown in Fig. 3 and Table 4.

The first plateau of urinary pregnanediol excretion corresponded to about 15.0 per cent. of the administered hormone. About the eighth day of the experiment the level began to rise and attained about 20.0 per cent. on the twelfth day. Thereafter, a steady /

steady plateau of excretion was maintained until the conclusion of the experiment on the twenty-seventh day.

Two interesting observations concerning the biological activity of progesterone were made in this series.

(a) Endometrial biopsy was performed by Dr. T.N. MacGregor two days after the last injection of progesterone to subject 'H'.

(The experiments in this series are not in chronological order and this was the last experiment in which pregnanediol excretion was studied in subject 'H')

Despite the administration of relatively large amounts of the hormone - 1.1 gm. in three weeks - there had been no withdrawal bleeding and although too little material was obtained for histological examination the scrapings were such as are obtained from an atrophic post-menopausal endometrium.

(b) During the experiments in which progesterone was administered by mouth in 60 mg. doses 'H' and 'N', the patients complained of progressive drowsiness. After ten days of progesterone therapy both subjects tended to fall asleep during the day and their relatives noted that they were less alert than usual. Subject 'H' who was accustomed to play cards in /

in the evenings became an unwelcome partner.

These findings are discussed at the end of this section.

Conclusion

In all four experiments the daily urinary pregnanediol excretion, after reaching a temporary initial plateau level, rose considerably despite the administration of a constant dosage of the hormone. The extent of this increase, which shall be referred to as a progesterone "priming" effect, expressed as the percentage increase of the final plateau level above the initial plateau level is summarised in the following table.

(The two preliminary experiments previous referred to (subjects 'F' and 'N') are included, but the percentage increase is only an interim figure since the experiments were interrupted before the second plateau levels were attained).

NORMAL POST-MENOPAUSAL WOMEN /

NORMAL POST-MENOPAUSAL WOMEN
Continued Daily Administration
of Progesterone

Subject	Route of Admin.	Dose of Progesterone	PREGNANEDIOL MG./24 HR.		INCREASE PER CENT.
			First plateau	Second plateau	
'F' (Prelim.)	Intra-muscular	60 mg. x 12	6.0	(10.5)	(75)
'H'	Intra-muscular	50 mg. x 22	5.0	11.2	125
'N' (Prelim.)	Oral	60 mg. x 15	10.5	(16.0)	(53)
'H'	Oral	60 mg. x 22	8.5	14.0	65
'G'	Oral	40 mg. x 27	6.0	8.5	42
'D'	Oral	40 mg. x 22	11.0	14.5	32

It will be seen that the percentage increase was greatest in the experiment in which progesterone was administered by intra-muscular injection. Furthermore, in the experiments in which oral administration was employed, a dose of 60 mg. per day produced a greater "priming" than was obtained with 40 mg. per day. These results suggested the following answers to the points which were in question.

(i) /

(i) In such experiments, the pregnanediol level can rise as high as 125 per cent. above the initial plateau level.

(ii) A second plateau is attained. The time taken depends on the route of administration, the rise being more rapid with intra-muscular administration. In one experiment (subject 'G') the plateau was maintained for sixteen days.

(iii) The progesterone "priming" effect appears to be related in some way to the physiological activity of the progesterone. The effect is still apparent, however, when the progesterone is administered by mouth and it is known that the hormone has a very much reduced biological activity when administered by that route. Furthermore, no structural change in the endometrium was observed in a subject receiving large amounts of progesterone by intra-muscular injection.

Figure 1.

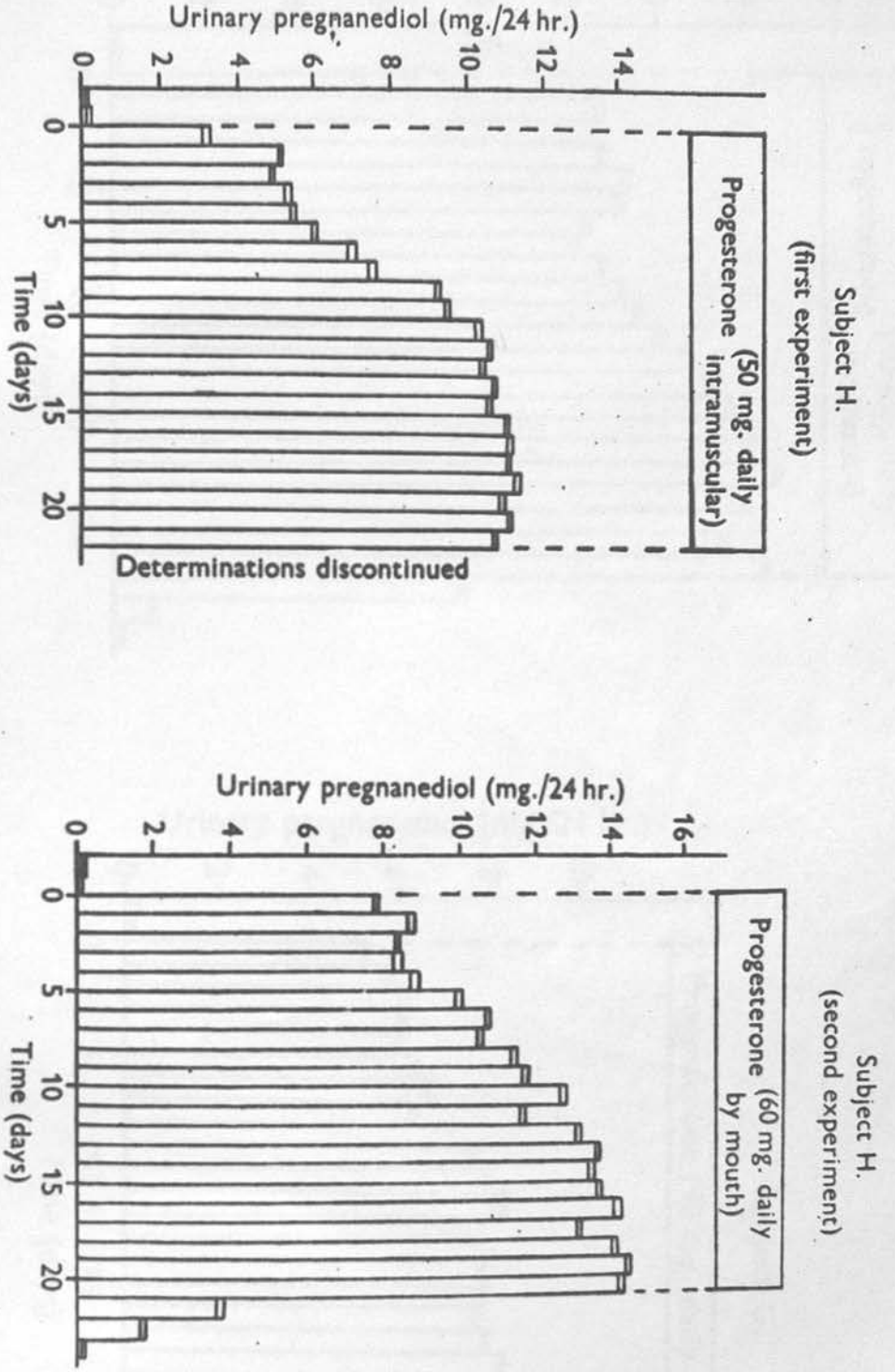

Urinary pregnanediol (mg./24 hr.)
0
2
4
6
8
10
12
14
Subject H.
(first experiment)
Progesterone (50 mg. daily intramuscular)
Time (days)
0
5
10
15
20
Determinations discontinued
Urinary pregnanediol (mg./24 hr.)
0
2
4
6
8
10
12
14
16
Subject H.
(second experiment)
Progesterone (60 mg. daily by mouth)
Time (days)
0
5
10
15
20

Figure 2.

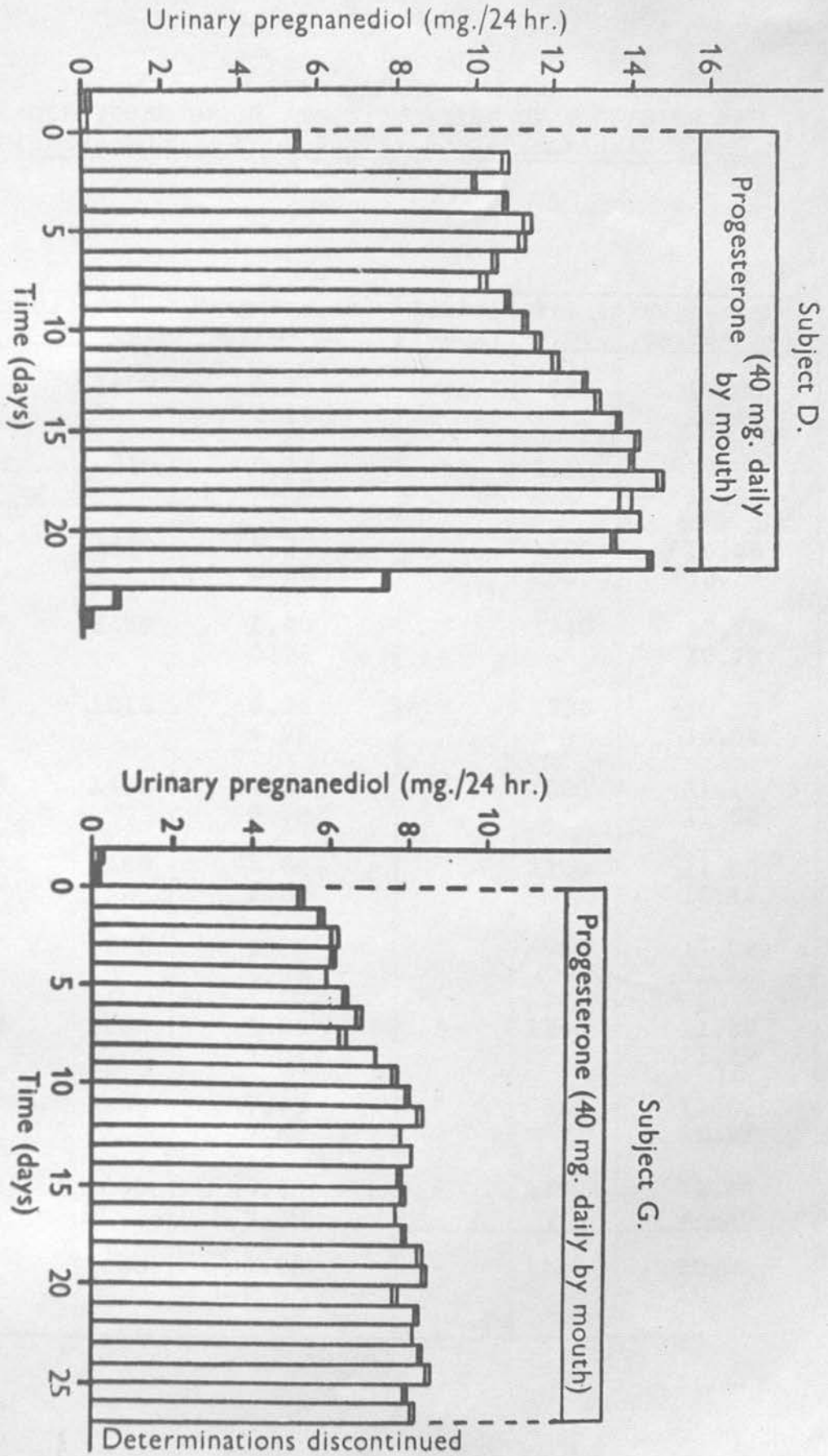

Urinary pregnanediol (mg./24 hr.)
0
2
4
6
8
10
12
14
16
Progesterone (40 mg. daily by mouth)
Subject D.
Time (days)
0
5
10
15
20
Urinary pregnanediol (mg./24 hr.)
0
2
4
6
8
10
Progesterone (40 mg. daily by mouth)
Subject G.
Time (days)
0
5
10
15
20
25
Determinations discontinued

TABLE 1

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (INTRA-MUSC.) TO A NORMAL POST-MENOPAUSAL WOMAN SUBJECT 'H'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	1900	0.11 0.12	Prog. 50 mg.	940	10.25 10.38
Prog. 50 mg.	910	0.13 0.16	"	1010	10.63 10.50
"	1600	3.20 3.05	"	600 (Comp.)	10.43 10.34
"	1460	5.20 5.08	"	1240	10.75 10.78
"	1210	4.98 4.95	"	1300	10.65 10.58
"	1450	5.28 5.38	"	1080	11.10 11.02
"	1480	5.45 5.53	"	1100	11.05 11.18
"	880	6.10 5.98	"	950	11.08 11.12
"	1380	6.88 7.08	"	1140	11.25 11.39
"	1250	7.43 7.58	"	880	11.00 10.85
"	1050	9.28 9.30	"	1200	11.10 11.16
"	1030	9.38 9.58	-	1120	10.80 10.75

TABLE 2

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO NORMAL POST-MENOPAUSAL WOMAN SUBJECT 'H'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	1600	0.19 0.15	Prog. 60 mg.	1240	13.20 13.10
Prog. 60 mg.	1320	0.13 0.13	"	1320	13.70 13.65
"	1210	7.85 7.73	"	1380	13.60 13.45
"	1290	8.80 8.65	"	1340	13.30 13.40
"	1500	8.40 8.35	"	1040	13.75 13.65
"	1230	8.50 8.30	"	1600	14.10 14.30
"	1180	8.70 8.90	"	1220	13.15 13.20
"	860	10.05 9.90	"	870 (cmc.)	14.05 14.20
"	1660	10.70 10.80	"	1375	14.45 14.53
"		10.50 10.60	-	1200	14.40 14.25
"	1530	11.40 11.50	-	1680	3.68 3.80
"	960	11.83 11.70	-	980	1.75 1.73
"	1300	12.83 12.63	-	1700	0.20 0.18
"	1550	11.60 11.75			

TABLE 3

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO A NORMAL POST-MENOPAUSAL WOMAN SUBJECT 'D'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	1480	0.18 0.16	Prog. 40 mg.	1145	12.75 12.80
Prog. 40 mg.	1260	0.15 0.16	"	1300	13.02 13.20
"	1100	5.13 5.20	"	1470	13.65 13.62
"	1260	10.65 10.78	"	1220	14.12 14.20
"	1080	9.90 9.95	"	900	14.00 13.92
"	920	10.70 10.75	"	980	14.62 14.77
"	1010	11.40 11.20	"	1005	13.65 14.00
"	1280	11.25 11.15	"	1240	14.20 -
"	1070	10.38 10.58	"	1580	13.45 13.55
"	1140	10.05 10.23	-	1395	14.42 14.50
"	1270	10.88 10.75	-	1100	7.70 7.67
"	1520	11.18 11.30	-	1120	0.99 0.92
"	1060	11.50 11.65	-	1230	0.13 0.11
"	980	11.98 12.15			

TABLE 4

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO A NORMAL POST-MENOPAUSAL WOMAN SUBJECT 'G'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	1630	0.27 0.24	Prog. 40 mg.	1800	7.55 7.50
Prog. 40 mg.	1590	0.18 0.21	"	1880	7.75 7.90
"	2170	5.23 5.12	"	1820	7.65 7.65
"	1990	5.80 5.68	"	2355	7.85 7.95
"	1780	6.30 6.10	"	1840	8.20 8.33
"	1735	6.05 6.07	"	2200	8.33 8.50
"	1780	5.90 -	"	1490	7.70 7.55
"	1540	6.45 6.53	"	2300	8.20 8.25
"	2040	6.75 6.85	"	1950	8.10 -
"	1900	6.20 6.33	"	1980	8.45 8.44
"	2075	- 7.23	"	1850	8.33 8.23
"	2450	7.55 7.65	"	1810	8.58 8.55
"	2425	7.95 8.00	"	2010	8.00 7.90
"	2300	8.15 8.33	-	1900	8.20 8.28
"	1850	7.80 -	-	2100	4.68 4.63
"	1980	7.95 8.10	-	2050	0.80 0.82

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

As indicated in the introduction, when progesterone is administered for one or two days to normal men, a proportion similar to that obtained in post-menopausal women is excreted as urinary pregnanediol. It might have been anticipated, therefore, that the prolonged administration of progesterone to men would result in a "priming" effect similar to that observed in post-menopausal women. This would also follow if the "priming" effect is a saturation phenomenon. On the other hand, in the preliminary experiment in which 40 mg. of progesterone was administered by mouth to a young man no "priming" effect was observed. Two further experiments were carried out, therefore, to determine whether this failure to observe the phenomenon was due to lack of response to the lower dose of the hormone administered by mouth or to an age difference.

1. Subject 'P':

Normal man aged 23 years.

Progesterone, 60 mg. daily for 18 days, in gelatin capsules by mouth.

The results are shown in Fig. 4 and Table 5.

The determination of urinary pregnanediol was not carried out on five days of the experiment but it is clear /

clear that a plateau level corresponding to about 18.0 per cent. of the administered hormone was maintained throughout the experiment.

2. Subject 'R':

Normal man aged 62 years.

The experiment was repeated (60 mg. x 18) in a subject of the same age group as the post-menopausal women.

The results are shown in Fig. 4 and Table 6.

A plateau corresponding to about 18.0 per cent. of the daily dose of progesterone was maintained throughout the experiment.

Conclusion

The results show clearly that there is a sex difference in the occurrence of the progesterone "priming" effect. It by no means followed that the absence of this phenomenon in men was attributable to the difference in reproductive anatomy, since the metabolism of tissues common to male and female - for example the liver - may differ in the sexes under hormonal influences. None the less the possibility had to be investigated that the post-menopausal uterus was in some way indispensable to the occurrence of the effect.

(c) /

Figure 3.

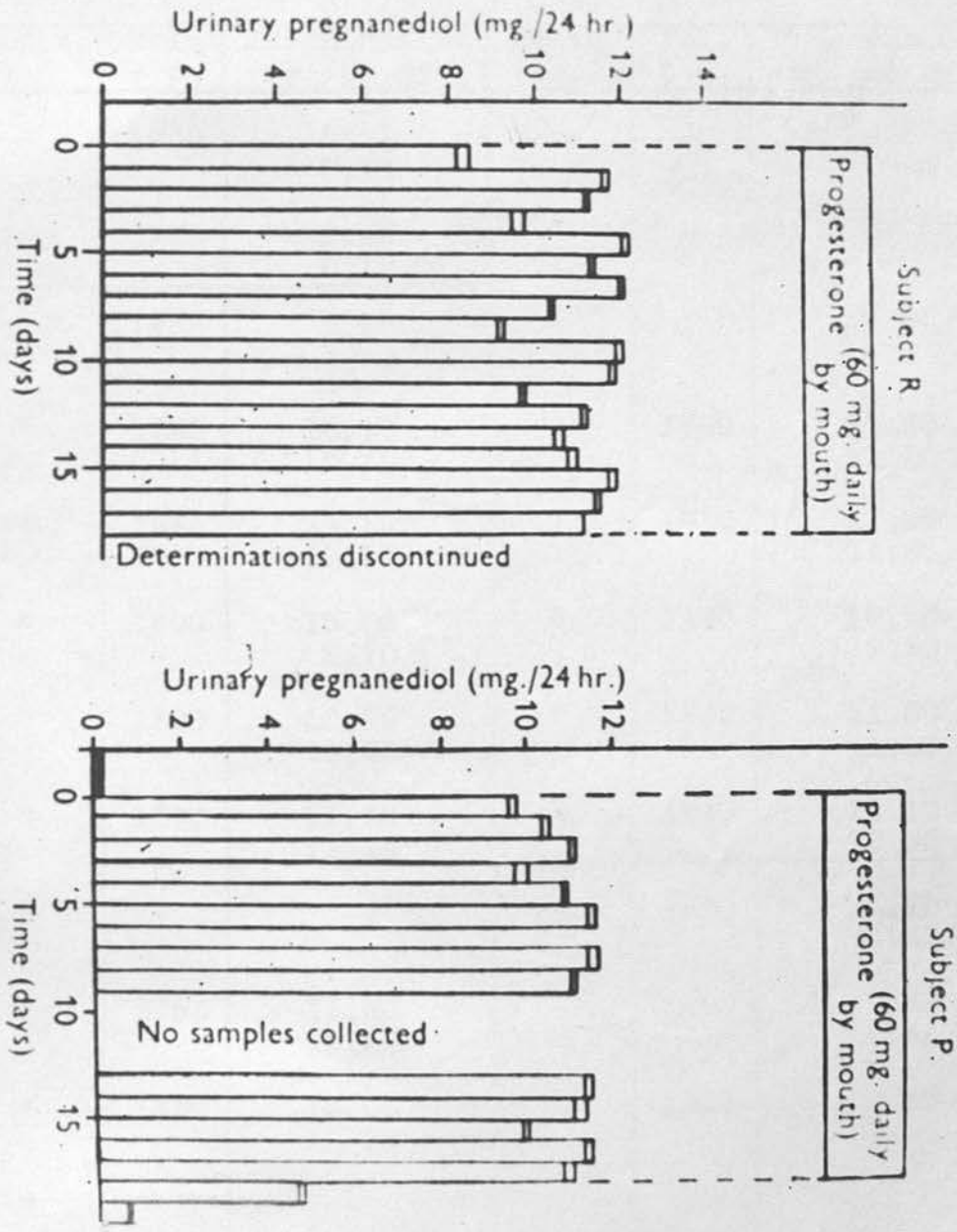

Urinary pregnanediol (mg /24 hr)
0
2
4
6
8
10
12
14
Time (days)
0
5
10
15
Progesterone (60 mg. daily by mouth)
Subject R.
Determinations discontinued
Urinary pregnanediol (mg./24 hr.)
0
2
4
6
8
10
12
Time (days)
0
5
10
15
Progesterone (60 mg. daily by mouth)
Subject P.
No samples collected

TABLE 5

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO A NORMAL MAN
SUBJECT 'P'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	1200	0.11 0.12	Prog. 60 mg.	-	-
Prog. 60 mg.	1100	0.10 0.12	"	-	-
"	1700	9.60 9.73	"	-	-
"	1820	10.50 10.35	"	1780	11.33 11.50
"	1825	11.10 11.00	"	1310	11.05 11.35
"	1200	10.00 9.70	"	1680	10.00 9.90
"	1600	10.90 10.85	"	1940	11.50 11.35
"	1500	11.40 11.55	-	1740	11.10 10.85
"	-	-	-	1840	4.73 4.63
"	2750	11.40 11.66	-	1800	0.63 0.66
"	1280	11.05 11.10	-	1420	0.12 0.15
"	-	-			

TABLE 6

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO NORMAL MAN
SUBJECT 'R'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	1800	0.16 0.17	Prog. 60 mg.	1360	11.90 11.75
Prog. 60 mg.	1770	0.19 0.18	"	1760	9.65 9.75
"	2000	8.50 8.20	"	1290	11.10 11.25
"	2315	11.60 11.75	"	1325	10.50 10.70
"	1680	11.20 11.25	"	1700	10.80 11.00
"	1460	9.45 9.75	"	2435	11.75 11.90
"	1750	12.25 12.10	"	1680	11.45 11.50
"	1400	11.30 11.40	"	1800	11.25 11.40
"	1700	12.15 12.00	-	2450	11.10 11.00
"	1430	10.40 10.45	-	1780	7.75 7.60
"	1310	9.10 9.35	-	1880	1.60 1.52
"	1620	12.10 11.95	-	1900	0.28 0.39

(c) Urinary pregnanediol excretion during continued daily administration of progesterone to hysterectomised (ovariectomised or post-menopausal) women

As indicated in the introduction, conflicting results have been reported concerning the possible importance of the uterus in the metabolism of progesterone and their discussion was postponed until this section. Venning and Browne (1938) were unable to recover "pregnanediol glucuronide" from the urine of two hysterectomised women after the administration of progesterone. In 1940 however, the same workers reported that following the administration of the hormone after supravaginal hysterectomy, 9.6 per cent. was recovered as "pregnanediol glucuronide" expressed as pregnanediol. Buxton (1940) recovered pregnanediol glucuronide from the urine of only two of four hysterectomised women similarly investigated. Seegar Jones and TeLinde (1941) reported the recovery of 15.6 per cent. of the administered hormone as pregnanediol from the urine of a woman after panhysterectomy and 12.9 per cent. and 10.9 per cent. after supravaginal hysterectomy. Hamblen (1939) had reported the absence of detectable pregnanediol excretion in a woman during the luteal phase of the menstrual cycle after curettage of the uterus. On the other hand, Seegar Jones and TeLinde recovered 6.1 mg. of "pregnanediol glucuronide" /

glucuronide" (expressed as pregnanediol) from the urine on the sixteenth day of a menstrual cycle in a woman in whom total hysterectomy had been performed on the first day of the cycle. After the administration of progesterone (60 mg.) on two successive days to three post-menopausal women after total hysterectomy, the present worker recovered 13.3 per cent., 14.1 per cent. and 14.9 per cent. as urinary pregnanediol (Sommerville, 1948; Sommerville and Marrian, 1950a). These results, and the results obtained after the administration of progesterone to men, prove beyond doubt that the uterus is not essential for the conversion of a proportion of administered progesterone to urinary pregnanediol.

In all these experiments, however, progesterone was administered for only one or two days and, therefore, it could not be assumed that such experiments gave a fair indication of the biochemical mechanism of progesterone disposal in action since, under physiological conditions - during the luteal phase of the menstrual cycle and during pregnancy - the human body is subjected to the influence of the hormone for considerably longer periods of time. Thus in view of the results obtained in the present work it seemed possible that a different state of affairs might exist when a comparison was made between normal and hysterectomised post-menopausal women after the

prolonged /

prolonged administration of progesterone.

Since it had been found that a higher proportion of administered progesterone may appear in the urine as pregnanediol when the hormone is injected in the immediate post-operative period than when the experiment is repeated in the same subject several weeks later (Sommerville, 1948), the cases in the present series were investigated not less than two months after total hysterectomy.

1. Subject 'H':

Hysterectomised post-menopausal woman aged 50 years (eight years after the menopause).

Progesterone, 60 mg. was administered daily for 22 days in gelatin capsules by mouth.

The results are shown in Fig. 5 and Table 7.

After an initial control period, the determination of urinary pregnanediol was carried out daily. A plateau of excretion corresponding to about 17.0 per cent. of the dose of administered progesterone was maintained throughout the experiment.

2. Subject 'K':

Hysterectomised-ovariectomised woman aged 45 years.

Progesterone, 60 mg. was administered daily for 18 days in gelatin capsules by mouth.

The results are shown in Fig. 5 and Table 8.

A plateau corresponding to about 16.0 per cent. of the dose of administered progesterone was maintained throughout the experiment.

3. /

3. Subject 'R':

Hysterectomised-ovariectomised woman aged 42 years.

Progesterone, 40 mg. was administered daily for 18 days in gelatin capsules by mouth.

The results are shown in Fig. 5 and Table 9.

It will be seen that a plateau of pregnanediol excretion corresponding to 19.0 per cent. of the administered hormone was maintained throughout the experiment.

Conclusion

In all three cases under investigation the excretion of urinary pregnanediol was maintained at a level corresponding to the temporary initial plateau observed in the series of normal post-menopausal women. There was no indication whatsoever of a progesterone "priming" effect. Under these experimental conditions, therefore, it is certain that the post-menopausal uterus is essential for the occurrence of the progesterone "priming" effect.

Figure 4.

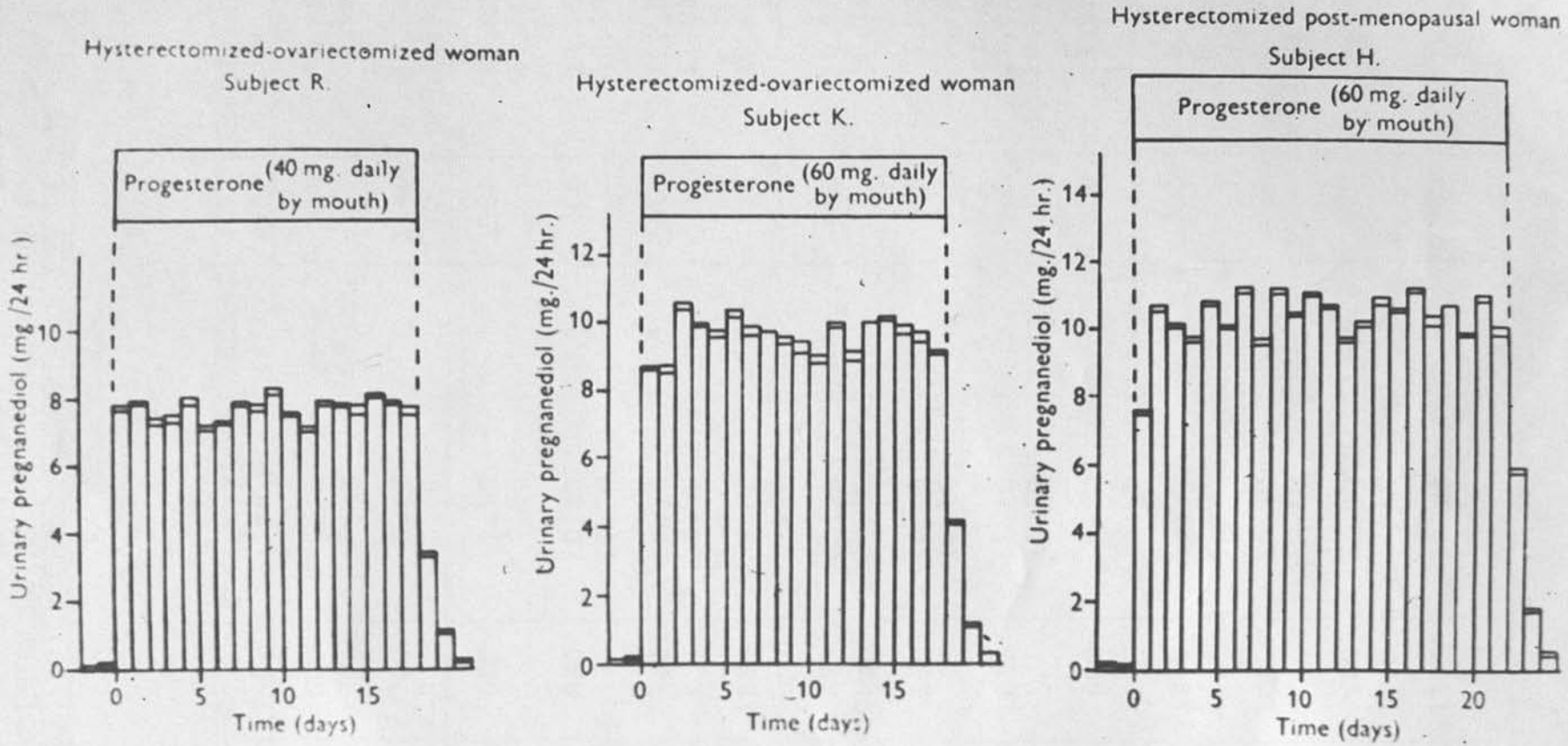

Hysterectomized-ovariectomized woman
Subject R.
Progesterone (40 mg. daily by mouth)
Urinary pregnanediol (mg./24 hr.)
Time (days)
Hysterectomized-ovariectomized woman
Subject K.
Progesterone (60 mg. daily by mouth)
Urinary pregnanediol (mg./24 hr.)
Time (days)
Hysterectomized post-menopausal woman
Subject H.
Progesterone (60 mg. daily by mouth)
Urinary pregnanediol (mg./24 hr.)
Time (days)

TABLE 7

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO HYSTERECTOMISED POST-MENOPAUSAL WOMAN SUBJECT 'Hy'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	850	0.19 0.18	Prog. 60 mg.	910	9.65 9.75
Prog. 60 mg.	740	0.15 0.16	"	1260	10.20 10.05
"	520	7.50 7.55	"	1120	10.55 10.50
"	980	10.50 10.70	"	600	10.75 10.95
"	740	10.15 10.05	"	1540	11.10 11.25
"	1280	9.65 9.75	"	950	10.40 10.15
"	470 (Comp.)	10.75 10.80	"	780	10.70 10.70
"	1180	10.05 10.10	"	960	9.80 9.75
"	820	11.10 11.25	"	980	11.00 10.80
"	600	9.70 9.55	-	850	9.85 10.10
"	1380	11.20 11.05	-	920	5.75 5.90
"	1080	10.40 10.50	-	1000	1.76 1.74
"	920	11.00 11.10	-	880	0.54 0.46
"	1195	10.70 10.65	-	750	0.20 0.21

TABLE 8

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO A HYSTERECTOMISED-OVARIECTOMISED WOMAN
SUBJECT 'K'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	2750	0.09 0.07	Prog. 60 mg.	3150	8.88 9.01
Prog. 60 mg.	2725	0.10 0.11	"	3500	9.87 10.01
"	2775	8.60 8.71	"	3010	8.85 9.21
"	2500	8.50 8.75	"	3200	10.05 10.05
"	3780	10.58 10.36	"	2750	10.12 10.23
"	3400	9.93 9.86	"	3100	9.98 9.61
"	3200	9.60 9.79	"	3250	9.63 9.75
"	4420	10.43 10.17	-	3580	9.13 9.19
"	3200	9.92 9.66	-	3400	4.25 4.18
"	3100	9.73	-	2950	1.24 1.24
"	3200	9.34 9.54	-	2550	0.42 0.39
"	2800	9.35 9.07			

TABLE 9

CONTINUED DAILY ADMINISTRATION OF PROGESTERONE (ORAL) TO HYSTERECTOMISED - OVARIECTOMISED WOMAN
SUBJECT 'W'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	750	0.10 0.11	Prog. 40 mg.	880	7.50 7.50
Prog. 40 mg.	1300	0.12 0.12	"	1005	7.00 7.15
"	1240	7.75 7.60	"	1140	7.75 7.90
"	1080	7.80 7.85	"	950	7.75 7.85
"	1100	7.40 7.10	"	1310	7.80 7.50
"	1310	7.50 7.25	"	1050	8.00 8.10
"	1130	8.00 7.75	"	885	7.95 7.80
"	720	7.05 7.15	-	1100	7.65 7.50
"	760	7.25 7.20	-	1020	3.45 3.38
"	1500	7.85 7.75	-	990	1.09 1.06
"	1600	7.80 7.60	-	1005	0.31 0.28
"	1200	8.05 8.15			

(d) Urinary pregnanediol excretion during continued daily administration of pregnane-3α,20α-diol to normal post-menopausal women

In view of the possibility, which will be discussed, 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. It was especially interesting to investigate this steroid in view of the possibility discussed in the introduction that all or most progesterone is largely reduced to this metabolite in the body and that the relatively small proportion appearing in the urine is due to further metabolism of the pregnanediol so formed or to excretion of pregnanediol by other routes. Furthermore, the result might throw some light on whether the progesterone "priming" effect is associated with the metabolic reduction of progesterone to pregnanediol or with the glucuronidic conjugation of the metabolite. Because of the low solubility of pregnane-3α,20α-diol in /

in oil it was not practicable to administer sufficient by injection to give an accurately measurable urinary excretion. Accordingly, administration in arachis oil in gelatin capsules by mouth was adopted.

400 mg. of pure pregnane-3α,20α-diol was dissolved in a minimal volume (about 10 ml.) of warm ethanol, 60 ml. of arachis oil (Organon) added, and the excess of ethanol boiled off by heating in a water-bath. After cooling the volume was made up to 100 ml. with more arachis oil. This solution contained 4.0 mg. per ml. and 40 mg. was administered daily in gelatin capsules by mouth.

1. Subject 'H':

Normal post-menopausal woman aged 70 years.

The experiment was carried out on the same subject who had shown marked "priming" after the administration of progesterone either by intramuscular or oral administration. A period of four months was allowed to elapse between this and the previous experiments.

Pregnane-3α,20α-diol, 40 mg. was administered daily for 17 days.

The results are shown in Fig. 6 and Table 10.

An irregular plateau corresponding to about 12.0 per cent. of the administered metabolite was maintained throughout the experiment.

2. /

2. Subject 'S':

Normal post-menopausal woman aged 58 years.

Pregnane-3α,20α-diol, 40 mg. was administered daily for 18 days.

The results are shown in Fig. 6 and Table 11.

As in the previous experiment a rather irregular plateau - in this case corresponding to about 10.0 per cent. of the administered metabolite was maintained throughout the experiment.

It was interesting to note that in subject 'H', who had previously complained of drowsiness after the administration of progesterone by mouth, but not after intra-muscular injection, observed the same effect in the present experiment.

Conclusion

There was no evidence of a "priming" effect after the prolonged administration of pregnane-3α,20α-diol to two post-menopausal women, one of whom had previously exhibited the effect during prolonged administration of progesterone.

DISCUSSION /

Figure 5.

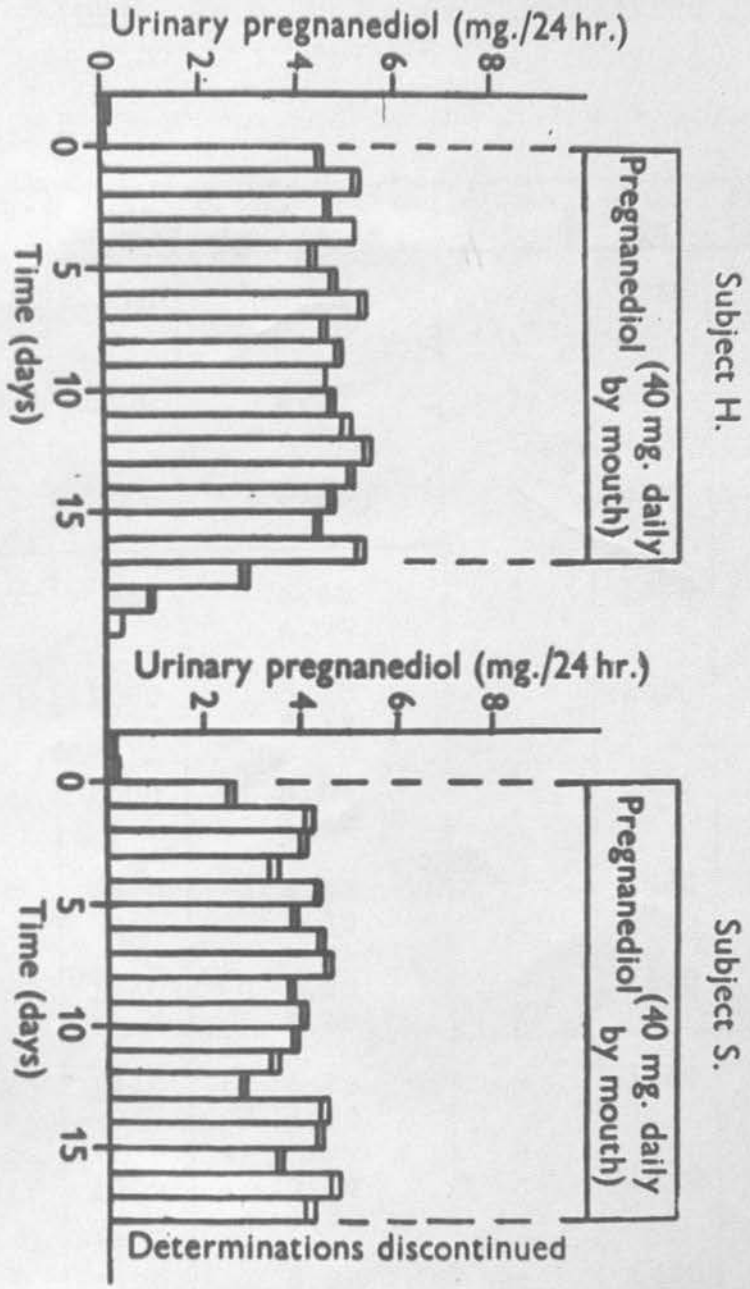

Urinary pregnanediol (mg./24 hr.)
0
2
4
6
8
0
5
10
15
Time (days)
Pregnanediol (40 mg. daily by mouth)
Subject H.
Urinary pregnanediol (mg./24 hr.)
0
5
10
15
Time (days)
Pregnanediol (40 mg. daily by mouth)
Subject S.
Determinations discontinued

TABLE 10

CONTINUED DAILY ADMINISTRATION OF PREGNANE-3α,20α-DIOL (ORAL) TO A NORMAL POST-MENOPAUSAL WOMAN
SUBJECT 'H'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
-	970	0.11 0.13	Preg. 40 mg.	1360	4.62
-	1200	0.15 0.14	"	1220	4.75 4.75
Preg. 40 mg.	1210	0.10 0.11	"	1280	4.90 5.05
"	940	4.43 4.50	"	1400	5.45 5.40
"	1580	5.22 5.12	"	1350	5.10 5.03
"	1500	4.62 4.68	"	1410	4.65 4.55
"	1440	5.12 5.10	"	1480	4.32 4.43
"	1340	4.28 4.38	-	1500	5.30 5.20
"	1140	4.78 4.72	-	1250	2.88 2.97
"	1700	5.38 5.30	-	1290	0.97 0.99
"	1120	4.38 4.50	-	1420	0.28 0.29
"	1390	4.90 4.82			

TABLE 11

CONTINUED DAILY ADMINISTRATION OF PREGNANE-3α,20α-DIOL (ORAL) TO A NORMAL POST-MENOPAUSAL WOMAN
SUBJECT 'S'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.	Admin. (con.)	Vol. c.c.	Pregnanediol mg./24 hr.
Preg. 40 mg.	1220	0.17 0.19	Preg. 40 mg.	1360	4.00 4.05
"	1400	2.52 2.58	"	1400	3.92 3.87
"	1420	4.17 4.25	"	1280	3.55 3.40
"	1280	4.00 4.10	"	1350	2.70 2.85
"	1500	3.42 3.50	"	1425	4.55 4.40
"	1240	4.37 4.30	"	1460	4.25 4.35
"	1520	3.90 3.87	"	1320	3.58 3.50
"	1560	4.33 4.43	"	1540	4.73 4.65
"	1500	4.50 4.55	"	1310	4.23 4.10
"	1320	3.70 3.80			

DISCUSSION

(a) The role of the uterus in the metabolism of progesterone

The results of the experiments in which progesterone was administered to men and hysterectomised women prove that the uterus is not essential for the conversion of the administered hormone to its urinary metabolite. On the other hand, it is clear from the present work that the enhanced power of the normal post-menopausal woman to excrete urinary pregnanediol after prolonged daily administration of progesterone is dependent upon the presence of the uterus. Accordingly, it may be concluded 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 an additional proportion of administered progesterone into urinary pregnanediol.

The fact that in men and hysterectomised women, a steady plateau of pregnanediol excretion is established in two to three days after the commencement of progesterone administration and is maintained for periods of up to twenty days indicates that the effect is not due to the saturation of tissues other than the uterus. The possibility that the progesterone "priming" effect is due to saturation of the uterine tissue with progesterone or its metabolites seems unlikely for two reasons. Firstly, if such saturation was to occur, then /

then in experiments of short duration, the pregnanediol excretion would be relatively lower in normal post-menopausal women than in hysterectomised women. This is not so. Secondly, if the "priming" effect was 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 was, in fact, observed.

The fundamental problem revealed by the present work is to determine in what way the progesterone "priming" phenomenon is related to histological or biochemical changes in the uterus.

At the present time there is no definite evidence to show whether the effect is associated with the physiological action of progesterone in inducing structural changes in the uterine endometrium or myometrium. The observation that the "priming" was greater with intra-muscularly than with orally administered progesterone, and the observation that in the three oral administration experiments the higher dose gave the greater "priming", suggest that this may be the case. It is unfortunate that more extensive studies of endometrial histology and vaginal

cytology /

cytology were not carried out in the subjects under study, but when the experiments were in progress it seemed unlikely that such doses of orally administered progesterone would induce detectable changes in the endometrium or vaginal epithelium. Furthermore, in previous studies in which progesterone was administered to three post-menopausal women pre-treated with oestrogen, a similar proportion of the hormone appeared as pregnanediol in the urine despite the varied responses of the endometria - in one case, atrophic, in another, proliferative; and in a third, secretory. These results suggested that differences in endometrial histology are not associated with alterations in the quantative relationship between progesterone and pregnanediol. There remain the possibilities, however, that biochemical changes are occurring in the endometrium or that histological and biochemical changes are occurring in the myometrium.

Little is known about the effect of progesterone on a uterus unprimed by oestrogen. The most detailed report is that of Crandall (1938), who administered progesterone daily by subcutaneous injection to ovariectomised rabbits and studied the uterine histology. The most interesting observation was the effect upon mitotic activity of the muscle cells. In untreated ovariectomised rabbits there were about 1.5 mitoses per sq. mm. of muscle tissue whereas after

three /

three days of progesterone administration (0.2 to 0.5 mg. daily) mitoses among these cells numbered about 25 to 30 per sq. mm. This effect was evanescent and the mitotic activity had returned to the control level after six days of progesterone administration.

It would be of considerable interest to correlate such structural changes in the uterus with the proportion of the hormone excreted as urinary pregnanediol. This could be carried out most satisfactorily in an experimental animal and preliminary work directed towards this end is described in section 5. Takometric studies might also contribute to the elucidation of this problem.

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 is due to the action of progesterone per se. The possibility must be borne in mind, however, that "priming" might result from other progesterone metabolites which cannot be tested. In this connection it may be pointed out that pregnanediol is by no means an "inert metabolite" of progesterone. In the present work, reference has been made to the soporific effect of 60 mg. of progesterone administered by mouth, and the lack of this effect when a similar dose was administered to the same woman ('H') by intra-muscular injection. /

injection. The effect was also noted when pregnane-3α,20α-diol was administered by mouth. These observations suggest that rapid absorption of the hormone or the metabolite from the intestine results in a higher serum concentration of either steroid than results from the gradual absorption of intramuscularly injected hormone. Selye (1941) was the first to investigate the anaesthetic property of steroids administered to rats and found that anaesthesia was produced by intra-peritoneal injection of progesterone, pregnanediol, desoxy corticosterone acetate, androsterone, testosterone and other steroids.

Hartman, Burge and Doctor (1947) showed that the term steroid anaesthesia is justifiable by studying the effect of progesterone on the brain potentials of rats. As might be expected from the present work, the anaesthetic potency of steroids administered to animals depends largely upon the route of administration. Injection of the steroid into the intestine below the pylorus gives rapid anaesthesia deep enough to allow prolonged abdominal operations whereas subcutaneous administration is much less effective. A second biological action attributable to pregnanediol is mucification of the vagina in ovariectomised mice (Freud, 1937).

Although it follows from the above remarks that histological studies of endometrium and myometrium ought /

ought to be carried out in animals receiving progesterone, it seems more likely to the present worker that the mechanism underlying the progesterone "priming" effect will be elucidated by a fuller understanding of the metabolism of uterine tissue. The part played by the uterus in the metabolism of progesterone may be concerned either with the metabolic reduction of progesterone to pregnanediol, with the further metabolism of pregnanediol or with its conjugation with glucuronic acid. In the absence of evidence to the contrary it seems not unreasonable to speculate that progesterone is largely reduced to pregnanediol in the body, that a considerable proportion of the metabolite is either further metabolised or excreted in the bile and that the proportion appearing in the urine is that fraction of the pregnanediol which is conjugated as a glucuronide. The role of the enzyme β-glucuronidase in glucuronide synthesis and hydrolysis is far from certain, but it would be surprising if it does not play a significant part in this process (Marrian, 1946). If then, progesterone were to inhibit the hydrolytic activity of the glucuronidase in uterine tissue then elevation of the urinary level of excretion of pregnanediol glucuronide might result. It has been shown that there is a correlation between β-glucuronidase activity and cell proliferation (Levvy, Kerr and Campbell, 1948). If the /

the high mitotic activity of the myometrium and a high glucuronidase activity are responsible for the priming then on the basis of the working hypothesis suggested above, the enzyme would require to be exerting a predominantly synthetic role.

Similarly, if the progesterone "priming" effect is associated with factors influencing the reduction and oxidation of progesterone and its metabolites a correlation might be found between the proportion of administered progesterone excreted as urinary pregnanediol and the activity of appropriate enzymes and coenzymes in the uterus. In this connection it is interesting to note that in ovarian tissues, correlations can be made between the functional state of the tissues and fluctuations in the activity of such enzymes as succinic dehydrogenase (Meyer et al., 1947), malic dehydrogenase and cytochrome oxidase (McShan et al, 1948). It is of the greatest interest to find that the uterus is a rich source of such vitamins as alpha-tocopherol and ascorbic acid since, as will be discussed, these substances might be expected to participate in such a reaction as the metabolic reduction of progesterone to pregnanediol. Experiments to investigate the possibility that alpha-tocopherol participates in the metabolism of progesterone are described in the section which follows. If the biochemical mechanism whereby the uterus exerts its

effect /

effect on the metabolism of progesterone is concerned with reduction-oxidation reactions or with glucuronide synthesis or hydrolysis, it would be anticipated that the uterus plays a similar role in the intermediary metabolism of other steroid hormones.

(b) Speculation as to possible clinical implications

The present results suggest the possibility that during pregnancy and, to a less extent, during the luteal phase of the menstrual cycle, the relationship of endogenous progesterone secretion to urinary pregnanediol excretion may be similar to that observed when the post-menopausal uterus is subjected to the influence of the hormone for prolonged periods of time. Thus, it may be that the rise in pregnanediol excretion which occurs after about the twelfth week of pregnancy is a manifestation of this changing relationship and is not an indication of a rising placental output of the hormone. It would be reasonable to expect that the effect observed in the atrophic post-menopausal uterus would be present to a greater degree in the physiologically active uterus. It may be, therefore, that during pregnancy the conversion of 50.0 per cent. of administered progesterone to pregnanediol, over and above that of endogenous origin, represents the "second plateau" observed after prolonged administration of the /

the hormone to normal post-menopausal women. Before any final conclusion can be reached as to the possibility of calculating the level of endogenous progesterone secretion from that of urinary pregnanediol excretion the effect of oestrogenic and gonadotrophic hormones upon the progesterone "priming" effect would require investigation.

The participation of the uterus in the metabolism of progesterone has the further implications that abnormalities of that metabolism may result from uterine dysfunction. The excretion of urinary pregnanediol is abnormally low in pre-eclamptic toxaemia (Browne et al., 1938; Smith and Smith, 1938, 1940, 1946). This may be due to an abnormally low level of progesterone secretion, to an abnormality of progesterone metabolism, or to impaired renal function. The first possibility is incalculable and the third is improbable. The possibility may be entertained, however, that there is an abnormality of progesterone metabolism in the toxaemias of pregnancy and that this reflects a fundamental and possibly pathogenetic disturbance of the enzyme systems of uterus and placenta.

SECTION II

TOCOPHEROL AND THE METABOLISM OF PROGESTERONE

> "Despite more than two decades of research, vitamin E still remains the mysterious intruder in the vitamin family, accepted at first with much reluctance and later reared somewhat as an outcast largely because of failure to satisfactorily establish its practical importance for man and beast. Its chemical nature is known, its laboratory synthesis accomplished, its wide distribution in the plant and animal world recognised, and the effect of its absence extensively studied in a large series of laboratory animals. Its physiological role in the animal body is still a matter of conjecture". (Mason, 1944).

The observation of structural and functional derangements characteristic of experimental vitamin E deficiency, and in particular the observation that intra-uterine death with resorption of the autolysed products of conception terminates pregnancy in vitamin E deficient rats (Evans and Burr, 1927) have long suggested the possibility that the vitamin may be of importance in human reproductive physiology. This contention has, however, lacked clear-cut confirmation from clinical and experimental studies and one is tempted to conclude that, either the vitamin does not play such a rôle or that it plays a rôle of such a fundamental nature as to remain untouched by the searching probe of experimental investigation. In the /

the latter connection, and in view of the speculations discussed in the previous section, it is of considerable interest to find in the literature references to the possibility that the tocopherols may participate as anti-oxidants in reversible oxidation-reduction systems in the body. Thus Houchin (1942) suggests that tocopherols, probably in phosphorylated form, may inhibit or regulate oxidative processes in muscle, and this worker may have provided a clue to the type of enzyme system involved, by the observation that the dystrophic muscle of vitamin E deficient animals exhibits a high succinoxidase activity and that this activity is inhibited by α-tocopheryl phosphate.

Since the observations of Zondek (1934) on the inactivation of oestrogens by the liver, there have been many reports indicating the importance of that organ in determining the biological activity of the steroid hormones, and in particular the dependence of this liver function on the presence of certain essential food factors (Biskind, 1942; Unna et al., 1944; Kochakian et al., 1944). The rôle of the liver in steroid metabolism, however, is still obscure although reference has already been made to the work of Masson and Hoffman (1945) on the metabolism of progesterone and to experiments in which the proportion of administered progesterone excreted as urinary pregnanediol was compared after oral and intra-muscular administration /

administration (Marrian, 1949; Sommerville and Marrian, 1949). Clark and Kochakian (1944) have studied the metabolites arising from the incubation of testosterone with rabbit liver slices in serum; Glass et al. (1940) found evidence for abnormal oestrogen metabolism in human subjects with impaired liver function, and Samuels and McCaulay (1948) have reported recently that human cirrhotic livers do not destroy testosterone as rapidly as normal livers. It is interesting, therefore, to consider whether certain aspects of experimental dietary necrosis of the liver may not be relevant to the present problem. It has been shown that this lesion is attributable to a deficiency of cystine (Glynn and Himsworth, 1945) and Schwarz (1944) found that its occurrence could be prevented by supplementing the diet with vitamin E. This effect has been confirmed and extended by György (1947) and it appears that tocopherol deficiency may sensitize the animal to the deficiency of the amino-acid.

In view of these indications of the rôle of vitamin E as an "antioxidant" in cell metabolism and as a factor in liver function attention was turned to previous studies in which the possible interaction of progesterone and vitamin E had been considered. No clear-cut evidence of an interdependence of hormone and vitamin had been adduced - whereas, in the case of /

of androgen there is some ground for the belief that a synergism exists between vitamin E deficiency and androgenic potency (Caridroit, 1942). The development of methods for the determination of urinary pregnanediol provided a new method of approach to this problem and one which has not yet been fully exploited.

In 1942, Winkler determined the excretion of "pregnanediol glucuronide" by the method of Venning, in the urine of women who were receiving synthetic d,l-α-tocopherol acetate throughout the menstrual cycle. The results indicated that an increase in the excretion of "pregnanediol glucuronide" accompanied the administration of tocopherol but the results were not convincing in view of the known variability in the amount of "pregnanediol glucuronide" excreted during the menstrual cycle and in view of the insensitivity of the method of determination employed. Winkler concluded that the apparent increase in pregnanediol excretion reflected a stimulation of endogenous progesterone secretion.

In 1948, de Watteville, Borth and Gsell questioned the validity of this assumption and suggested that the effect - if it existed - might equally well result from a "shift in the intermediary metabolism of progesterone". To investigate this possibility these workers administered progesterone to post-menopausal /

post-menopausal women and determined the proportion excreted as urinary pregnanediol by a modification of the method of Huber (1947). The vitamin, d,l-α-tocopherol acetate ("Ephynal", Roche) was then administered for seven days after the disappearance of pregnanediol from the urine, and the progesterone administration and pregnanediol determination were then repeated. The experiment was carried out in twelve post-menopausal women, and in two of these the administration of the vitamin was omitted. The results showed that the proportion of administered progesterone excreted as urinary pregnanediol was increased by the previous administration of synthetic α-tocopherol. Thus, before treatment the proportion of progesterone excreted as urinary pregnanediol ranged from 7.3 per cent. to 13.8 per cent. (mean 9.66 per cent.) whereas after treatment the results represented 10.4 per cent. to 19.9 per cent. (mean 14.17 per cent.) of the administered hormone. There is no suggestion in the description of these experiments that the women investigated were receiving diets deficient in vitamin E; and the authers cautiously refrain from relating their results to the possible therapeutic value of tocopherol therapy in pregnancy but suggest that the effect might be due to "diminished oxidative destruction of progesterone" due to the antioxidant action of the vitamin.

The /

The results obtained by de Watteville *et al*.(1948) gained a new significance in view of our speculation that the mechanism of the progesterone "priming effect" might depend upon the activity in uterine tissue of vitamin E or of some similar substance. Accordingly, tentatively assuming that the results obtained by these workers were valid, it seemed important to repeat their experiments but to investigate men and, if necessary, hysterectomised women, instead of post-menopausal women.

In the present work, the selection of normal human subjects, the collection of twenty-four-hour urine specimens and the determination of urinary excretion of pregnanediol by the method of Sommerville, Gough and Marrian (1948) were as in the previous section. Apart from the choice of subjects and the choice of method of pregnanediol assay, the experimental conditions were identical with those of de Watteville *et al*.

(i) Progesterone, 100 mg., was administered by intra-muscular injection and urinary pregnanediol determined on the day preceding the injection and until the excretion had returned to the control "blank" value.

(ii) "Ephynal" (Roche), 60 mg., was administered by mouth for seven days.

(iii) On the seventh day, pregnanediol determinations were resumed and on the eighth day a second /

second intra-muscular injection of 100 mg. of progesterone was administered.

The proportion of the administered hormone excreted as urinary pregnanediol was calculated after correction of the apparent pregnanediol excretion for the control period "blank" value. This "blank" value which may be due to pregnanediol of adreno-cortical origin or to traces of other substances which are chromogenic in the sulphuric acid reaction does not amount to more than about 0.2 mg. per twenty-four-hour specimen in normal men or normal post-menopausal women. The pattern of excretion in such experiments is shown in Fig. 6 which illustrates the urinary excretion of pregnanediol after the administration of progesterone to subject 'R'. The arrow indicates the intra-muscular injection of 100 mg. of progesterone and the values obtained in the duplicate determinations are indicated by the double lines at the tops of the columns.

(a) Proportion of administered progesterone excreted as urinary pregnanediol by normal men after the administration of d,l-α-tocopherol acetate

The experiment was carried out as above in three normal men, and to a third man ('S') the progesterone was administered in gelatin capsules by mouth. The results /

results are shown in the following table, and in Tables 12 and 13.

Subject	Recovery of Pregnanediol from Administered Progesterone: Per Cent.	
	Before Tocopherol	After Tocopherol
'M'	8.29	8.48
'P'	15.83	15.74
'R'	8.43	8.50
'S'	10.69	9.87
	mean: 10.81	mean: 10.15

Conclusion

These experiments show very clearly that pre-treatment with synthetic vitamin E has no effect upon the proportion of administered progesterone excreted as urinary pregnanediol. Before concluding, however, that there is a sex difference in the occurrence of the effect observed by de Watteville et al., it was obviously necessary to confirm the occurrence of the effect in normal post-menopausal women.

(b) /

Figure 6.

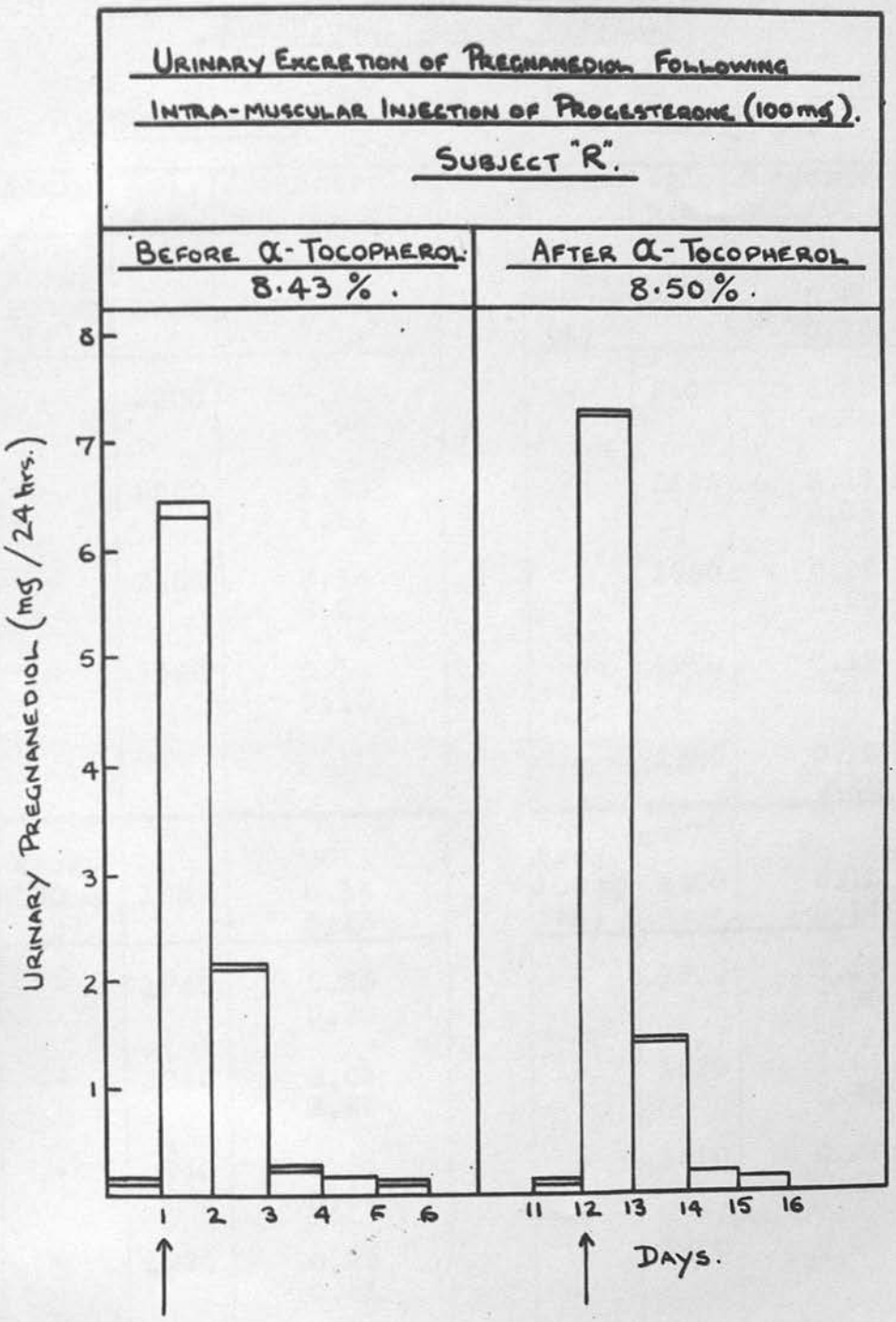

URINARY EXCRETION OF PREGNANEDIOL FOLLOWING
INTRA-MUSCULAR INJECTION OF PROGESTERONE (100 mg).
SUBJECT "R".
BEFORE α-TOCOPHEROL
8·43 %.
AFTER α-TOCOPHEROL
8·50 %.
URINARY PREGNANEDIOL (mg/24 hrs.)
1
2
3
4
5
6
7
8
1
2
3
4
5
6
11
12
13
14
15
16
DAYS.

TABLE 12

ADMINISTRATION OF PROGESTERONE (100 mg. INTRA-MUSC.) TO NORMAL MEN BEFORE (A) AND AFTER (B) ADMINISTRATION OF "EPHYNAL"

SUBJECT 'M'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
Prog. 100 mg (A)	1500	0.19 0.16
-	3200	7.04 6.94
-	2520	1.63 1.64
-	3250	0.18 0.27
-	1660	0.15 0.15
Prog. 100 mg (B)	1020	0.16 0.18
-	1740	5.25 5.30
-	1340	3.05 2.99
-	940	0.56 0.59
-	1820	0.28 0.21
-	1520	0.14 0.15

SUBJECT 'R'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
Prog. 100 mg (A)	2315	0.15 0.13
-	2600	6.29 6.36
-	2265	2.17 2.18
-	1980	0.28 0.26
-	1900	0.18 -
-	1800	0.16 0.18
Prog. 100 mg (B)	1400	0.13 0.14
-	1600	7.25 7.30
-	1820	1.44 1.42
-	1250	0.20 0.21
-	1400	0.15 -

TABLE 13

ADMINISTRATION OF PROGESTERONE (INTRA-MUSC. TO SUBJECT 'P' AND ORAL TO SUBJECT 'S') TO NORMAL MEN BEFORE (A) AND AFTER (B) ADMINISTRATION OF "EPHYNAL"

SUBJECT 'P'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
Prog. 100 mg (A)	1310	0.10 0.11
-	1500	8.98 9.08
-	1420	6.60 6.75
-	1500	0.41 0.49
-	1480	0.09 0.14
Prog. 100 mg (B)	1620	0.16 0.13
-	1800	10.35 10.23
-	1520	5.35 5.43
-	1680	0.52 0.49
-	1750	0.15 0.13

SUBJECT 'S'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
Prog. 100 mg (A)	1440	0.13 0.13
-	1640	9.58 9.63
-	1350	0.99 0.96
-	1290	0.52 0.46
-	1920	0.17 0.15
-	1530	0.10 0.12
Prog. 100 mg (B)	1720	0.12 0.11
-	1920	8.83 8.85
-	1220	1.02 1.03
-	1640	0.43 0.40
-	1400	0.11 0.09

(b) Repetition of the experiment in normal post-menopausal women

Up to the present time, two cases have been investigated.

1. Subject 'C': aged 60 years:- The proportion of injected progesterone excreted as urinary pregnanediol before administration of d,l-α-tocopherol acetate was 10.22 per cent. and after administration was 9.71 per cent.

2. Subject 'H': aged 70 years:- In this case 12.23 per cent. was recovered before and 12.79 per cent. after pre-treatment with d,l-α-tocopherol-acetate.

Conclusion

The results in these two cases are sufficiently clear-cut to justify the conclusion that, under these experimental conditions, pre-treatment with synthetic vitamin E has no effect upon the proportion of administered progesterone excreted as urinary pregnanediol by normal post-menopausal women.

The results of the twelve metabolic experiments described above are illustrated in Fig. 7, in which the total excretion of urinary pregnanediol (corrected for the "blank" value) is represented as a percentage of the administered hormone.

Figure 7.

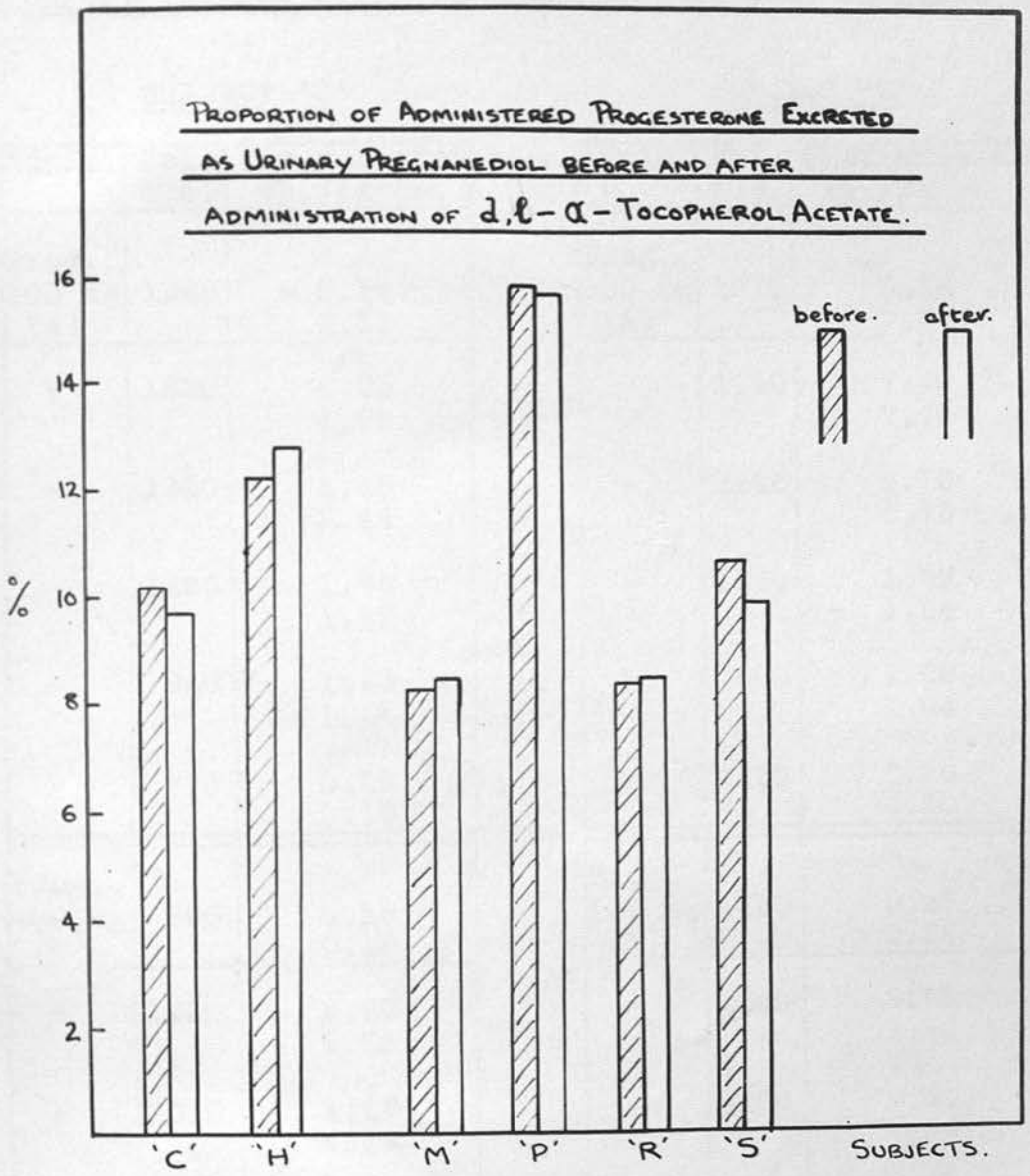

Proportion of Administered Progesterone Excreted
as Urinary Pregnanediol before and after
administration of d,l-α-Tocopherol Acetate.
before.
after.
16
14
12
10
8
6
4
2
%
'C'
'H'
'M'
'P'
'R'
'S'
Subjects.

TABLE 14

ADMINISTRATION OF PROGESTERONE (100 mg. INTRA-MUSC.) TO NORMAL POST-MENOPAUSAL WOMEN BEFORE (A) AND AFTER (B) ADMINISTRATION OF "EPHYNAL"

SUBJECT 'C'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
Prog. 100 mg (A)	1320	0.15 0.11
-	1830	4.83 4.95
-	1320	2.45 2.46
-	1420	1.98 1.99
-	960	1.33 1.34
-	770	0.18 0.19
Prog. 100 mg (B)	800	0.18 0.18
-	1400	5.60 5.75
-	1050	4.17 4.19
-	1200	0.35 0.36
-	1020	0.19 0.23

SUBJECT 'H'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
Prog. 100 mg (A)	1010	0.23 0.20
-	1040	7.78 7.70
-	1640	2.70 2.73
-	1360	1.52 1.54
-	800	1.08 1.04
-	1150	0.26 0.30
Prog. 100 mg (B)	1420	0.29 0.28
-	1350	9.93 9.98
-	1200	2.80 2.68
-	1600	0.94 0.92
-	1480	0.31 0.33

DISCUSSION

The schedule of dosage and timing of the experiments in the present work and in the investigation by de Watteville *et al.* were identical, and it is reasonable to suppose that the factors responsible for the contradictory results were associated either with the choice of subjects or with the choice of method for the determination of urinary pregnanediol.

It is a criticism of the investigation of de Watteville *et al.* that all of their cases were suffering from either carcinoma of the cervix (nine cases) or carcinoma of the breast (three cases). The former had received radiation therapy and in many of the cases the disease was well established with, in at least two cases, cachexia and general metastases. Whereas it is unlikely that the cases studied in either set of experiments were receiving a diet deficient in vitamin E, it does seem possible that a relative deficiency of the vitamin might be associated with the clinical condition of the cases of de Watteville *et al.* Alternatively, the neoplastic tissue may have an increased affinity for the administered vitamin and, since the neoplasms were confined to secondary sex organs, it is not impossible (in view of the results in section 1) to visualise a consequent /

consequent effect on steroid metabolism. In this connection it would seem more logical to investigate the hypothesis that α-tocopherol is acting as an anti-oxidant in progesterone metabolism by the simultaneous rather than by the previous administration of the vitamin. It is a considerable assumption to relate the anti-oxidant activity of the vitamin - a function which has been studied in other fields of biochemistry, to the intermediary metabolism of the steroids but a recent discovery has greatly strengthened the status of α-tocopherol as a participant in oxidation-reduction systems.

Until recently it was difficult to reconcile such a rôle for α-tocopherol with the nature of its oxidation product, α-tocopheryl quinone (Fig. 8). The reversibility of this reaction has not been demonstrated and the quinone is biologically inactive when administered to rats (Wright and Drummond, 1940) and guinea pigs (Golumbic, 1940). Since the tocopherol molecule has only one unsubstituted phenolic hydroxyl group, the bivalent oxidation to the quinone involves an opening of the side-ring and presumably explains the irreversibility of the oxidation. In 1949, however, Michaelis and Wollman obtained evidence for a reversible univalent oxidation of the vitamin by the demonstration of a semiquinone arising by the removal of only one electron from α-tocopherol. Although

this /

this process would undoubtedly involve a large amount of free energy, these workers suggest that the vitamin may be present as a prosthetic group of an enzyme and that the process may take place intramolecularly within the enzyme-substrate complex. This finding suggests new possibilities in relation to cell metabolism in general and dehydrogenations in particular.

None the less even if the vitamin does participate in the metabolism of progesterone, there are many more ways in which it might act than by diminishing the "metabolic oxidation" of the hormone as suggested by de Watteville et al. It is less difficult to imagine how the univalent oxidation of the vitamin might enable it to participate as a co-enzyme in the metabolic reduction of progesterone to pregnanediol and the possibility that the activity of the vitamin might favour the formation of pregnanolone is especially relevant to the present problem.

The modified method of Huber (1947) - used by de Watteville et al. - and based upon that of Astwood and Jones (1941), has the disadvantage that the estimation of the steroid depends upon a gravimetric determination. This diminishes the specificity of the procedure and it is possible that pregnanolone and allopregnanolone would be determined as pregnanediol by this method. A shift in the metabolism of progesterone /

progesterone favouring the formation of such ketonic glucuronides to the detriment of non-ketonic glucuronides such as sodium pregnanediol glucuronidate would account for the discrepancy between the results obtained in the two sets of experiments.

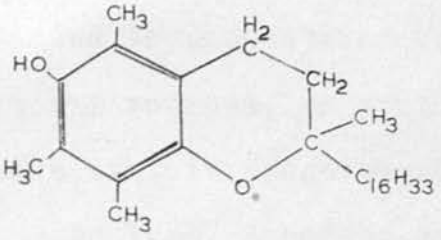
In view of these considerations, two lines of action are indicated.

(i) Normal subjects should be investigated by the modified method of Huber and/or patients with carcinoma should be investigated by the method of Sommerville, Gough and Marrian.

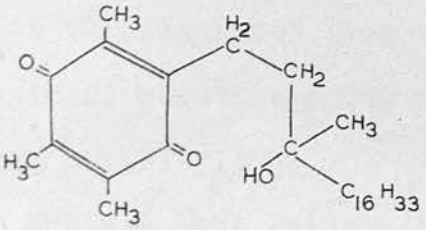
(ii) Normal subjects should be investigated in experiments in which both methods for the determination of urinary pregnanediol are applied to aliquots of each twenty-four hour specimen.

Whatever the outcome of such studies, it may be concluded from the present work, and the work of de Watteville et al. does not constitute evidence to the contrary, that, under these experimental conditions, the proportion of administered progesterone excreted as urinary pregnanediol by normal men or normal post-menopausal women is not influenced by pre-treatment with d,l-α-tocopherol acetate.

Figure 8.

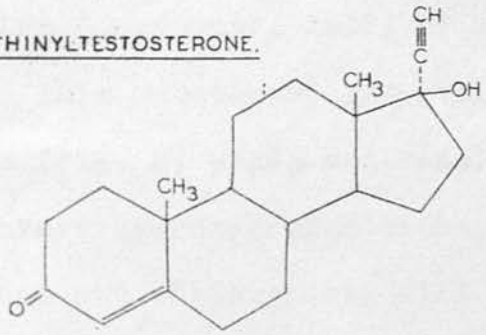


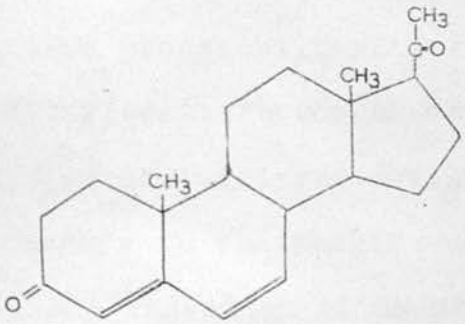
α-TOCOPHEROL



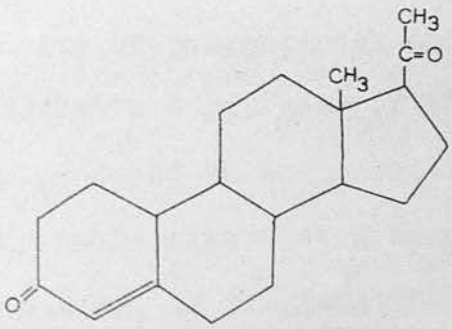
α-TOCOPHERYL QUINONE

17α-ETHINYLTESTOSTERONE.





6-DEHYDROPROGESTERONE



10-NORPROGESTERONE

SECTION III

THE ADMINISTRATION OF 17α-ETHINYLTESTOSTERONE AND ITS IMPLICATIONS TO URINARY PREGNANEDIOL DETERMINATION

Although a very large number of steroids closely related to progesterone have been isolated from natural sources, or synthesised, relatively few of these exhibit progestational activity.

In 1938, Inhoffen and Hohlweg, and Ruzicka et al. prepared a new synthetic progestogenic substance by the oxidation (Oppenauer, 1937) of an ethinylandrostenediol. This substance, 17α-ethinyl-Δ^4-androsten-17-ol-3-one (Fig. 8) which has received many names, notably anhydro-hydroxyprogesterone, pregneninolone, pregneninonol and ethisterone, will be referred to in the present work as ethinyltestosterone. A comparison of the progestational activity of progesterone and of ethinyltestosterone showed that a 4 mg. dose of the latter administered orally produced an endometrial response in the rabbit comparable with that produced by the injection of about 0.6 mg. of progesterone. In 1939, Courrier and Jost maintained pregnancy in an ovariectomised rabbit by the administration of ethinyltestosterone, and in the same year, Zondek and Rozin induced intracyclic bleeding by the administration of the steroid to women with normal menstrual habit /

habit and observed that the effective dose was about six times greater than that of intra-muscularly injected progesterone. The progestational activity of orally administered ethinyltestosterone has received ample confirmation (Emmens and Parkes, 1939; Salmon and Salmon, 1940; Weisbader, 1941; Zondek, 1942) and in addition, it has been shown to possess weak androgenic and oestrogenic activity (Emmens and Parkes, 1939).

Other steroids such as desoxycorticosterone and testosterone are much less active progestationally than ethinyltestosterone but a synthetic unsaturated derivative of progesterone prepared by Wettstein (1940) (Fig. 8) is more active, having approximately one-half of the biological activity of progesterone while 10-norprogesterone (Fig. 8) prepared by Ehrenstein in 1944 is claimed to be as active or even more active than the natural hormone. These substances differ from ethinyltestosterone, however, in that they do not possess its remarkable activity on oral administration and, therefore, lack its clinical possibilities.

A study of the literature on intermediary steroid metabolism reveals the surprising fact that almost nothing is known about the fate of ethinyltestosterone in the body. Hamblen, Cuyler and Hirst (1940) administered 320 mg. of ethinyltestosterone to a young woman /

woman with "uterine bleeding of an oestrogenic nature" and were unable to demonstrate the presence of pregnanediol glucuronide in the urine. Goldberg and Hardegger (1941) were also unable to detect pregnanediol glucuronide in such urine. In 1944, however, Allen, Viergiver and Soule administered 200 mg. of ethinyltestosterone daily for six days to patients with primary and secondary amenorrhoea and recovered about 10.0 per cent of the administered steroid as "pregnanediol". The method of determination (Allan and Viergiver, 1941) depends upon the estimation of the amount of glucuronic acid liberated by acid hydrolysis of urinary glucuronides and these workers admit that the final product determined as "pregnanediol glucuronide" might be the glucuronide of a related steroid.

In 1948, Dorfman, Ross and Shipley administered 300 mg. of ethinyltestosterone orally for two to four days to three human subjects - two men, one with Addison's disease, the other with diabetes and hypopituitarism, and one woman with secondary amenorrhoea. Neither "pregnanediol glucuronide" as determined by the method of Venning nor any other steroid conjugate was demonstrated in the urine.

Ethinyltestosterone is frequently employed in obstetrics and gynaecology as a substitute for progesterone. /

progesterone. It is important, therefore, to establish whether administered ethinyltestosterone is metabolised to urinary pregnanediol in view of the implications to the clinical use of pregnanediol determination, since, if this conversion does not occur the pregnanediol excreted by cases receiving ethinyltestosterone must be entirely of endogenous origin. In view of the contradictory results obtained by previous workers and in order to establish whether the method employed in the present work determines an excretion of urinary pregnanediol after ethinyltestosterone administration, the synthetic steroid was administered to three normal men. The shorter method described by Sommerville, Marrian and Kellar (1948) is commonly employed for clinical studies in pregnancy, and since this method is less specific than the method of Sommerville, Gough and Marrian (1948) it was important to compare the results obtained when both methods were applied to the same urine specimens.

1. Subject 'B':

Ethinyltestosterone, 150 mg. was administered by mouth on two consecutive days and the twenty-four hour urine specimens examined by the method of Somerville, Gough and Marrian (1948) on the day before the administration and until the level had returned to the control "blank" value

The results are shown in Table 15.

Apparent /

The results show that using method (i) from 0.1 to 0.6 per cent of administered ethinyltestosterone was determined as urinary pregnanediol and using method (ii) less than 1.0 per cent was so determined. The nature of the colour obtained with concentrated sulphuric acid indicates that the chromogenic substance is not pregnane-3α,20α-diol, and it was very interesting to observe that numerous large crystals appeared during the incubation of the precipitation mixture formed by the addition of N/10 sodium hydroxide to an ethanolic solution of the neutral toluene-soluble fraction. It was not possible to examine these crystals as the total final product was necessary for the determination, but they were seen to become less numerous after the second and third precipitations of the longer method. The large amount of crystals and the low chromogenicity of the final product after one precipitation (shorter method) indicate that this substance is weakly chromogenic with sulphuric acid. The administration of large amounts of ethinyltestosterone to subjects with negligible endogenous progesterone secretion should permit of the isolation and identification of this interesting substance which is presumably steroid in nature and may be a metabolite of ethinyltestosterone. If it is excreted as a glucuronide this would afford a reasonable explanation for the /

the results obtained by Allen, Viergiver and Soule (1944).

Conclusion

Assuming that the metabolism of ethinyltestosterone is qualitatively similar in pregnant and non-pregnant subjects these experiments indicate that when urinary pregnanediol excretion is studied in cases receiving ethinyltestosterone during pregnancy, using either the method of Sommerville, Gough and Marrian or that of Sommerville, Marrian and Kellar, the values obtained will not be confused by the presence of significant amounts of pregnanediol of exogenous origin.

In addition, the fact that this progestogen is not excreted as urinary pregnanediol could be applied to the further investigation of at least two problems discussed in the present work.

(a) The assumption that progesterone administration does not affect the level of excretion of urinary pregnanediol of endogenous origin could be investigated as far as progestational activity is concerned by determining the level of pregnanediol excretion during pregnancy following the administration of ethinyltestosterone.

(b) /

(b) The dependence of the progesterone "priming" effect upon the physiological activity of the hormone might be clarified by experiments in which ethinyltestosterone was administered to human subjects.

TABLE 15

ADMINISTRATION OF ETHINYLTESTOSTERONE (150 mg. x 2) TO NORMAL MEN

SUBJECT 'B'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
150 mg Oral	1580	0.20 0.25
150 mg Oral	2360	0.41 0.44
-	1360	0.49 0.46
-	1060	0.32 0.36
-	1590	0.26 0.23

SUBJECT 'R'

Admin.	Vol. c.c.	Pregnanediol mg./24 hr.
150 mg Oral	1350	0.18 0.14
150 mg Oral	1660	0.21 0.18
-	1580	0.27 0.30
-	970	0.11 0.11

TABLE 16

ADMINISTRATION OF ETHINYLTESTOSTERONE (200 mg.) TO NORMAL MAN

SUBJECT 'P'

Administration	Volume	Pregnanediol mg./24 hr.	
		Method (i)	Method (ii)
200 mg. Oral	940	0.11	0.28 0.32
-	1360	0.92	1.36 1.06
-	1120	0.35	0.95 0.90
-	900	0.21	0.22 0.17

Method (i) :- Sommerville, Gough and Marrian (1948)

Method (ii):- ----, Marrian and Kellar (1948)

SECTION IV

URINARY EXCRETION OF PREGNANEDIOL DURING NORMAL PREGNANCY

Following the pioneer work of Venning and Browne (Venning, 1937, 1938; Browne et al., 1937) on the quantitative determination of the urinary excretion of "pregnanediol glucuronide" during pregnancy, other workers, using the method of Venning confirmed and extended their observations (Bachman et al., 1940; Smith and Smith, 1940; Hain, 1942; Delfs and Jones, 1948).

These workers are in general agreement that there is a progressive increase in the excretion of the "pregnanediol glucuronide" (expressed as pregnanediol) from the eighth week of gestation when the level is about 8 to 10 mg. per twenty-four hours to the thirty-sixth week when it attains from about 70 mg. to 110 mg. per twenty-four hours. In many cases there then follows a gradual or swinging fall in the excretion of the steroid until immediately before labour it reaches a level 10 mg. to 20 mg. lower than its maximum. This pre-labour fall in pregnanediol excretion requires confirmation especially in view of the significance attached to it by Smith and Smith (1948) in connection with their theory of the interdependence of progesterone and oestrogen metabolisms. During labour there is

a /

a sudden fall in the level of excretion which reaches a non-pregnant level by about the third day of the puerperium.

Bachman et al. (1940) obtained a mean excretion curve which showed monthly cyclic fluctuations, but this has not been a general finding. Davis and Fugo (1947) studied the excretion of pregnanediol during normal pregnancy using a semi-quantitative modification of the method of Guterman (1946). When this method and the method of Venning were applied to the same urine specimen, similar results were obtained, and it was not surprising therefore that the curve of excretion of "pregnanediol" during normal pregnancy obtained by Davis and Fugo (1947) resembled that obtained for "pregnanediol glucuronide" by Venning (1938).

In view of the evidence, discussed in the introduction, that the Venning procedure determines as pregnanediol the glucuronides of substances other than pregnanediol, it seemed of interest to investigate whether the excretion of pregnanediol during pregnancy differed markedly from the excretion of the "pregnanediol glucuronide" determined by the method of Venning. In the method employed in the present work (Sommerville, Gough and Marrian, 1948) pregnanediol is isolated in relatively pure form before the application of the sulphuric acid reaction and, as has been discussed in the introduction, this method, being more nearly

specific /

specific for pregnanediol than any hitherto described does not determine pregnanolone or allopregnanolone in human pregnancy urine.

As a preliminary investigation, one hundred and fifty determinations were carried out throughout the course of three normal pregnancies and, in addition, thirty-two determinations were carried out during the first and second trimesters of a further sixteen normal pregnancies.

Twenty-four hour specimens of urine were collected from the patients in their homes until a few days before term when the patients were transferred to hospital. The urine was diluted snd its pregnanediol content determined in duplicate. The results are shown in Fig. 9.

RESULTS

It will be seen that in two of the cases studied- 'M' and 'L' - the patterns of excretion were very similar throughout pregnancy and that the levels in all cases during the first two trimesters were closely related. Until the fifteenth week of pregnancy, the levels ranged from 7 mg. to 14 mg. of pregnanediol per twenty-four hours and showed a rather flat but gradually ascending curve. The excretion then began to rise more rapidly until at mid-term a level of about 20 mg. /

20 mg. per twenty-four hours was attained. At the twenty-third week, the levels ranged from 22 mg. to 28 mg. per twenty-four hours but at this stage the excretion by case 'F' rose rapidly so that by the thirty-fourth week it had reached a plateau of about 90 mg. per twenty-four hours, whereas at that stage the other two cases had attained only 50 mg. per twenty-four hours. In cases 'L' and 'F' there was a fluctuating plateau from the thirty-third week until term but in case 'M' there was a steadily climbing excretion which did not reach a maximum until the thirty-eighth week. There was a swinging pre-labour fall in the former cases but not in the latter. In all three cases the excretion feel rapidly during labour and had reached a non-pregnant level of 0.2 to 0.5 mg. per twenty-four hours by the fourth day of the puerperium. Frequent determinations were carried out in case 'M' from the sixth week of pregnancy and the results which show some interesting features are illustrated in Fig. 10. There are few detailed reports of the level of pregnanediol excretion during the first trimester, and these have been characterised by the irregularity of the excretion from day to day. It is probable that such apparent irregularities of excretion at levels below about 15 mg. per twenty-four hour specimen were due to technical difficulties in the quantitative determination of the steroid. In this /

this case the level of pregnanediol excretion remained relatively constant from the sixth to the tenth week of pregnancy, from the tenth to the twelfth weeks there was a slight but definite depression and it was not until the succeeding weeks that the excretion level showed a definite rise.

DISCUSSION

Apart from the more regular pattern of excretion during early pregnancy, the trend of excretion of urinary pregnanediol is similar to that based upon the excretion of the glucuronides determined by the method of Venning.

There is, however, in the present work a suggestion that there is a quantitative difference between the results obtained by the two methods of determination. When the mean curves of excretion obtained by Venning (1938), Bachman et al., (1940) and Delfs and Jones (1948) were compared with a tentative mean for the excretion in the present work it was found that throughout pregnancy the latter was about 20 to 30 per cent. lower than the former. This finding would require confirmation by the study of a much larger series, but it is in excellent agreement with the observations of Marrian and Gough (1946). As discussed in the introduction, these workers found that samples of "sodium pregnanediol glucuronidate" obtained by the method /

method of Venning contain about 20.0 per cent of ketonic glucuronides notably sodium pregnanolone glucuronidate (Sutherland and Marrian, 1947). It is also compatible with the finding of Dobriner et al. (1948) that the urinary excretion of pregnanolone during pregnancy follows a similar trend to that of "pregnanediol glucuronide". The level of excretion of pregnanolone determined by Dobriner et al. is higher than would be expected even if the difference between the curves of excretion of "pregnanediol glucuronide"(expressed as "pregnanediol") and of pregnanediol is mainly due to the determination of sodium pregnanolone glucuronidate by the method of Venning. On the other hand it is most unlikely that pregnanolone glucuronide is recovered quantitatively by the method of Venning and the results of Dobriner et al. may reflect the physiological relationship. If pregnanolone is present in pregnancy urine in more than one conjugated form this would afford an alternative explanation for the apparent anomaly.

It is interesting to speculate that the absence of a significant rise in pregnanediol excretion in case 'M' (Fig. 10) from between the sixth and twelfth weeks of pregnancy, and the gradual rise in the excretion level which followed, reflect the smooth transfer of the main site of progesterone production from the corpus /

corpus luteum of pregnancy to the maturing placenta.

The quantitative determination of urinary pregnanediol excretion is receiving increasing attention as a potential prognostic criterion in pregnancies complicated by pre-eclamptic toxaemia or by threatened or habitual abortion. In assessing the significance of a pregnanediol determination in such cases, there is little doubt that previous determinations at an earlier stage of the same pregnancy constitute a better standard for comparison than does a mean curve of excretion for a series of normal pregnancies. This is frequently impossible, however, and precious time is lost in determining whether the level is rising or not. It is essential, therefore, that the scatter of normal values should be established, and it is clear from the preliminary results reported here and from the theoretical considerations previously discussed that, when a method reasonably specific for the determination of pregnanediol is employed, curves of excretion based upon the method of Venning are unsuitable for this purpose. It follows, that a large number of determinations should be carried out during normal pregnancy by a routine laboratory using the same method which is to be employed in the study of pregnanediol excretion in complicated pregnancies.

Figure 9.

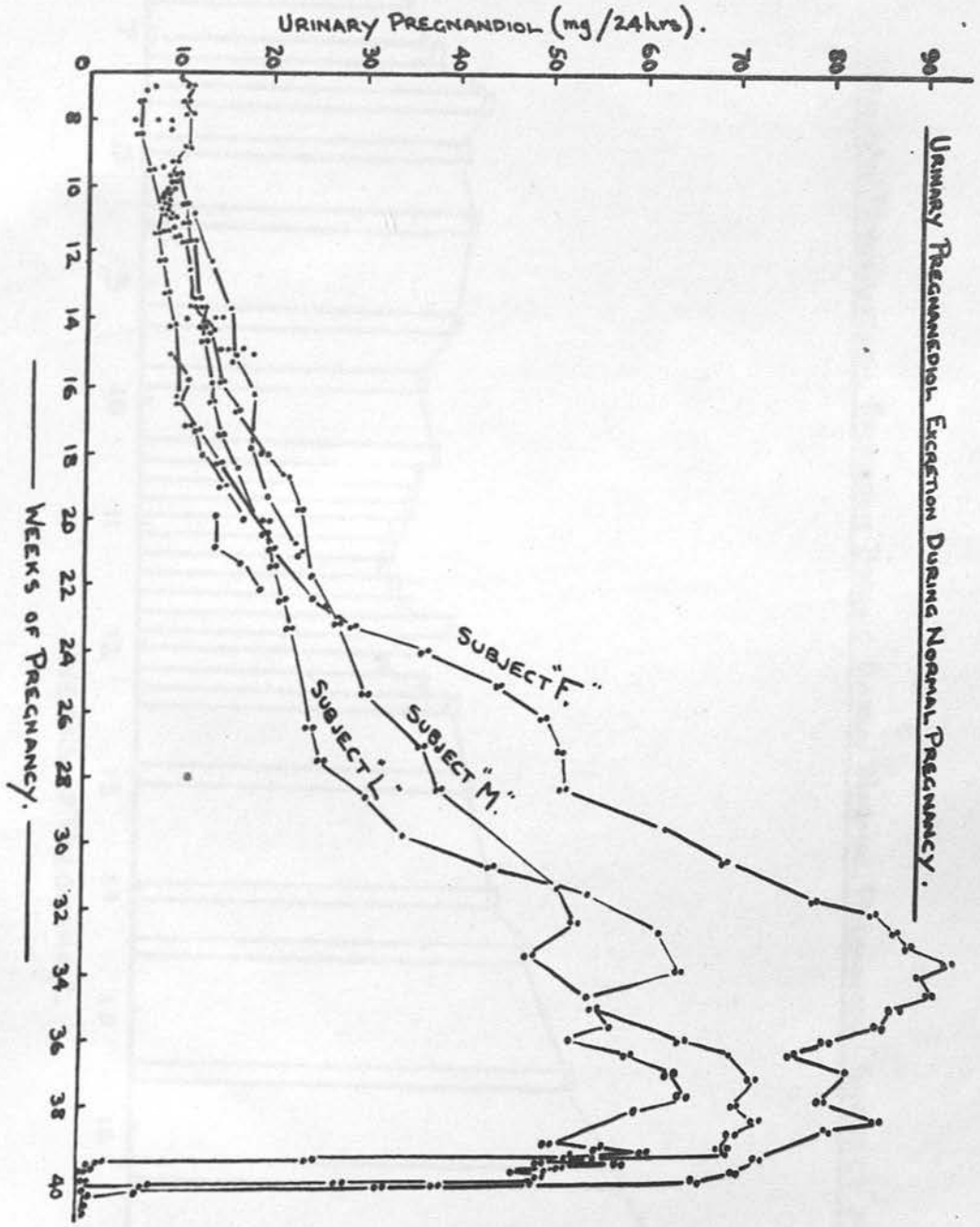
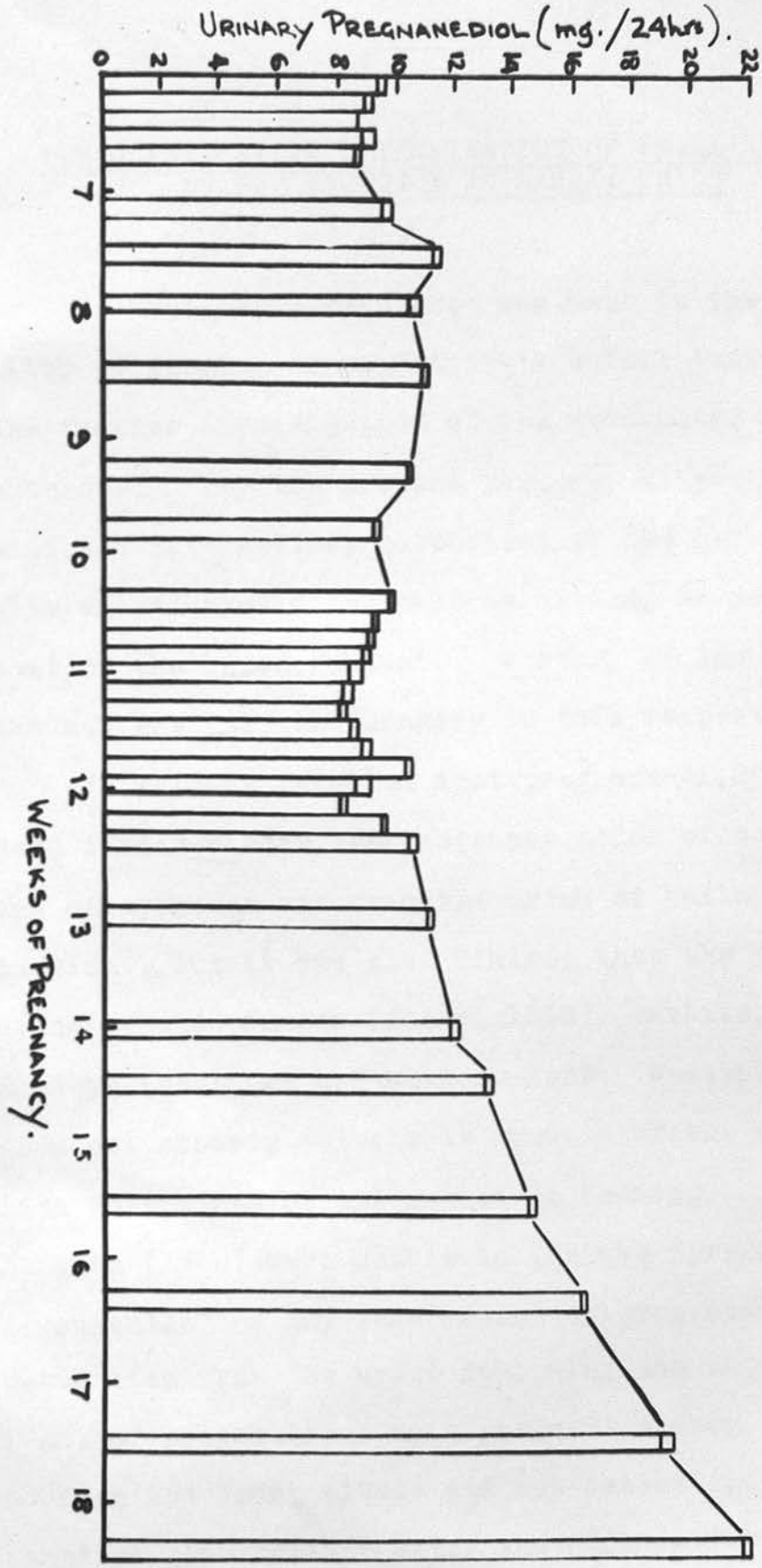

URINARY PREGNANDIOL (mg/24hrs).
0
10
20
30
40
50
60
70
80
90
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
WEEKS OF PREGNANCY.
URINARY PREGNANEDIOL EXCRETION DURING NORMAL PREGNANCY.
SUBJECT "F."
SUBJECT "M."
SUBJECT "L."

Figure 10.



URINARY PREGNANEDIOL EXCRETION DURING EARLY NORMAL PREGNANCY. SUBJECT "M".

SECTION V

THE QUANTITATIVE DETERMINATION OF SMALL AMOUNTS OF PREGNANEDIOL IN RABBIT URINE

In section I, reference was made to the desirability of finding an experimental animal suitable for the further investigation of the metabolism of progesterone. For the present purpose, it was necessary that the intermediary metabolism of the hormone in this animal should resemble as closely as possible that of the human subject. A study of the literature was not entirely encouraging in this respect.

It will be recalled that pregnane-3α,20α-diol had been isolated from the pregnancy urine of cows, mares and chimpanzees and from the urine of bulls (Introduction), but it was also claimed that the pregnancy urine of chimpanzees (Elder, 1940), rabbits, cats and monkeys (Westphal and Buxton, 1939; Westphal, 1942) does not contain detectable amounts of the glucuronides determined by the method of Venning. Marker and Hartman (1940) were unable to isolate "pregnanediol glucuronide" or any end-product of progesterone metabolism from the urine following the injection of 1 gm. of progesterone to a pregnant monkey, and Fish, Dorfman and Young (1942) did not detect urinary "pregnanediol glucuronide" after the administration of progesterone /

progesterone to guinea pigs. On the other hand, it has been clearly shown that "pregnanediol glucuronide" can be determined by the method of Venning in the urine of male and female rabbits after the administration of very large doses of the hormone (Heard, Bauld and Hoffman, 1941; Hoffman and Browne, 1942; Hoffman, 1942 and Westphal, 1942). It seemed possible that the apparent anomaly afforded by these results and by repeated failure to detect pregnanediol of endogenous origin in the urine of pregnant rabbits might be due to the low level of urinary excretion attained in these animals and to the relatively insensitive methods of determination employed.

Accordingly, an attempt was made to discover whether the more sensitive method of pregnanediol determination used in the present work was applicable to the determination of the steroid in rabbit urine.

Experimental Conditions

The rabbits were housed in metabolic cages, received 200 to 300 ml. of water daily and were fed on a pellet diet advocated by Dr. A.S. Parkes (Diet "43", Association of London Flour Millers). The urine was collected over twenty-four periods in vessels containing 0.5 ml. toluene as a preservative. The volume of each specimen was measured and the specimen was then diluted /

diluted by cage washings. Each specimen was made faintly acid to litmus with HCl in order that the pH. should be similar to that of human urine. Each specimen was then filtered through glass wool, made up to 500 ml. with water, and divided into two 250 ml. samples for the duplicate determinations. Since this volume was half that employed in the original method (Sommerville, Gough and Marrian, 1948) all volumes of reagents and solvents used in the determinations were halved. The only other modifications of the original method were the following:-

(i) In the precipitation procedure for the purification of the pregnanediol the times of incubation at 37°C were increased to twenty-four hours in each case.

(ii) The charcoal treatment for the elimination of interfering pigment was thought to be unnecessary with rabbit urine and was replaced by a simple filtration in ethanolic solution through a Whatman No. 1 paper.

Although it was anticipated that gums and resins foreign to human urine might interfere in the quantitative precipitation of the steroid, the following preliminary experiments were carried out with the method as above.

(a) Hydrolysis of the conjugated pregnanediol in rabbit urine

On the basis of the observation that a similar result is obtained when the pregnanediol excreted after progesterone administration to a rabbit is determined by the method of Venning, as when it is determined in the free form from acid-hydrolysed urine, Hoffman (1942) concluded that the steroid is present "for the most part if not entirely" as the glucuronide. It seemed probable that conditions of acid hydrolysis giving the maximum yield of free pregnanediol from human urine would also be optimal for rabbit urine containing conjugated pregnanediol. However as it could not be assumed that this was the case experimental verification was sought.

Progesterone (30 mg. intra-muscularly) was administered to two male rabbits on two consecutive days. Twenty-four hour specimens were collected on the second and third days after the first injection, the four specimens were pooled and diluted to 2 litres, and 8 samples each of 250 ml. were taken. Two samples of the pooled urine were each boiled for 5, 10, 15 and 20 minutes after the addition of 10 per cent. (v/v) HCl and the pregnanediol determined in the usual manner. The results shown in the following table indicate that, as in the case of human urine /

urine (Astwood and Jones, 1941), 10 minutes boiling gives optimal hydrolysis of the conjugated pregnanediol.

Time of Hydrolysis Minutes	Pregnanediol Recovered Mg.
5	0.250 0.265
10	0.291 0.289
15	0.255 0.235
20	0.236 0.184

(b) Accuracy of procedures for the extraction and purification of the pregnanediol

The sensitivity of the method was investigated in a series of recovery experiments in which pure pregnane-3α,20α-diol was added to acid-hydrolysed urine from male rabbits. Three twenty-four hour urine specimens were collected from three male rabbits, pooled, made up to 1,500 ml. with water and divided into six samples of 250 ml. Each sample was boiled under reflux for ten minutes after the addition of 25 ml. concentrated HCl and then cooled. Two of the acid-treated /

acid-treated samples were retained as male urine "blanks", while to the remaining four, pure pregnane-3α,20α-diol in ethanolic solution was added in two different known amounts in duplicate. This procedure was repeated on five occasions and the amounts of added pregnanediol were varied between 0.13 and 2.51 mg. The results of these experiments are shown in Table 17. It will be seen that whereas the recovery was unsatisfactory when 0.13 mg. was added to half a twenty-four hour specimen, good recoveries resulted when 0.23 mg. or more were added. A supply of highly purified sodium pregnane-3α,20α-diol glucuronidate was not available, but it has been shown (Sommerville, Gough and Marrian, 1948) that the loss incurred in the acid-hydrolysis of the glucuronide is negligible in the original method and it may be concluded that as little as 0.25 mg. of pregnanediol will be determined with a satisfactory degree of accuracy in one half of a twenty-four hour specimen of rabbit urine.

The urinary pregnanediol excretion following the intra-muscular injection of progesterone to a male rabbit is illustrated in Fig. 11. It will be seen that the pattern of excretion is very similar to that obtained after administration of progesterone to human subjects with minimal endogenous progesterone production. The proportion of administered progesterone excreted as urinary pregnanediol is also of the same order /

order as in such human subjects - in this case 6.9 per cent. The most striking feature of this experiment, however, is the agreement of the duplicate determinations and the knowledge that they accurately represent the excretion of the steroid at levels far below the range of excretion which could be determined by previous methods.

The importance of the availability of a method of determination of urinary pregnanediol of such sensitivity became apparent when consequent upon the present work, the urine of pregnant rabbits was investigated (Verly, Sommerville and Marrian, 1950). Not only was it now possible to detect pregnanediol in such urine - and to identify it as consisting almost entirely of pregnane-3α,20α-diol - but it was possible to follow the daily excretion of the steroid by duplicate determinations even although the plateau of pregnanediol excretion which was maintained throughout the greater part of each gestation did not rise above a level of about 0.8 mg. per twenty-four hour specimen. New possibilities are, therefore, offered for the investigation of the relationship between the intermediary metabolism and the physiological activity of the hormone.

Figure 11.

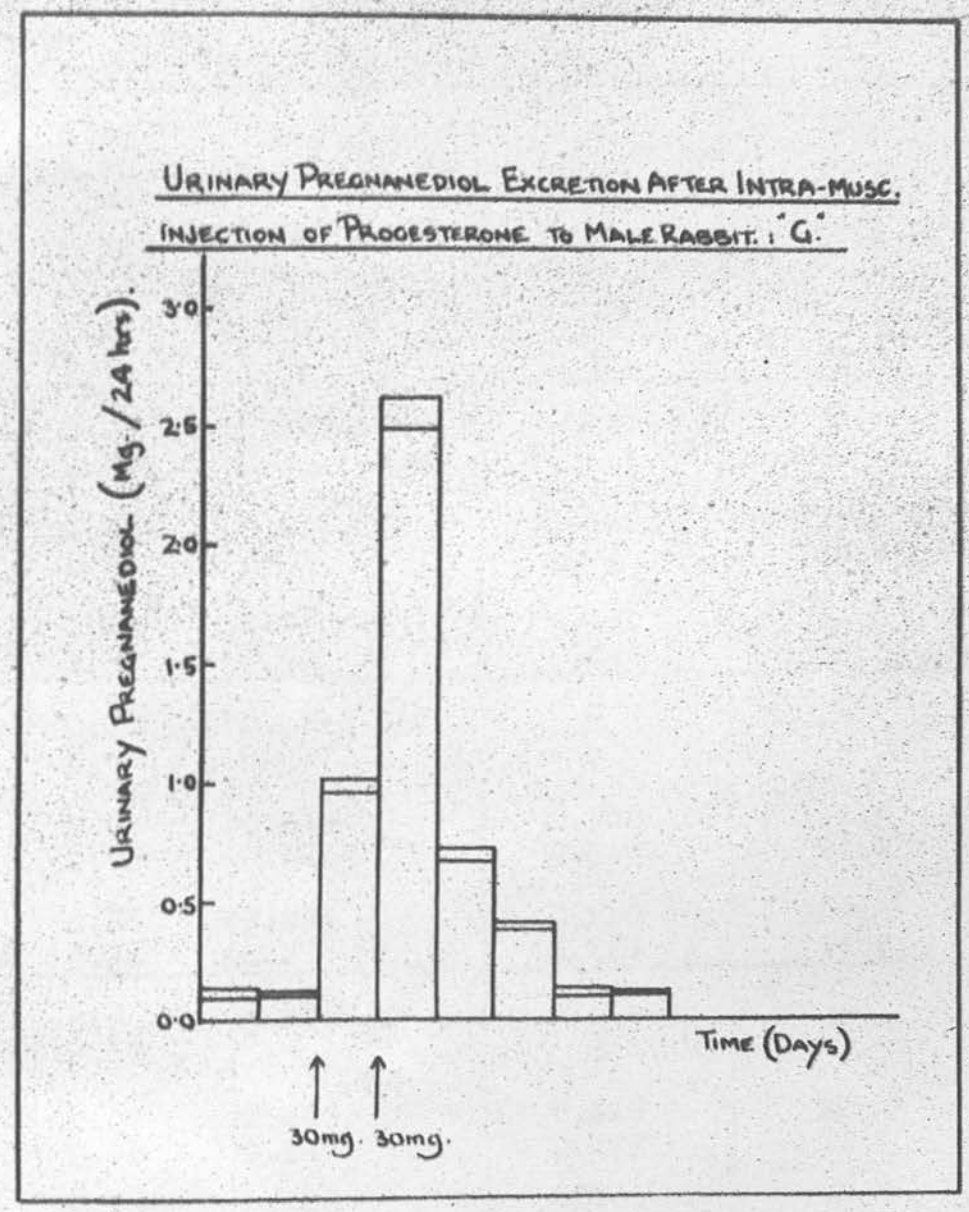

URINARY PREGNANEDIOL EXCRETION AFTER INTRA-MUSC.
INJECTION OF PROGESTERONE TO MALE RABBIT. : G.
URINARY PREGNANEDIOL (Mg./24 hrs).
3·0
2·5
2·0
1·5
1·0
0·5
0·0
TIME (Days)
30mg. 30mg.

TABLE 17

RECOVERY OF PREGNANEDIOL ADDED TO ACID-HYDROLYSED MALE RABBIT URINE

Pregnanediol added to equivalent of half of 24-hr. urine (mg.)	Apparent pregnanediol recovery (mg.)	Pregnanediol recovery corrected for "blank" (mg.)	Percentage pregnanediol recovery (corrected)
0.00	0.045) av.	-	-
0.00	0.030) 0.037	-	-
2.51	2.50	2.46	98.4
2.51	2.47	2.43	97.1
1.26	1.28	1.24	99.0
1.26	1.24	1.20	96.2
0.00	0.018) av.	-	-
0.00	0.023) 0.020	-	-
1.00	0.99	0.98	97.5
1.00	1.05	1.03	102.5
0.50	0.491	0.471	94.2
0.50	0.484	0.464	92.8
0.00	0.033) av.	-	-
0.00	0.037) 0.035	-	-
0.05	0.540	0.505	101.0
0.50	0.523	0.488	97.6
0.25	0.285	0.250	100.0
0.25	0.272	0.237	94.8
0.00	0.030	-	-
0.00	-	-	-
0.04	0.448	0.418	92.1
0.45	0.447	0.417	92.1
0.23	0.236	0.206	90.0
0.23	0.235	0.205	90.0
0.00	0.060) av.	-	-
0.00	0.037) 0.048	-	-
0.25	0.266	0.218	87.2
0.25	0.275	0.222	88.8
0.13	0.120	0.072	57.6
0.13	0.118	0.070	56.0

PART II

THE MECHANISM OF CORTISONE ACTION;
A STUDY OF THE METABOLISM OF PROGESTERONE
IN RHEUMATOID ARTHRITIC SUBJECTS

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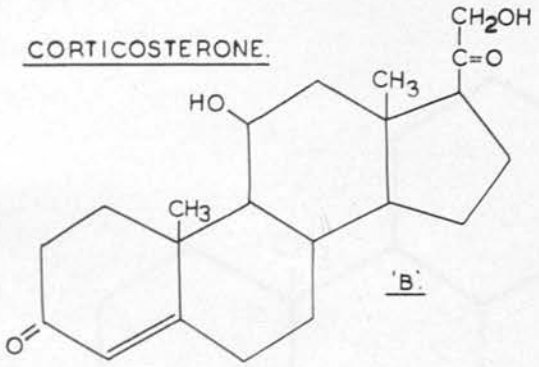
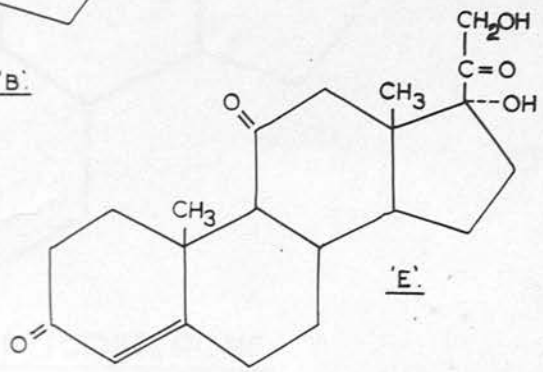
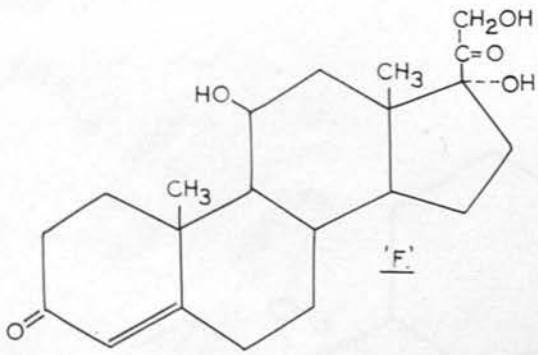
Figure 12.



CORTICOSTERONE.



17- HYDROXY- 11- DEHYDROCORTICOSTERONE



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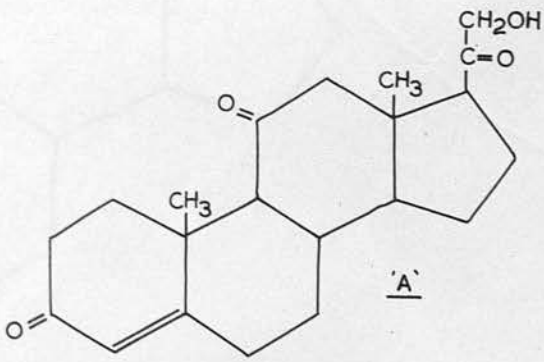
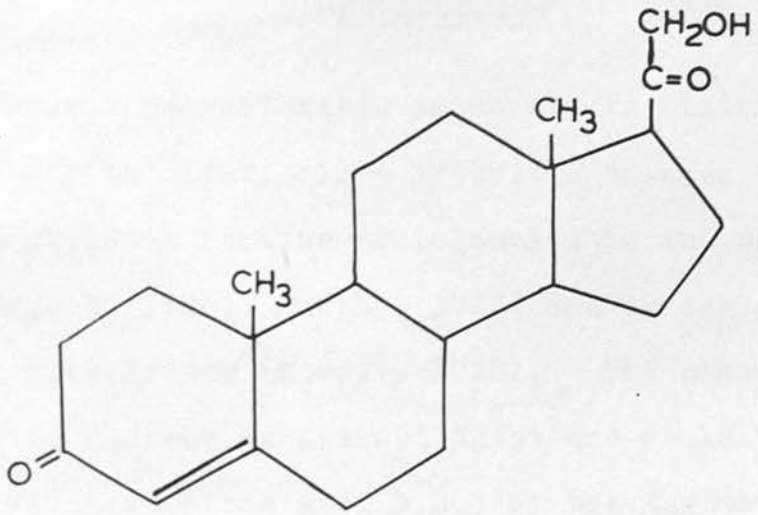
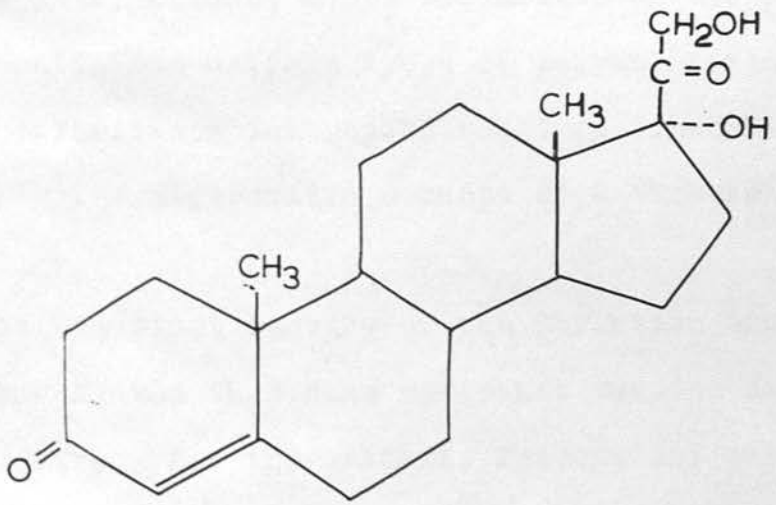


11 – DEHYDROCORTICOSTERONE

Figure 13.



11-DESOXYCORTICOSTERONE.



17-HYDROXY-11-DESOXYCORTICOSTERONE

[Reichstein's substance 'S'.]

PART II

INTRODUCTION

Chronic polyarthritis is as old as history, or indeed may be older, since arthritic changes are found in the skeletal remains of palaeolithic and neolithic man (Osgood, 1940; Murphy, 1943) and in the early fossil vertebrates (Moodie, 1923). The disease was common in ancient Persia and Egypt and would undoubtedly be studied (circa 2950 B.C.) by the Egyptian physician, Imhotep (Ruffer, 1921). Its clinical features were clearly described by Hippocrates, Asclepiades, Celsus, Galen and Aretaeus, and Galen - differentiating certain types of polyarthritis from gouty arthritis - introduced the term "rheumatism" reflecting the Hippocratic concept of a "humoral" aetiology.

In the first century of the Christian era, Aretaeus stated that "the medicines for the disease are innumerable; for the calamity renders the patients themselves expert druggists" and that if the disease "has acquired strength from time, all treatment is useless". An accurate account of the attempts which have been made to influence the onset or modify the course of arthritic diseases, especially of the rheumatic group, would form a fascinating record of human /

human endeavour over a period of some six thousand years.

That these attempts have, until very recently, been largely unsuccessful is shown by the fact that, at the present time, the rheumatic diseases and their sequelae are responsible for more disability, higher economic loss and greater human suffering than any other group of medical disorders (Comroe, 1949).

Countless theories concerning the aetiology of the diseases of this group have been formulated in the millennia during which they have been prevalent, and it is natural that, in more recent times, dysfunction of the endocrine glands should be considered as a possible aetiological factor. In fact, almost two thousand years ago, Celsus had suggested a correlation between reproductive function and the incidence of chronic polyarthritis.

> "Ea (vitia articulorum) raro vel castratos, vel pueros ante feminae coitum, vel mulieres, nisi quibus menstrua suppressa sunt, tentant".
>
> De Medicina; Book IV.

The observation of the rarity of articular disease in gonadectomised men is of especial interest since Celsus was able to study a larger series than is ever likely to be available in modern times.

The occurrence of menopausal arthralgia, the rarity of rheumatoid arthritis in hyperthyroidism, the arthralgia /

arthralgia of myxoedema, the spondylitis of acromegaly (Marie, 1886) and the recognition of endocrine factors in osteoporosis, all suggested possible inter-relationships between arthritis and dysfunction of the ovaries, the thyroid, the parathyroid and pituitary glands. The results of thyroid medication and parathyroidectomy were discouraging, however (Oppel, 1929; Shkurov, 1935), and although beneficial effects have been observed following oestrogen administration these have been mainly confined to menopausal arthralgia (Kuipers, 1935; Siemelink, 1945; Laroche & Hochfeld, 1948). None the less it is doubtful if adequate doses of oestrogen have ever been administered in clinical trials of oestrogen therapy in rheumatoid arthritis.

In 1890, Garrod noted that a remission in the progress of rheumatoid arthritis might occur during pregnancy. On the other hand, pregnancy has sometimes been regarded as a misfortune for such cases since the disease may have its onset or appear to be severely aggravated during pregnancy or the puerperium (Hench et al., 1940; Gil, 1944). The only large series of pregnancies studied in rheumatoid arthritics is that described by Hench (1938) from which it was concluded that pregnancy is more commonly beneficial than detrimental /

detrimental to the course of the disease.

In 1933, Hench had reported that hepatitis and jaundice had an ameliorating effect upon the course of chronic arthritis, and in 1938, was able to summarise studies on forty-five cases. He concluded that this effect of jaundice was invariably observed in cases with marked hyperbilirubinaemia. In the cases observed by Hench, the jaundice was of the obstructive type or due to infective or cincophen hepatitis.

From these studies made over a period of twenty years (1929 - 1949), Hench concluded that the "inherent reversibility of rheumatoid arthritis is activated more effectively by the intercurrence of jaundice and pregnancy than by any other condition or agent thus far known. Regardless of the superficial validity of the microbic theory, rheumatoid arthritis can be profoundly influenced by phenomena which are primarily biochemical" and that the lesion is "some basic biochemical disturbance which is transiently corrected by some incidental biologic change common to a number of apparently unrelated events".

For many years, attempts to derive therapeutic benefit from this concept met with repeated failure. Thus attempts to reproduce the ameliorating effects of pregnancy by the administration of female sex hormones (Hench, 1938) or the serum of pregnant women (Barsi, 1947) or to reproduce the effects of jaundice by

experimental /

experimental hyperbilirubinaemia (Hench, 1938; Thomson and Wyatt, 1947; Hanssen, 1942) were largely unsuccessful. Attention was then turned by Hench and his colleagues to the possibility of a clinical trial of the steroid hormones of the adrenal cortex since it had been clearly shown that the activity of the adrenal cortex is markedly increased during pregnancy (Venning, 1946; 1948).

Such an investigation could not have been encouraged by the knowledge that the incidence of rheumatoid arthritis is not significantly different in subjects with Addison's disease or Cushing's syndrome from that in previously normal individuals, nor by the observations of Selye (1944) on the induction of experimental "arthritis" by the administration of 11-desoxycorticosterone to rats sensitised by unilateral nephrectomy and a high salt diet. It is interesting to note that de-Gennes et al. (1947) have observed "subacute arthritis" which they attribute to implantation of 11-desoxycorticosterone acetate in two cases of Addison's disease. There would appear, therefore, to have been some evidence against the possibility that administration of the most readily available corticoid - 11-desoxycorticosterone - would be beneficial.

At /

At least twenty-eight crystalline steroids have been isolated from the adrenal cortex and of these six have been found to possess adreno-cortical activity. The residual amorphous fraction retains about 14 to 30 per cent. of the activity (based on life maintenance) of the total cortical extract (Heard, 1948). Four of these steroids have marked effects on carbohydrate metabolism (gluconeogenesis) but are relatively ineffective in life maintenance or in causing retention of sodium or chloride ions, whereas the remaining two steroids have a powerful action on electrolyte and water balance, but little effect on carbohydrate metabolism. The former group, possessing high gluconeogenic activity may be designated the gluco-corticoids and since they all possess a carbonyl or hydroxyl group in the C_{11} position may be termed the 11-oxygenated series. These steroids which are illustrated in Fig. 12 have the following structural characteristics.

(i) Corticosterone: (Reichstein, 1937).

Δ^4-pregnene-11β,21-diol-3,20-dione.

(ii) 11-dehydrocorticosterone: (Kendall, 1937)

Δ^4-pregnene-21-ol-3,11,20-trione. (Kendall's compound 'A').

(iii) 17-hydroxycorticosterone: (Reichstein, 1937)

Δ^4-pregnene-11β,17α,21-triol-3,20-dione (Kendall's compound 'F').

(iv) 17-hydroxy-11-dehydrocorticosterone: (Reichstein, 1936)

Δ^4-pregnene-17α,21-diol-3,11,20-trione (Kendall's compound 'E').

The steroids of the latter group which may be referred to as the mineralo-corticoids and which constitute the 11-desoxy series are illustrated in Fig. 13 and have the following structural characteristics.

(i) 11-desoxycorticosterone: (Reichstein and von Euw, 1938)

Δ^4-pregnene-21-ol-3,20-dione.

(ii) 17-hydroxy-11-desoxycorticosterone: (Reichstein and von Euw, 1938)

Δ^4-pregnene-17α,21-diol-3,20-dione: (Reichstein's compound 'S')

Thus if Selye's results in experimental "arthritis" were valid and applicable to the problem in man, and if none the less, the adrenal cortex was implicated in the "basic biochemical disturbance" postulated by Hench, it was reasonable that in the clinical trial one of the gluco-corticoids should be administered.

Whatever the reason for their choice in 1948, Hench in collaboration with Kendall began a clinical trial of 17-hydroxy-11-dehydrocorticosterone. This substance had been isolated from cortical extracts almost simultaneously by Reichstein; Mason, Myers and Kendall; and Wintersteiner and Pfiffner, in 1936. It was renamed "cortisone" for clinical purposes by Kendall in 1949.

In the series studied by Hench et al. (1949) cortisone and later cortisone acetate were administered to /

to fourteen cases of rheumatoid arthritis in doses of 100 mg. daily. The dramatic clinical response, which occurred within a few days of the first injection, included diminution in muscular and articular stiffness, disappearance of pain, swelling and deformity, increase in weight and appetite, correction of anaemia, fall in erythrocyte sedimentation rate and reversion of abnormal albumin/globulin ratios. Also included in this now famous series were two cases to whom the adreno-corticotrophic hormone of the anterior pituitary gland (A.C.T.H.) was administered. The clinical response was essentially similar to that obtained following the administration of cortisone. Certain implications of the administration of the adreno-corticotrophic hormone are considered in section III.

The report of Hench et al. aroused world-wide interest and, in the year which has elapsed, their observations on rheumatoid arthritis have been generally confirmed and attempts have been made to extend the use of this new form of therapy to related diseases. These clinical studies with their wide implications lie outwith the scope of this thesis but two related facts have emerged which colour the background of the present work. In the first place, it is clear that a relatively large dose of cortisone or A.C.T.H. is required to elicit the clinical response and that if this /

this therapy is not maintained a relapse to the pre-treatment clinical status occurs. Secondly, it is certain that in many if not the majority of cases prolonged administration of cortisone or A.C.T.H. is complicated by the advent of serious toxic effects. It would be unwise at the present time to attempt to assess the severity of these effects which include disturbances of electrolyte and water balance, of carbohydrate metabolism and of pituitary function, but they appear to be of such a nature as to preclude the prolonged replacement therapy necessary for permanent cure. This appears to constitute an even more serious bar to the application of the therapy than does the acute shortage of supply which will undoubtedly continue until methods for the synthesis of one or other hormone have been devised.

A search is being made for a suitable substitute for cortisone or A.C.T.H. but so far no non-toxic substance with comparable activity to cortisone has been described. The use of 11-desoxycorticosterone in combination with ascorbic acid is discussed in section IV.

It is reasonable to suppose that the probability of resolving these difficulties would be greatly increased by an elucidation of the mechanism of cortisone action. Furthermore, an understanding of this /

this mechanism should throw light upon the fundamental biochemical disorder which determines the occurrence and course of rheumatoid arthritis and of other responding diseases. The present work has been directed towards this end.

From a study of the papers of Hench et al. (1949) and of subsequent writings on cortisone and A.C.T.H. therapy, it appears that it is tacitly assumed that the success of this therapy indicates the existence of a deficiency of endogenous adreno-cortical hormones in rheumatoid arthritis. On the other hand, rheumatoid arthritic patients do not exhibit any of the generally recognised symptoms of adrenal insufficiency such as occur in Addison's disease, and conversely patients with Addison's disease are not especially prone to rheumatoid arthritis. Furthermore, much larger doses of cortisone are required to elicit the clinical response in rheumatoid arthritis than had been previously found to effect replacement therapy in Addison's disease (Sprague et al., 1947, 1948; Perara, 1941). These facts suggested to Professor Marrian and the author the possibility that the biochemical error in rheumatoid arthritis might consist of an abnormality in the intermediary metabolism of the adreno-cortical hormones rather than in a deficiency of their endogenous secretion.

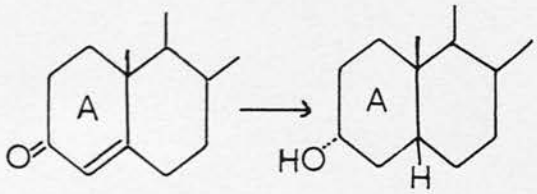
Apart /

Apart from the aetiological implications of such a theory its therapeutic implication must be obvious since, if this hypothetical abnormal metabolism could be corrected minimal amounts of cortisone would be required and these requirements might even be met by endogenous secretion. It is not possible to study quantitatively the metabolism of cortisone in rheumatoid-arthritic subjects since not only are insufficient quantities of the steroid available but its metabolites have not yet been established nor is it certain that methods for their quantitative determination could be devised.

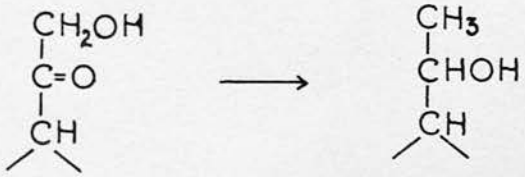
The related steroid, progesterone, (Δ^4pregnene-3,20-dione) has also been isolated from the adrenal cortex (Beall, 1938) and, as has been described in Part I, not only has the status of its metabolic reduction product - pregnanediol - been securely established and a method devised for its quantitative determination in human urine, but a considerable amount of information has been accumulated concerning the conversion of administered progesterone into urinary pregnanediol in normal people. It was of the greatest interest, therefore, to consider the possibility that the metabolic pathways followed by cortisone might be similar to those followed by progesterone, and that a study of the metabolism of progesterone /

progesterone in rheumatoid arthritic subjects might contribute to the present problem.

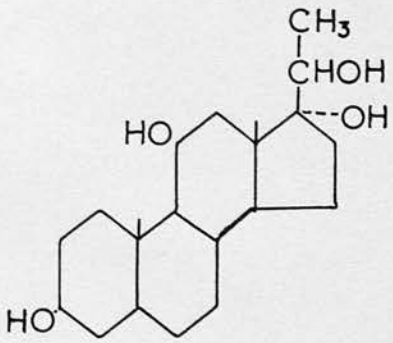
As illustrated in Fig. 14, the conversion of 11-desoxycorticosterone (as its acetate) to urinary pregnanediol can be accomplished by the human subject (Cuyler et al., 1940; Horwitt, et al. 1944). Thus the ring A of 11-desoxycorticosterone can be reduced in the same way as the ring A of progesterone.



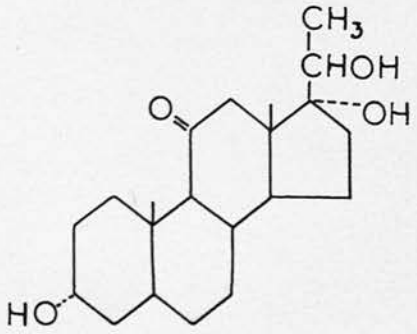
Similarly the side-chain of 11-desoxycorticosterone can be reduced in the same way as that of progesterone.



Similar metabolic reductions may, therefore, occur in other adreno-cortical hormones and thus, by analogy, 17-hydroxy-11-dehydrocorticosterone (cortisone) might yield a compound with the structure of the hypothetical pregnane-3α,11,17,20-tetrol (Fig. 15).

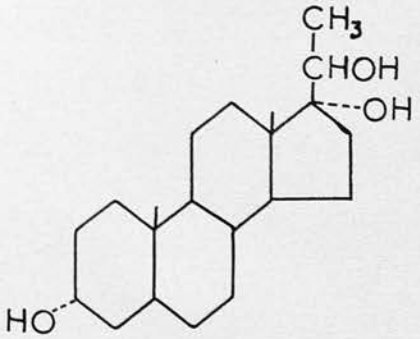


In addition, 20-ketosteroids analogous to pregnanolone or 11-ketosteroids such as the hypothetical pregnane-3α,17,20-triol-11-one might be formed (Fig. 15).

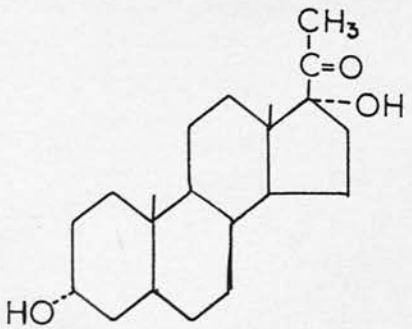


These speculations on the metabolic reduction of adreno-cortical hormones assume a certain probability in view of the isolation and characterisation of steroids from the urine of cases of adrenal hyperplasia. There is no doubt that these compounds could arise by the metabolic reduction of adreno-cortical hormones in a manner analogous to the metabolic reduction of progesterone to pregnanediol. These relevant steroids are:-

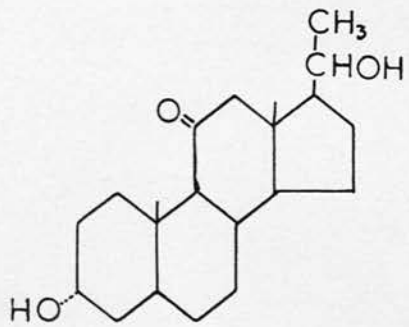
(i) pregnane-3α,17,20-triol: Isolated from the urine of women with adreno-cortical hyperplasia by Butler and Marrian (1937, 1938) and by Marker and Rohrmann (1939).



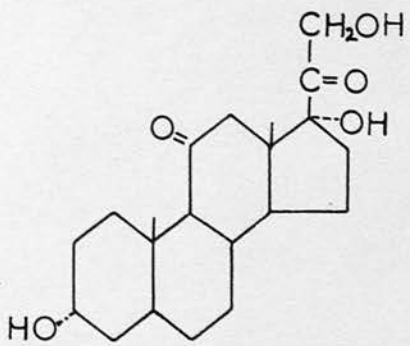
(ii) pregnane-3α,17-diol-20-one: Isolated from the urine of patients with adrenal hyperplasia, adrenal tumour and cryptorchidism, by Lieberman and Dobriner (1945).



(iii) pregnane-3α,20α-diol-11-one: Isolated from the urine of two cases of Addison's disease after the administration of large doses of 11-dehydrocorticosterone by Mason (1948).



(iv) pregnane-3α,17-21-triol-11,20-dione: Isolated from the urine of a man receiving A.C.T.H., by Lieberman, Hariton and Dobriner (1950).



The possible relationships of these steroids to the adreno-cortical hormones are shown in Fig. 15.

It /

It would not be surprising that if such hypothetical metabolites such as pregnane-3α,11,17,20-tetrol and pregnane-3α,17,20-triol-11-one had escaped isolation in the urine of cases with adreno-cortical hyperfunction since chance isolation would be rendered difficult by the acid-lability and high degree of water solubility of steroids with a tertiary hydroxyl group in the C 17 position.

All this evidence supports the contention that cortisone may to some extent be metabolised to analogues of pregnanediol or of pregnanolone. If this is so, then it would be reasonable to suppose that the enzymatic reducing system involved in the reduction of progesterone to pregnanediol would also participate in the metabolic reduction of cortisone. Accordingly it might reasonably be expected that an abnormality of the metabolism of cortisone would be associated with an abnormality of the metabolism of progesterone. The following experiments were carried out to investigate this hypothesis.

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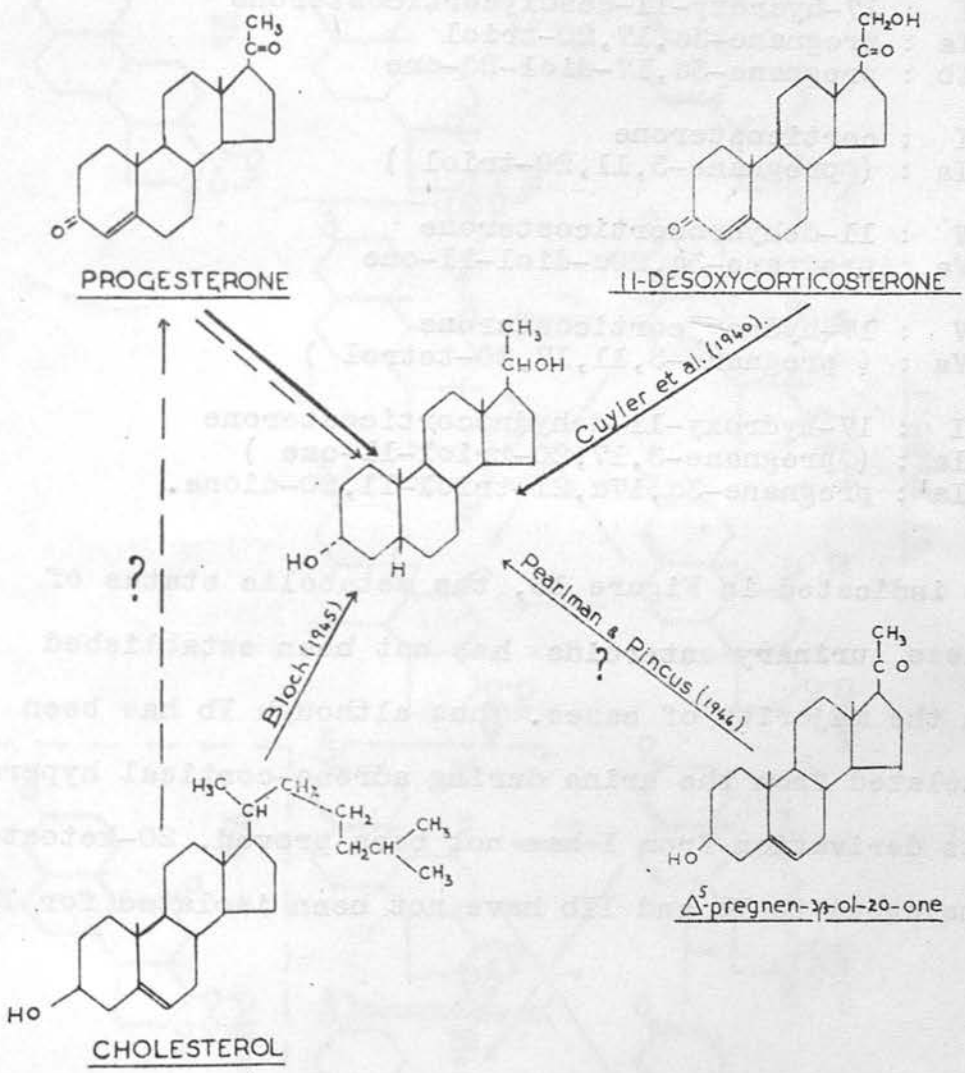
Figure 14.

METABOLIC PRECURSORS OF PREGNANE-3α,20α-DIOL:

[— all demonstrated in human subjects.]



KEY TO FIGURE 15.

I : 11-desoxycorticosterone
Ia : pregnane-3α,20α-diol
Ib : pregnan-3α-ol-20-one

II : 17-hydroxy-11-desoxycorticosterone
IIa : pregnane-3α,17,20-triol
IIb : pregnane-3α,17-diol-20-one

III : corticosterone
IIIa : (pregnane-3,11,20-triol)

IV : 11-dehydrocorticosterone
IVa : pregnane-3α,20α-diol-11-one

V : 17-hydroxycorticosterone
Va : (pregnane-3,11,17,20-tetrol)

VI : 17-hydroxy-11-dehydrocorticosterone
VIa : (pregnane-3,17,20-triol-11-one)
VIa' : pregnane-3α,17α,21-triol-11,20-dione.

(As indicated in Figure 15, the metabolic status of these urinary steroids has not been established in the majority of cases. Thus although Ib has been isolated from the urine during adreno-cortical hyperactivity its derivation from I has not been proved. 20-ketosteroids analagous to Ib and IIb have not been isolated for III to VI)

FIGURE 15.

SECTION I

PROPORTION OF ADMINISTERED PROGESTERONE EXCRETED AS URINARY PREGNANEDIOL BY RHEUMATOID ARTHRITIC SUBJECTS

The conversion of administered progesterone to urinary pregnanediol was investigated in a series of twenty-three rheumatoid arthritic subjects. Post-menopausal women (thirteen cases) and men (ten cases) were selected in order to ensure that the urine would not contain significant amounts of pregnanediol derived from progesterone of endogenous origin. The patients who were under the care of Dr. J.J.R. Duthie at the Rheumatic Unit of the Northern General Hospital, Edinburgh, were all suffering from rheumatoid arthritis and in no case was the diagnosis in doubt. The clinical status of these patients will be discussed later in this section.

The cases were divided into two series. In series (a), which comprised nine post-menopausal women and four men, 120 mg. of progesterone (60 mg. on two successive days) were administered by intra-muscular injection. This was a procedure identical to that previously employed by the author in a study of six normal post-menopausal women and four normal men. /

men. In series (b), which comprised three post-menopausal women and six men, 60 mg. of progesterone was administered by intra-muscular injection. Apart from this difference in progesterone dosage the experimental procedure was identical in all cases and was as follows.

Experimental Procedure

Progesterone dissolved in arachis oil was administered to the subjects in daily doses of 60 mg. Twenty-four hour urine specimens (preserved with toluene) were collected from each subject during a preliminary control period of one or two days, during the day or days on which the hormone was administered and for the following three to four days until the excretion of urinary pregnanediol had returned to the control period "blank" value. Each specimen was made up to a volume of 2,500 ml. with distilled water, and pregnanediol determinations in duplicate were carried out on 500 ml. samples by the method of Sommerville, Gough and Marrian (1948). The total pregnanediol excreted as a result of the administration of the hormone was calculated after correction of the daily apparent pregnanediol excretion for the average control-period "blank".

(a) Administration /

(a) Administration of progesterone (intra-muscular) to rheumatoid arthritic post-menopausal women and rheumatoid arthritic men

The results of thirty-three such metabolic experiments, comprising 210 pregnanediol determinations (in duplicate), are shown in Table 18, in which a comparison is made between the rheumatoid arthritic subjects in series (a) and the series of normal post-menopausal women and normal men. This comparison is illustrated in Fig. 16. The pattern of pregnanediol excretion following the administration of progesterone to a typical normal subject is compared with that in a typical rheumatoid arthritic patient in Fig. 17. Satisfactory agreement was obtained between the duplicate determinations in all cases, and the values given are calculated from the mean of the duplicates.

Table 18 /

TABLE 18

PERCENTAGE OF ADMINISTERED PROGESTERONE RECOVERED AS URINARY PREGNANEDIOL

Type of Subject	Normal	Rheumatoid Arthritic	
Post-Menopausal Women		19.0	(C)
	12.1	20.7	(L)
(a)	13.7	22.0	(M)
	15.2	25.1	(Y)
120 Mg. Intra-Musc.	15.6	25.7	(A)
	15.6	26.8	(D)
	16.0	31.0	(H)
		33.0	(McG)
		36.4	(Ca)
		18.8	(S)
(b)		20.5	(W)
		24.7	(Bo)
60 Mg. Intra-Musc.		36.5	(B)
Men	9.3	21.7	(F)
	10.0	23.8	(C)
(a)	12.2	26.1	(L)
120 Mg. Intra-musc.	14.7	27.4	(R)
		19.9	(Mu)
(b)		20.9	(M)
		22.4	(K)
60 Mg. Intra-Musc.		22.9	(Mo)
		32.8	(Be)
		37.3	(McN)

Each of these experiments is detailed below, and an attempt is made to assess the activity of the disease at the time of each progesterone metabolism experiment. This impression of "activity" (expressed as "quiescent", "active") is based mainly upon the acuteness of the articular lesions (pain, swelling, mobility), course of the disease (deteriorating, static, improving) and upon such features as elevation of erythrocyte sedimentation rate (E.S.R. Westergren), malaise, loss of weight, lack of appetite and degree of anaemia. This impression is open to the criticisms discussed in sub-section (c) but is to some extent supported by the independent assessment of the expert rheumatologists in charge.

Rheumatoid Arthritic Post-menopausal Women

Series (a)

Subject 'C': Age 46; duration of disease - 4 years.
Admitted with active disease but now quiescent, E.S.R. has fallen from 87 mm./ hr. to 56 mm./hr.
Anaemia persisting (Hb. 72 per cent.)
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 23.95 mg.
Corrected for control "blank": 22.75 mg.
Recovery from administered progesterone- 19.0 per cent.

Subject 'L': /

Subject 'L': Aged 65; duration of disease - 10 years.
Old quiescent case with acute exacerbation one year ago. Admitted to ward six weeks ago with active disease.
General condition improving but E.S.R. is stationary at 70 mm./hr.
Hb. 85 per cent.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 25.98 mg.
Corrected for control "blank": 24.78 mg.
Recovery from administered progesterone- 20.7 per cent.

Subject 'M': Aged 60; duration of disease - 9 years.
Quiescent case of long standing with steady level of E.S.R. which was 35 mm./hr. on admission and is now 28 mm./hr.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 27.46 mg.
Corrected for control "blank": 26.38 mg.
Recovery from administered progesterone- 22.0 per cent.

Subject 'Y': Aged 53; duration of disease - 5 years.
Admitted eight weeks previously with acute arthritis of both knee joints and with E.S.R. 71 mm./hr. Improving steadily. E.S.R. now 25 mm./hr. but still active.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 31.42 mg.
Corrected for control "blank": 30.12 mg.
Recovery from administered progesterone- 25.1 per cent.

Subject 'A': Aged 57; duration of disease - 10 weeks.
Admitted one month previously with very active disease. Condition improved but still active with acute involvement of one joint after another. E.S.R. - 120 mm./hr. Appetite poor. Patient looks toxic.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 31.73 mg.
Corrected for control "blank": 30.89 mg.
Recovery from administered progesterone- 25.7 per cent.

Subject 'D':/

Subject 'D': Aged 61; duration of disease - 7 years.
Moderately active disease for several years with prolonged immobilisation. E.S.R. 30 mm./hr. Complains of almost continuous pain even at rest. Obesity (14 st. 2 lb.)
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 33.82 mg.
Corrected for control "blank": 32.20 mg.
Recovery from administered progesterone- 26.8 per cent.

Subject 'H': Aged 68; duration of disease - 15 years.
Active disease with fluctuating course. Condition steadily improving. E.S.R. rising slightly from 25 mm./hr. on admission to 40 mm./hr. at time of metabolic experiment. No anaemia.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 38.68 mg.
Corrected for control "blank": 37.18 mg.
Recovery from administered progesterone- 31.0 per cent.

Subject 'McG': Aged 58; duration of disease - 1 year.
Subacute onset following delayed healing of fracture of right wrist. Left knee swollen, quadriceps atrophic. Complains of sacral pain. E.S.R. falling, now 50 mm./hr., and otherwise disease moderately quiescent.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 40.49 mg.
Corrected for control "blank": 39.59 mg.
Recovery from administered progesterone- 33.0 per cent.

Subject 'Ca': Aged 49; duration of disease - 4 years.
Very active disease especially affecting knee joints with E.S.R. fluctuating between 24 mm./hr. and 52 mm./hr. Anaemia responding to ferrous sulphate with Hb. rising from 66 per cent. to 80 per cent. As with subject 'D' the patient complains of severe pain at rest which has been increased by the withdrawal of salicylates.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 44.86 mg.
Corrected for control "blank": 43.72 mg.
Recovery from administered progesterone- 36.4 per cent.

Series (b)

Subject 'S': Aged 50; duration of disease - 9 months. Subacute onset with generalised stiffness and joint pain. Disease quiescent and patient "much improved" with few complaints. None the less E.S.R. is 62 mm./hr. A resistant anaemia has responded to intra-venous iron therapy but is still present. (Hb. 84 per cent.) May be significant that the patient has had three severe attacks of acute rheumatic fever since childhood.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 9.93 mg.
Corrected for control "blank": 9.42 mg.
Recovery from administered progesterone- 18.8 per cent.

Subject 'W': Aged 69; duration of disease - 6 months. A recent case with minimal articular damage but very high E.S.R. (117 mm./hr) and anaemia (Hb. 58 per cent.) Improving. Joints relatively pain-free but splinted.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 13.05 mg.
Corrected for control "blank": 12.29 mg.
Recovery from administered progesterone- 20.5 per cent.

Subject 'Bo': Aged 50; duration of disease - 29 years. Multiple arthritis with gross articular damage. Disease quiescent with exception of hip joints.. E.S.R.22mm/hr. and Hb. 68 per cent.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 15.63 mg.
Corrected for control "blank": 14.79 mg.
Recovery from administered progesterone- 24.7 per cent.

Subject 'B':/

Subject 'B': Aged 58; duration of disease - 2 years.
Multiple arthritis, active, but with minimal articular damage. Condition improving with E.S.R. falling from 47 mm./hr. on admission to 23 mm./hr. Hb. 80 per cent. Patient found to be suffering from hitherto undiagnosed diabetes mellitus.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 22.69 mg.
Corrected for control "blank": 21.89 mg.
Recovery from administered progesterone- 36.5 per cent.

Rheumatoid Arthritic Men

Series (a)

Subject 'F': Aged 58; duration of disease - 14 years.
Steady clinical improvement since admission with correction of anaemia - (Hb. 87 per cent.) and falling B.S.R. (48 mm./hr.) Disease still moderately active.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 27.45 mg.
Corrected for control "blank": 26.05 mg.
Recovery from administered progesterone- 21.7 per cent.

Subject 'C': Aged 60; duration of disease - 8 years.
Gross changes in knee joints with effusion and marked wasting of quadriceps. Knees very painful even at rest. Active disease but condition static. E.S.R. 63 mm./hr.
Hb. 56 per cent.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery:29.88 mg.
Corrected for control "blank": 28.50 mg.
Recovery from administered progesterone- 23.8 per cent.

Subject 'L':/

Subject 'L': Aged 45; duration of disease - 1 year.
Subacute onset with pain and stiffness in neck and shoulders. Fluctuating course with ankles and knees mainly affected. Left knee swollen and painful even at rest. E.S.R. 40 mm/hr. Active disease. No anaemia.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 32.49 mg.
Corrected for control "blank": 31.34 mg.
Recovery from administered progesterone- 26.1 per cent.

Subject 'R': Aged 40; duration of disease - 4 years.
Moderately active disease with pain mainly extra-articular.
E.S.R. 39 mm./hr. but rising.
Anaemia has responded to ferrous sulphate.
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 34.45 mg.
Corrected for control "blank": 32.89 mg.
Recovery from administered progesterone- 27.4 per cent.

Series (b)

Subject 'Mu': Aged 62; duration of disease - 14 years.
Quiescent case of very long standing.
E.S.R. 15 mm./hr. Arteriosclerosis.
History of trauma to the right shoulder- the most severely affected joint.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 13.16 mg.
Corrected for control "blank": 11.92 mg.
Recovery from administered progesterone- 19.87 per cent.

Subject 'M':/

Subject 'M': Aged 46; duration of disease - 7 years.
E.S.R. has remained elevated (45 mm./hr.) despite excellent improvement in clinical condition following a recent attack of scarlatina. No anaemia and minimal damage to joints. Residual disability in the wrist joints which were the most severely affected. Interesting to note occupation - baker. Quiescent.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 11.10 mg.
Corrected for control "blank": 10.47 mg.
Recovery from administered progesterone- 20.94 per cent.

Subject 'K': Aged 24; duration of disease - 6 months.
Moderately active case with rising E.S.R. which was only 12 mm./hr. at the time of the metabolic experiment but rose to 54 mm./hr. within a month. Improving slowly. General condition excellent.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 14.22 mg.
Corrected for control "blank": 13.46 mg.
Recovery from administered progesterone- 22.43 per cent.

Subject 'Mo': Aged 57; duration of disease - 1 yr. 3mth
Very acute exacerbation occurred four months ago. There is now multiple arthritis but articular changes are not severe. Patient appears toxic and complains of continuous pain at rest after withdrawal of salicylates. E.S.R. 83 mm./hr. Anaemia (Hb. 70 per cent.) - i.e. active case: Very active.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 14.42 mg.
Corrected for control "blank": 13.70 mg.
Recovery from administered progesterone- 22.9 per cent.

Subject 'Be':/

Subject 'Be': Aged 46; duration of disease - 2 years. Acute onset affecting left tarsal joints and left knee. Responded well to gold therapy one year ago but left knee now very painful with effusion. Anaemia following gold therapy has been corrected. E.S.R. 30 mm./hr. Active disease.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 20.36 mg.
Corrected for control "blank": 19.68 mg.
Recovery from administered progesterone- 32.8 per cent.

Subject 'McN': Aged 59 - duration of disease - 3 years. Multiple arthritis with marked limitation of movement especially of hips and much pain on walking especially after withdrawal of salicylates. Condition static but disease still very active. E.S.R. 90 mm./hr. Anaemia (Hb. 74 per cent.)
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 23.66 mg.
Corrected for control "blank": 22.38 mg.
Recovery from administered progesterone- 37.3 per cent.

Conclusion

The results indicate clearly that rheumatoid arthritic subjects of both sexes excrete in the urine as pregnanediol an abnormally high proportion of intramuscularly injected progesterone. The percentage conversion by normal subjects ranged from 9.3 per cent. to 16.0 per cent whereas that of rheumatoid arthritic subjects ranged from 18.8 per cent. to 37.3 per cent.

Although only ten identical metabolic experiments have so far been carried out in normal human subjects, the normal range indicated by these experiments is supported by a larger number of similar experiments previously /

previously carried out by the author. Furthermore, in previous experiments the proportion of administered progesterone excreted as urinary pregnanediol did not appear to be influenced by the dosage employed, since similar values were obtained following the administration of doses varying from 40 mg. to 240 mg. (Sommerville and Marrian, 1950a, b). Thus, in the experiments in which 100 mg. of progesterone were administered prior to α-tocopherol therapy (Part I, Section II) and in the preliminary plateaux of pregnanediol excretion obtained during prolonged administration of the hormone (Part I, Section I) it may be deduced that the proportion of administered hormone excreted as urinary pregnanediol fell within this range. The total amount so excreted could not be accurately assessed, however in the experiments of prolonged duration and in experiments in which the progesterone was administered by mouth the proportion which would have been excreted following intra-muscular administration could only be tentatively deduced by the substraction of 2 to 3 per cent., assuming that the proportion excreted after oral administration is about 2 to 3 per cent. higher than after intra-muscular administration (Part I, Introduction) (Sommerville and Marrian, 1950a). None the less, with one exception, all these studies in normal human subjects are compatible with the normal range in the present series.

The exceptional case is that of Subject 'D' who was studied in Part I, Section I(a). This was an apparently "normal" post-menopausal woman to whom 40 mg. of progesterone was administered orally for twenty-two days. The first plateau of pregnanediol excretion corresponded to approximately 25 per cent. of the administered hormone and thus to about 22 per cent. of intra-muscularly administered progesterone. This value is outwith the suggested normal range, and it was of the greatest interest to recall that at the time of the experiment the patient was complaining of vague arthralgias and of "arthritis" of the right knee. No significance had been attached to these features at that time, but at a recent visit the patient volunteered the information that the "menopausal arthritis" had now resolved.

As illustrated in Figs. 16 and 17, the duration of the excretion of pregnanediol following the administration /

administration of progesterone to rheumatoid arthritics is more prolonged than that observed in normal subjects, as would be expected in view of the greater amount of the steroid excreted. In the present series the increase in the proportion of the administered hormone converted to urinary pregnanediol over that converted by normal subjects (mean value: 13.4 per cent.) varied from 40 per cent. to 180 per cent., and the results clearly indicate an abnormality of progesterone metabolism associated with rheumatoid arthritis.

Some salicylates are known to be excreted in the urine in conjugation with glucuronic acid and the possibility had to be considered that salicylates might in some way influence the excretion of pregnanediol by interfering with the formation of pregnanediol glucuronide. Accordingly, the precaution was taken of witholding all salicylates for one to eight weeks prior to the metabolic experiment. There remained, however, the theoretical possibility that previous prolonged therapy by salicylates might have affected the metabolism of progesterone. To investigate this possibility, the following experiment was carried out.

Subject 'J': /

Subject 'J': Man aged twenty-five. Admitted to hospital five weeks previously with acute rheumatic fever. The patient had received acetyl salicylic acid ('Dysprin') 30 gr. daily during that time and the disease was now quiescent. The salicylate was continued throughout the metabolic experiment, which was carried out as described above. The proportion of administered progesterone converted to urinary pregnanediol by this convalescent patient was 10.2 per cent.

This result clearly indicates that a normal proportion of administered progesterone can be excreted as urinary pregnanediol despite the concurrent administration of large doses of salicylates, and that the high recoveries observed in rheumatoid arthritics - some of whom had received no such therapy during the previous two months - cannot be attributable to such therapy.

(b) Administration of progesterone (intra-muscular) to atypical cases of rheumatoid arthritis

The cases reported above exhibited the characteristic clinical features of active rheumatoid arthritis. Two further cases were investigated which do not fall into this category and the results were ofespecial interest.

Subject 'B':/

Subject 'B': A man aged forty-one years, with rheumatoid arthritis of eight years' duration. The disease had an acute onset and a virulent course. It was now completely quiescent, however, with E.S.R. 2 mm./hr (Westergren), leaving the patient with marked limitation of movement and gross damage to shoulder, elbow and hip joints.
Progesterone (60 mg.) was administered by intra-muscular injection.
Apparent pregnanediol recovery: 9.24 mg.
Corrected for control "blank": 8.32 mg.
Recovery from administered progesterone- 13.87 per cent.

Subject 'S': A post-menopausal woman, aged 48 years, with arthritis of twelve years' duration. A diagnosis of osteo-arthritis had been made and arthroplasty of both hip joints was advocated following a poor response to deep X-ray therapy. It was noted, however, that the E.S.R. was raised - 16 mm./hr. at the time of the investigation and the possibility was considered that there might be latent or superimposed rheumatoid arthritis (there appeared to be no other cause for the elevated E.S.R.)
Progesterone (60 mg. intra-muscular) was administered
Apparent pregnanediol recovery: 12.14 mg.
Corrected for control "blank": 11.69 mg.
Recovery from administered progesterone- 19.5 per cent.

Conclusion

The result in subject 'B' confirms the clinical impression of a case in which the disease - initially acute and destructive - had "burned itself out". In subject 'S' the progesterone metabolism experiment appears to afford a criterion of the activity of the disease /

disease and a possible means of differentiating such complicated cases from authentic osteo-arthritis.

(c) Repetition of progesterone metabolism experiment in rheumatoid arthritic subjects and attempt to correlate the clinical status with the abnormality of steroid metabolism

The experimental procedure was repeated later in the course of the disease in seven of the rheumatoid arthritic subjects investigated in the original series. The results of these progesterone metabolism experiments are shown in the following table.

Repetition of Progesterone Metabolism Experiment
Progesterone, 60 mg. Intra-musc.

Case	Per Cent. Recovered as Pregnanediol		Time Interval (weeks)
	1st Experiment	2nd Experiment	
'F'	21.7	24.4	5
'McG'	33.0	29.2	5
'H'	31.0	24.9	5
'C'	36.4	34.6	6
'McN'	37.3	36.2	6
'A'	25.7	25.2	12
'D'	26.8	28.1	12

Conclusion /

Conclusion

This series affords striking evidence that in the majority of cases the abnormality of steroid metabolism associated with rheumatoid arthritis remains remarkably unaffected by the fluctuations in clinical status which are so characteristic of the disease. The following paragraphs summarise changes in the clinical condition of the patients since the previous progesterone metabolism experiments.

Subject 'F': No marked change in clinical condition during the intervening five weeks but E.S.R. has fallen from 48 mm./hr. to 10 mm./hr.

Subject 'McG': No marked change in clinical condition. Anaemia persists (Hb. 83 per cent.) E.S.R. 33 mm./hr.

Subject 'H': Definite improvement in general condition Movements freer and much less painful. Patient now fully ambulatory. E.S.R. has fallen from 40 mm./hr. to 9 mm./hr.

Subject 'C': Moderate improvement in general condition, but disease still active and patient complains of pain in the splinted joints.

Subject 'McN': Definite improvement in general condition but disease still active and pain and stiffness have been increased following withdrawal of salicylates.

Subject 'A':/

Subject 'A': No marked change in clinical status since the first experiment although there have been several transient exacerbations during the intervening three months. Disease is still very active.

Subject 'D': During the intervening six months there was some clinical improvement but recently the patient complained increasingly of pain on weight-bearing. E.S.R. has not fallen (now 45 mm./hr) and anaemia has increased (Hb. 70 per cent.).

It is obviously of some importance to determine whether the deviation from normal of the metabolism of progesterone in rheumatoid arthritics reflects their clinical condition.

Attempts to find a correlation between clinical status and the metabolic abnormality studied in the present work are rendered difficult by the inadequacy of quantitative objective criteria in rheumatoid arthritis. The functional capacity of rheumatoid arthritics may be measured in various ways but active movement may be limited more by pain than by muscular stiffness or articular changes and thus the functional capacity is profoundly influenced by the psychological factors which determine the threshold for pain. Where tests are devised to afford a quantitative measurement of functional capacity skill increases with practice. Furthermore, a complicating factor in the present series was the necessity to withdraw salicylate therapy, thereby intensifying pain and stiffness. /

stiffness in many cases.

Similarly, it is difficult to derive a quantitative expression of the activity of the disease. The criteria employed have been listed in sub-section (a) and although these of themselves may be valid they are not readily converted into an "activity index".

Reviewing the cases studied, it is apparent that no correlation exists between the degree of steroid metabolic abnormality and the age of the patient, the duration of the disease, the height of the E.S.R. or the degree of anaemia. There is, however, a suggestion that in cases with minimal metabolic abnormality the disease is more "quiescent" and in cases with maximal metabolic abnormality the disease is more "active". Since this impression is not based upon elevation of E.S.R. or the degree of anaemia it must refer to the articular changes and to the general condition of the patient. Accordingly, it seems to the author that a more detailed study of the local changes in the joints of such patients may throw some light upon the basis of the abnormality of steroid metabolism. The "burned out" case (Subject 'B') is of especial interest. In such a case the gross joint lesions represent the ravaged aftermath of a disease process which is no longer active, and in this case the metabolism of administered progesterone was normal.

The /

The fact that in the present study the severity of the metabolic abnormality was not proportional to the number of joints involved is not discouraging to this hypothesis since the number involved does not necessarily reflect the intensity of the articular pathology. A study of joint temperature changes or histological studies by biopsy might be of value in this connection, and it will be of interest to study the steroid metabolism of patients suffering from forms of acute arthritis.

Figure 16.

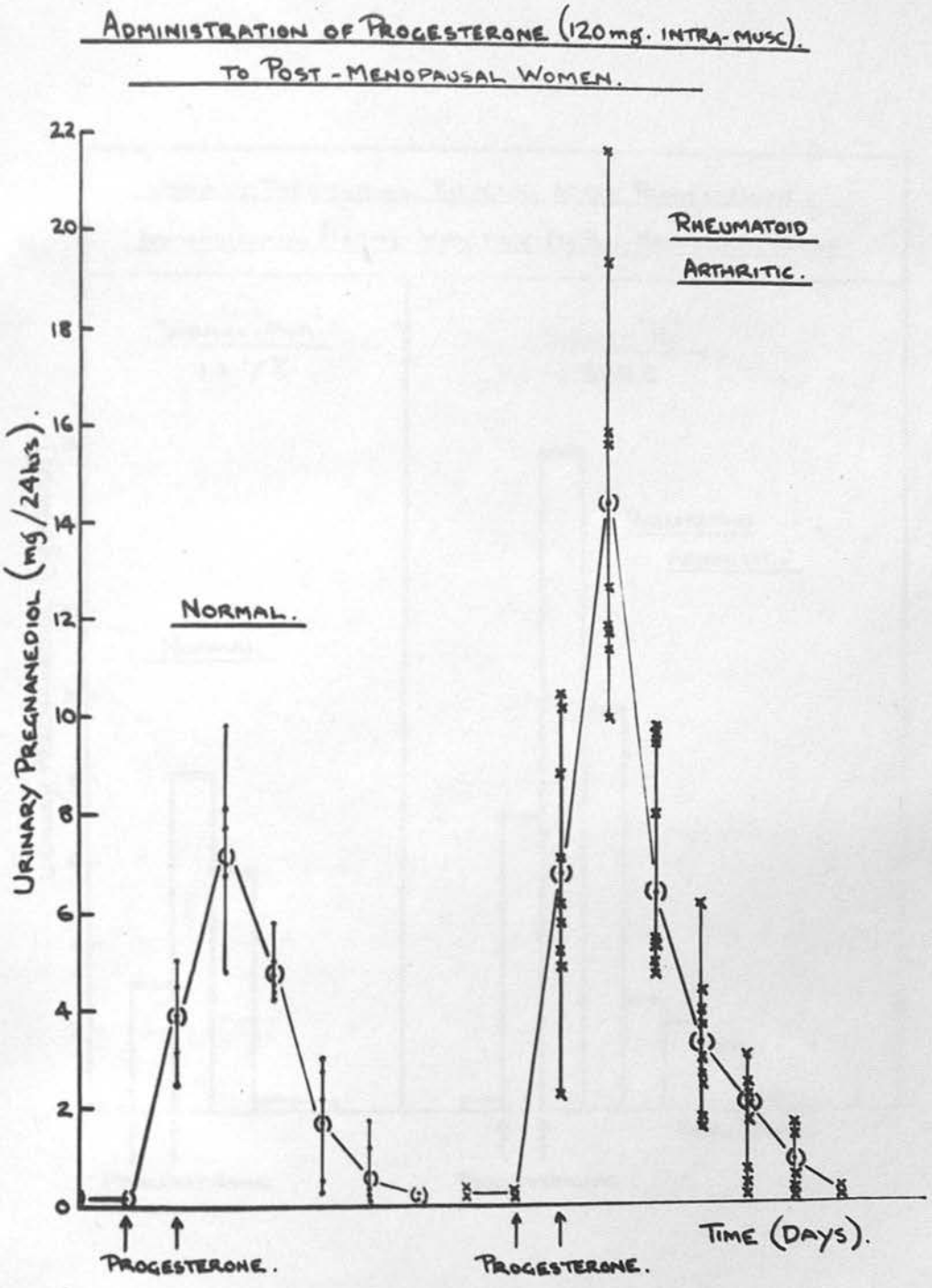

ADMINISTRATION OF PROGESTERONE (120 mg. INTRA-MUSC).
TO POST-MENOPAUSAL WOMEN.
URINARY PREGNANEDIOL (mg/24hrs).
22
20
18
16
14
12
10
8
6
4
2
0
NORMAL.
RHEUMATOID
ARTHRITIC.
PROGESTERONE.
PROGESTERONE.
TIME (DAYS).

Figure 17.

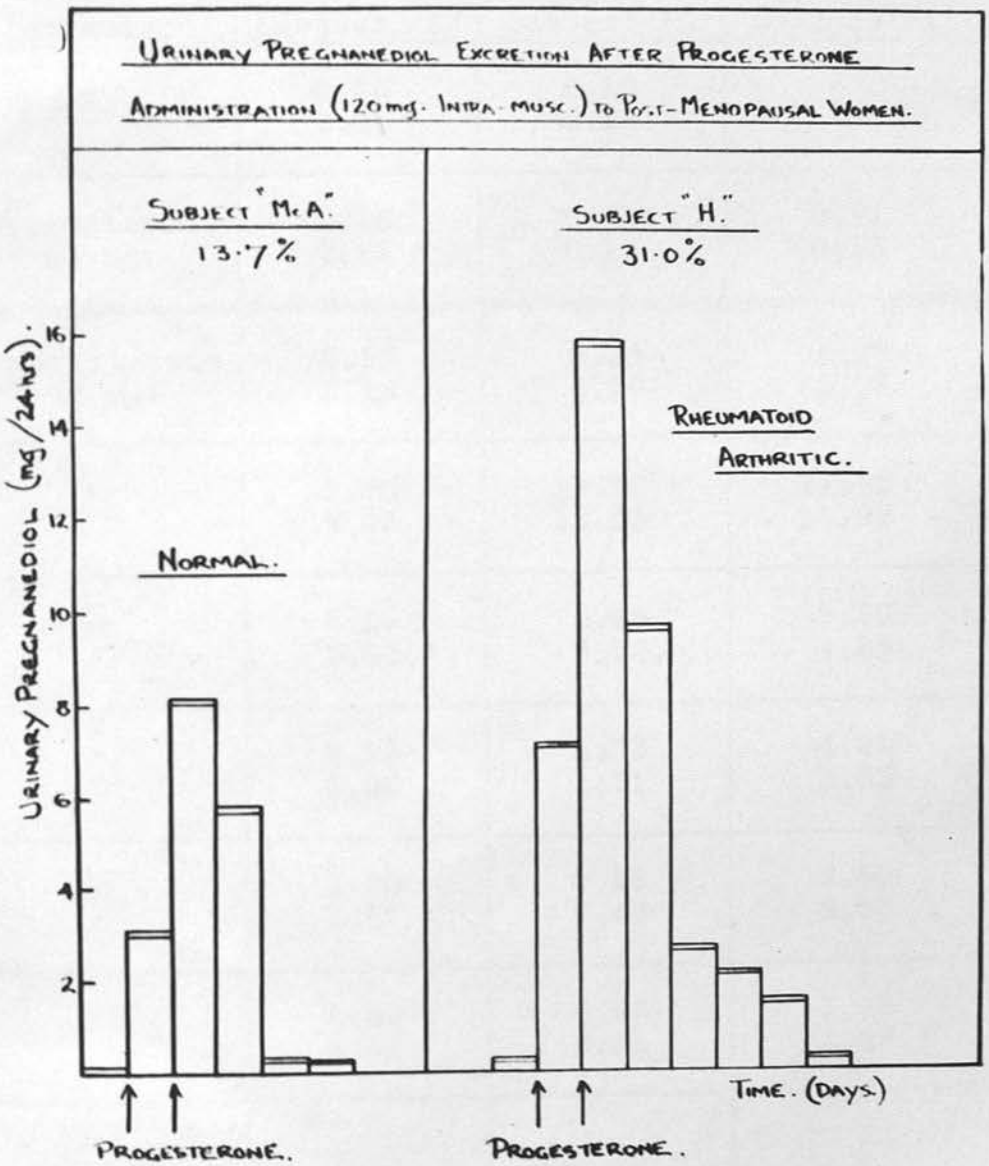

URINARY PREGNANEDIOL EXCRETION AFTER PROGESTERONE ADMINISTRATION (120 mg. INTRA-MUSC.) TO POST-MENOPAUSAL WOMEN.
SUBJECT "M. A."
13·7%
SUBJECT "H."
31·0%
NORMAL.
RHEUMATOID ARTHRITIC.
URINARY PREGNANEDIOL (mg/24 hrs).
16
14
12
10
8
6
4
2
PROGESTERONE.
PROGESTERONE.
TIME. (DAYS.)

TABLE 19

PROGESTERONE (120 mg. INTRA-MUSC.) TO RHEUMATOID ARTHRITIC POST-MENOPAUSAL WOMEN

Admin-istration	Pregnanediol mg./24 hr.		
	Subject 'M'	Subject 'L'	Subject 'A'
-	0.18 0.15	0.20 0.18	-
Progesterone 60 mg.	0.16 0.19	0.21 0.19	0.11 0.13
Progesterone 60 mg.	5.23 5.13	5.25 5.18	2.09 2.07
-	9.70 9.83	12.70 12.63	11.75 11.93
-	7.18 7.00	5.45 5.53.	9.20 9.25
-	2.96 3.00	1.73 1.71	4.35 4.33
	1.70 1.69	0.59 0.63	2.50 2.53
	0.59 0.62	0.23 0.31	1.43 1.46
			0.28 0.26

TABLE 20

PROGESTERONE (120 mg. INTRA-MUSC.) TO RHEUMATOID ARTHRITIC POST-MENOPAUSAL WOMEN

Admin-istration	Pregnanediol mg./24 hr.		
	Subject 'Y'	Subject 'C'	Subject 'D'
-	- 0.26	0.21 0.24	0.27 0.25
Progesterone 60 mg.	0.28 0.23	0.26 0.25	0.28 0.26
Progesterone 60 mg.	10.58 10.45	5.63 5.70	5.00 4.90
-	- 11.30	11.55 11.63	15.85 16.05
-	4.98 5.15	4.60 4.73	5.40 5.50
-	3.88 4.08	1.69 1.64	3.95 3.83
-	0.57 0.53	0.34 0.36	2.13 2.14
-			1.46 1.42

TABLE 21

PROGESTERONE (120 mg. INTRA-MUSC.) TO RHEUMATOID ARTHRITIC POST-MENOPAUSAL WOMEN

Administration	Pregnanediol mg./24 hr.		
	Subject 'Ca'	Subject 'H'	Subject 'Mc'
Progesterone 60 mg.	0.20 0.18	0.25 0.24	0.20 0.18
Progesterone 60 mg.	10.30 10.38	7.05 7.15	8.90 9.05
-	19.30 19.65	15.75 15.85	21.60 21.75
	9.60 9.63	9.53 9.65	6.10 6.08
	2.67 2.70	2.64 2.69	3.08 3.00
	2.01 2.04	2.10 2.08	0.71 0.69
	0.72 0.68	1.48 1.45	
		0.22 0.28	

TABLE 23

PROGESTERONE (60 mg. INTRA-MUSC.) TO RHEUMATOID ARTHRITIC POST-MENOPAUSAL WOMEN

Admin.	Pregnanediol mg./24 hr.			
	Subject 'S'	Subject 'B'	Subject 'W'	Subject 'Bo'
Progesterone 60 mg.	0.13 0.14	0.22 0.18	0.19 0.18	0.21 0.20
-	5.68 5.60	9.70 9.50	5.50 5.75	7.10 7.25
-	3.75 3.80	8.45 8.35	4.50 4.65	5.45 5.60
-	0.45 0.48	3.50 3.55	2.48 2.60	2.62 -
-	0.20 0.17	1.15 1.16	0.28 0.32	0.29 0.30

TABLE 24

PROGESTERONE (60 mg. INTRA-MUSC.)
TO RHEUMATOID ARTHRITIC MEN

Admin-istration	Pregnanediol mg./24 hr.		
	Subject 'K'	Subject 'McN'	Subject 'Mu'
Progesterone 60 mg.	0.16 0.21	0.31 0.33	0.31 0.31
-	9.05 9.10	15.50 15.10	6.25 6.38
-	3.53 3.45	5.75 5.50	4.30 4.13
-	1.34 1.31	1.71 1.68	2.25 2.30
-	- 0.32	1.05 1.01	0.35 0.38
-	-	0.29 0.28	-

TABLE 25

PROGESTERONE (60 mg. INTRA-MUSC.)
TO RHEUMATOID ARTHRITIC MEN

Administration	Pregnanediol mg./24 hr.		
	Subject 'Mo'	Subject 'Be'	Subject 'Ma'
Progesterone 60 mg.	0.18 0.16	0.23 0.28	0.22 0.21
-	8.40 8.70	7.75 8.00	8.70 8.80
-	4.50 4.88	9.90 10.15	2.00 1.96
-	0.95 0.98	2.48 2.42	0.35 0.38
-	0.20 0.21	0.28 0.33	0.18 0.20

TABLE 26

PROGESTERONE ADMINISTRATION AND SALICYLATE THERAPY

SUBJECT 'J'

Administration	Pregnanediol mg./24 hr.
Progesterone 60 mg. plus "Dysprin" (30 gr.)	0.21 0.24
"Dysprin" (30 gr.)	3.05 3.13
"Dysprin" (30 gr.)	2.95 2.80
"Dysprin" (30 gr.)	0.80 0.88
"Dysprin" (30 gr.)	0.19 0.23

TABLE 27

PROGESTERONE (60 mg. INTRA-MUSC.) TO ATYPICAL CASES OF RHEUMATOID ARTHRITIS

Administration	Pregnanediol mg./24 hr.	
	Subject 'Stk'	Subject 'Bin'
Progesterone 60 mg.	0.15 0.14	0.24 0.21
-	8.75 9.05	5.00 5.15
-	3.00 3.05	3.05 3.08
-	0.18 0.23	0.85 0.81
-	-	0.25 0.26

SECTION II

PROPORTION OF ADMINISTERED PROGESTERONE EXCRETED AS URINARY PREGNANEDIOL BY SUBJECTS WITH DISEASES WHICH EXHIBIT ONE OR MORE OF THE CLINICAL FEATURES OF RHEUMATOID ARTHRITIS

In the previous section, it has been shown that an abnormality of the intermediary metabolism of progesterone is associated with rheumatoid arthritis. It was not claimed that the occurrence of this abnormality is limited to rheumatoid arthritis, to the rheumatic group of diseases or even to arthritic disease in general, and this section is concerned with an attempt to investigate the distribution of the phenomenon. The erythrocyte sedimentation rate (E.S.R.) is elevated in many conditions other than rheumatoid arthritis - for example, tuberculosis and neoplasm, and it seemed possible that the abnormality of progesterone metabolism might be no more specific.

Seven subjects were investigated in this connection and the experimental procedure was identical to that described in Section I.

Subject 'C':/

Subject 'C': Man, aged 26 years.
Diagnosis: Tuberculosis of spine.
Bilateral pulmonary tuberculosis, and tuberculosis of the third and fourth lumbar vertebrae. The patient has recently had a recurrence of a right psoas abscess. E.S.R. 44 mm./hr. (Westergren).
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 7.84 mg.
Corrected for control "blank": 7.18 mg.
Recovery from administered progesterone- 12.0 per cent.

Subject 'H': Man, aged 58 years.
Diagnosis: Unresolved lobar pneumonia - ? neoplasm.
Patient has an unresolved lobar pneumonia with opacity at the base of the right lung. Very high E.S.R. 110 mm./hr. Pneumonic signs are resolving slowly and the possibility that there is an underlying neoplasm is being investigated.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 7.41 mg.
Corrected for control "blank": 6.93 mg.
Recovery from administered progesterone- 11.6 per cent.

Subject 'G': Man, aged 51 years.
Diagnosis: Osteoarthritis of spine.
Duration of disease - 3 years.
History of trauma. Unequivocal radiological evidence of hypertrophic osteoarthritic changes. No elevation of E.S.R. (5 mm./hr.). No anaemia (Hb. 102 per cent.).
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 8.59 mg.
Corrected for control "blank": 8.11 mg.
Recovery from administered progesterone- 13.5 per cent.

Subject 'Ba':/

Subject 'Ba': Man, aged 39 years.
Diagnosis - Spondylitis Ankylopoietica.
Duration of disease - 2 years.
Early case of spondylitis with stiffness and limitation of movement of the sacro-iliac joints and lower lumbar region of spine. Radiological changes are diagnostic but minimal. (E.S.R. 20 mm./hr.).
Progesterone: 120 mg. intra-muscular.
Apparent pregnanediol recovery: 15.66 mg.
Corrected for control "blank": 14.41 mg.
Recovery from administered progesterone- 12.0 per cent.

Subject 'S': Girl, aged 9 years.
Diagnosis - Still's disease.
Duration of disease - 6 years.
Characteristic multiple articular involvement. History of splenic enlargement. Anaemia (Hb. 80 per cent.) E.S.R. has fallen from 50 mm./hr. to 23 mm./hr. during past two months.
Progesterone: 55 mg. intra-muscular.
Apparent pregnanediol recovery: 5.03 mg.
Corrected for control "blank": 4.67 mg.
Recovery from administered progesterone- 8.5 per cent.

Subject 'McR': Boy, aged 15 years.
Diagnosis - Rheumatic Carditis.
Admitted for rest following the diagnosis of active carditis following acute rheumatic fever. E.S.R. 50 mm./hr. Hb. 84 per cent.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 6.50 mg.
Corrected for control "blank": 6.06 mg.
Recovery from administered progesterone- 10.1 per cent.

Subject 'Cu':/

Subject 'Cu': Man, aged 20 years.
Diagnosis - Atypical Juvenile Rheumatism.
Duration of disease - 3½ years.
Endocrine type of obesity and gynaecomastia. No involvement of small joints or spindling of fingers. Generalised joint stiffness and effusion in right knee. Cannot be classified as Still's disease. E.S.R. - 59 mm./hr. Hb. 84 per cent.
Progesterone: 60 mg. intra-muscular.
Apparent pregnanediol recovery: 10.08 mg.
Corrected for control "blank": 9.20 mg.
Recovery from administered progesterone - 15.3 per cent.

Conclusion

The proportions of administered progesterone excreted as urinary pregnanediol by these cases of Tuberculosis, Osteoarthritis, Spondylitis, Rheumatic Carditis and Still's Disease, fall within the range: 8.5 per cent. to 15.3 per cent. A comparison of these values with those of normal subjects (9.3 per cent. to 16.0 per cent.) leaves no doubt as to the normality of progesterone metabolism in these cases, despite clinical features similar to those found in rheumatoid arthritis. The number of cases of any one type of disease is too small to permit the conclusion that an abnormality of steroid metabolism does not occur in subjects affected by such a disease but the series as a whole strongly suggests that the abnormality of steroid metabolism may be strictly limited /

limited in its distribution, and affords ground for speculation that the abnormality is not merely associated with, but is intimately concerned in, the pathology of rheumatoid arthritis. Spondylitis ankylopoietica and Still's disease have been shown to respond to A.C.T.H. therapy (Elkinton, 1949; Boland, 1950) and this apparent anomaly will be discussed.

TABLE 28a

PROGESTERONE (60 mg. INTRA-MUSC.) TO CASES WITH CLINICAL FEATURES IN COMMON WITH RHEUMATOID ARTHRITIS

Admin.	Pregnanediol mg./24 hr.			
	Subject 'Ha'	Subject 'Cu'	Subject 'Ge'	Subject 'Co'
Prog. 60 mg.	0.18 0.15	0.23 0.21	0.18 0.14	0.26 0.29
-	5.25 5.00	4.30 4.25	5.30 5.50	2.30 2.13
-	1.70 1.90	2.54 2.65	3.00 2.90	0.66 0.71
-	0.50 0.46	0.98 0.94	0.28 0.20	0.35 0.39
-	0.15 0.14	0.23 0.24	-	0.23 0.24

TABLE 28c

PROGESTERONE (120 mg. INTRA-MUSC.) TO CASE OF SPONDYLITIS ANKYLOPOIETICA

Administration	Pregnanediol mg./24 hr. Subject 'Box'
Progesterone 60 mg.	0.25 0.24
Progesterone 60 mg.	4.08 4.04
-	7.68 7.75
-	1.73 1.75
-	1.70 1.68
-	0.43 0.47

SECTION III

INVESTIGATION OF THE EFFECT OF THE ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.) UPON THE METABOLISM OF PROGESTERONE IN RHEUMATOID ARTHRITIC SUBJECTS

A relationship between the activity of the anterior pituitary gland and hypertrophy of the adrenal cortex was first observed by Evans (1924). This observation was confirmed and extended by Smith (1926) and in 1933, Collip et al. obtained pituitary extracts with high adrenocorticotrophic activity. The isolation of the pure protein hormone from swine pituitary was reported by Sayers, White and Long in 1943, and from sheep pituitary by Li, Evans and Simpson in the same year.

The purified hormone preparations obtained by these two groups were closely similar in chemical, physical and biological properties. Thus elementary analysis revealed a similar content of carbon, hydrogen, sulphur, nitrogen and phosphorus, and electrophoretic studies indicated that the two proteins had almost identical isoelectric points. Li, Evans and Simpson found that sheep A.C.T.H. contained cystine (7 per cent), tyrosine (4.5 per cent.) and methionine (2 per cent.). In 1943, these workers carried out partial (50 per cent.) hydrolysis of the hormone with crude /

crude pepsin and were able to demonstrate that the hormonal activity resides in the hydrolysed fragments (polypeptide residues) of the original protein molecule. From later studies Li has concluded that these biologically active peptide fragments have an average amino-acid content of eight, that the pure hormone has a molecular weight of about 20,000 whereas these peptides have a molecular weight of about 1,200, and that these partial peptide hydrolysates are many times more active than the protein hormone (Li and Evans, 1948; Li, 1949; Li, 1950). These observations have been largely confirmed by Henly, Morris and Morris (1949), and by Morris and Morris (1950), who have prepared a similar polypeptide fraction by ultra-filtration and chromatography of fractionated extracts of beef anterior pituitary. This polypeptide is approximately ten times more active than the protein hormone of Sayers, White and Long.

Adrenocorticotrophic activity has also been detected in the urine of women (Blumenthal, 1940, 1945) and in the serum of pregnant mares (Golla and Reiss, 1942).

In 1948, Thorn et al. described in detail the effect of the administration of A.C.T.H. on the level of circulating eosinophils and lymphocytes and upon electrolyte balance, and Conn et al. (1948) stressed the /

the importance of the changes in carbohydrate metabolism which follow administration of the hormone. The interesting studies of Li and others upon the administration of A.C.T.H. to animals, are outwith the scope of the present work, but in connection with the work of Conn et al. it may be mentioned that it has been clearly shown that the hormone enhances diabetogenecity in alloxan-treated animals (Li and Evans, 1947) and inhibits the insulin effect on glycogenesis in isolated rat diaphragm (Li, 1949, 1950).

Reference was made in the Introduction to the dramatic results which followed the clinical trial of A.C.T.H. in rheumatoid arthritis by Hench et al.(1949). Since that trial, it has been claimed that the hormone has beneficial results in a galaxy of conditions. At least sixty distinct clinical entities have been studied including rheumatic fever, Still's disease, disseminated lupus and rare diseases of the collagen group, nephrosis, ulcerative colitis, leukaemia, asthma, and other allergic disturbances. (Li, 1950; Davidson, 1950; Boland, 1950).

In addition, interesting studies have been made of the effect of the adrenocorticotrophic hormone (A.C.T.H.) or of its polypeptide components (A.C.T.P.) on the excretion of urinary steroids. From these studies it is clear that an administration of the hormone /

hormone results in a marked increase in the excretion of 17-ketosteroids and corticoids. Thus, Mason (1949, 1950) administered A.C.T.H. (100 mg. daily) to a rheumatoid arthritic woman for twelve days and observed a rise in the 17-ketosteroids from a control period level of 4.0 to 4.3 mg. per twenty-four hours to a level of 43.8 mg. per twenty-four hours on the twelfth day. The corticoid excretion rose from 1 mg. per twenty-four hours to 17.5 mg. per twenty-four hours during this period. A sharp fall in the urinary level of these steroids occurred within twenty-four hours of withdrawal of the hormone. Mason estimated that 30 to 50 per cent. of the corticoids chemically determined in these experiments consisted of 17-hydroxycorticosterone (Kendall's compound 'F').

This recent work by Mason et al. confirms the observation of Mason and Sprague (1948) that 17-hydroxycorticosterone was excreted in the urine of a case of Cushing's syndrome with severe diabetes mellitus. Unpublished work by Mason et al., quoted by Boland (1950) appears to indicate that the urinary excretion of 17-ketosteroids falls during cortisone therapy.

The immediate relevance of A.C.T.H. to the present work lies in the fact that its administration appears to offer a method of testing the validity of the original working hypothesis, namely that the

biochemical /

biochemical defect in rheumatoid arthritis consists of an abnormality in cortisone metabolism, rather than in a deficiency of cortisone secretion. If the former is true, then it might be anticipated that administration of A.C.T.H. would not ameliorate the hypothetical abnormality of cortisone metabolism and by analogy would not affect the demonstrated abnormality of progesterone metabolism. Furthermore, it may be argued that if the administration of A.C.T.H. leads to correction of the metabolic defect, then during prolonged administration of the hormone the metabolic abnormality should gradually resolve and the dose required to elicit the clinical response should diminish. There is no evidence to suggest that this is so.

To investigate the effect of A.C.T.H. on the abnormal progesterone metabolism of rheumatoid arthritics, the following procedure was adopted.

Experimental Procedure

(1) Control metabolic experiment in which progesterone (60 mg. intra-muscular) was administered and the proportion excreted as urinary pregnanediol was determined as in Section I.

(2) /

(2) A.C.T.H. plus Progesterone: (seven days later)

(a) Intra-muscular injections as follows:-

(i) Saline: 2 ml. daily for three days.

(ii) A.C.T.H. (Organon) 75 mg. daily for five days in divided doses of 25 mg.

(iii) Progesterone: 60 mg. along with the first A.C.T.H. injection on the third day of A.C.T.H. administration.

(iv) Saline: 2 ml. daily for three days.

The saline injections were employed in order to minimise psycho-somatic effects.

(b) Determination of urinary pregnanediol carried out daily (in duplicate), throughout the experiment.

(3) Repetition (ten days later) of the progesterone metabolic experiment as in (1)

This procedure has so far been carried through in only two cases (subjects 'Ca' and 'McN') but the results are sufficiently clear-cut to permit a definite conclusion. In subject 'Ca' the post-A.C.T.H. progesterone metabolic experiment was again repeated after a lapse of ten days.

Results

Improvement in the clinical condition of both patients was apparent by the second day of A.C.T.H. administration.

Subject 'McN' /

Subject 'McN', who had been walking with great difficulty due to limitation of movement and much pain in the hip and knee joints experienced complete relief from pain and was able to walk freely. Movements, such as getting out of bed or rising from a chair, were changed overnight from penance to pleasure. Subject 'Ca' also experienced dramatic relief from pain and stiffness and awoke with the sensation that her joints had been miraculously "released". This patient had been confined to bed for many months but she was able to walk a few steps by the fifth day of the administration. The changes in electrolyte balance were similar to those previously reported and the excretion of urinary 17-ketosteroids which was determined in 'McN' was markedly increased. These findings will not be described in more detail since neither the ancillary biochemical determinations nor the assessment of clinical improvement were carried out by the author.

The results of the progesterone metabolism experiments are summarised in the following table in which the proportion of administered progesterone excreted as urinary pregnanediol before, during, and after A.C.T.H. therapy, is expressed as a percentage of the dose. A marked diuresis followed withdrawal of the adreno-corticotrophic hormone in both cases.

Percentage /

Percentage of Administered Progesterone Recovered as Urinary Pregnanediol

	Subject 'Ca' Per Cent.	Subject 'McN' Per Cent.
Before A.C.T.H. administration	34.6	37.3
During A.C.T.H. administration	32.0	34.5
After A.C.T.H. administration (a)	27.8	36.2
After A.C.T.H. administration (b)	31.0	-

These results are illustrated in Fig. 17 (subject 'McN') and detailed in Tables 29a, 29b, 29c.

Conclusion

The results show clearly that no significant alteration in the abnormality of the intermediary metabolism of progesterone, associated with rheumatoid arthritis, resulted from concurrent administration of progesterone and of the adrenocorticotrophic hormone. It is doubtful if any significance should be attached to the slight fall in the percentage conversion which was detected ten days after cessation of A.C.T.H. therapy /

therapy in subject 'Ca' but this point will be elucidated by other identical experiments which are in progress. In fact the absence of a significant effect is especially convincing in this case in whom the proportion of administered progesterone excreted as urinary pregnanediol has gradually fallen from 36.4 per cent. (Section I) to 31.0 per cent. over a period of five months. The orthodoxy of the changes induced in the clinical condition, electrolyte balance and 17-ketosteroid excretion indicate the potency of the preparation employed and the reversibility of the lesions. It seems probable, therefore, that these two cases represent a typical response to A.C.T.H. in rheumatoid arthritis and this enhances the value of the observations on progesterone metabolism.

As was indicated in the introduction to this section, proof of the inability of A.C.T.H. to influence the abnormality of progesterone metabolism adds support to the original working hypothesis concerning the fundamental rôle of an abnormality of steroid metabolism in rheumatoid arthritis and strengthens the conclusions reached in the final discussion.

Figure 18.

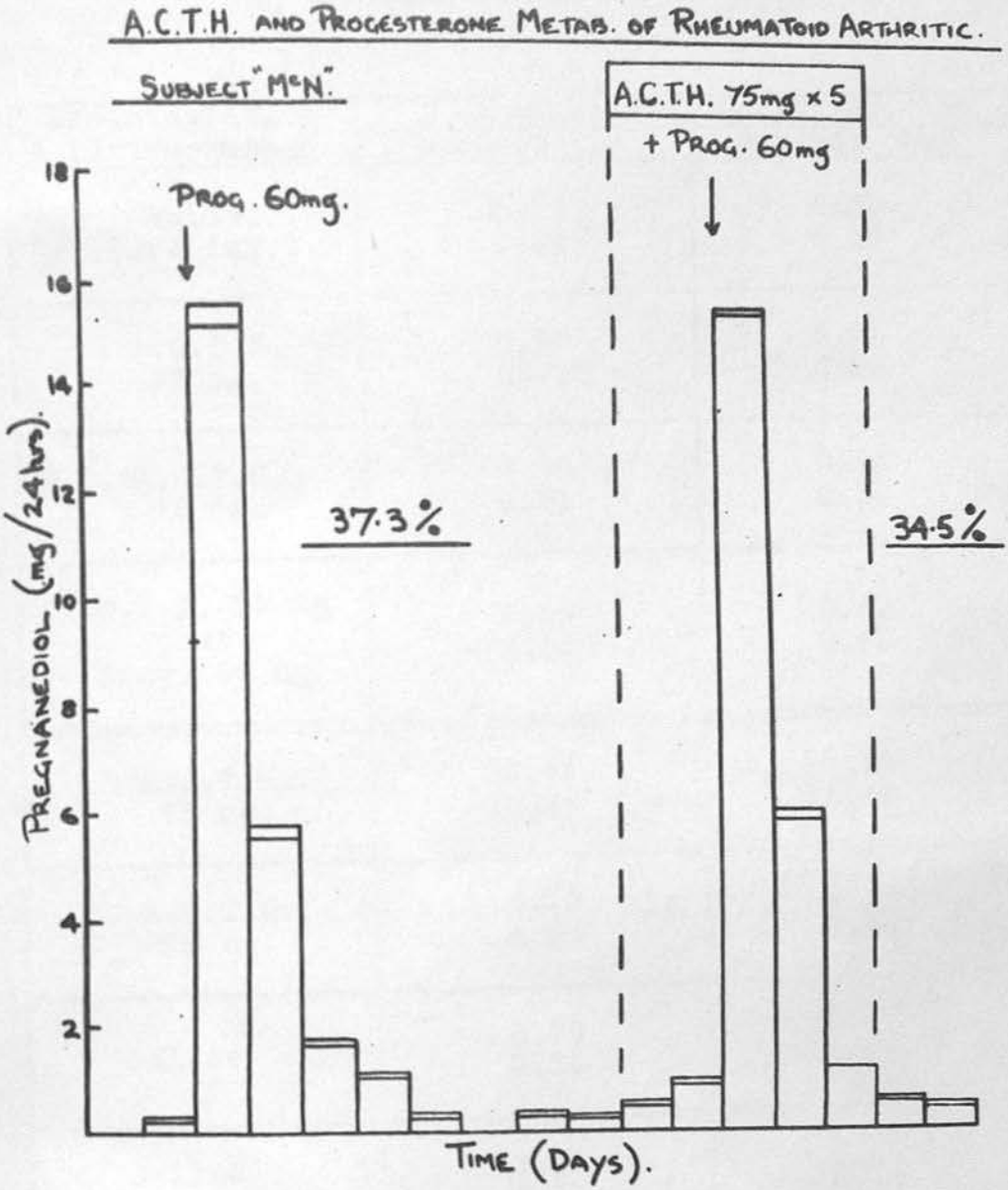

A.C.T.H. AND PROGESTERONE METAB. OF RHEUMATOID ARTHRITIC.
SUBJECT "McN."
A.C.T.H. 75mg x 5
+ PROG. 60mg
PROG. 60mg.
PREGNANEDIOL (mg/24 hrs).
18
16
14
12
10
8
6
4
2
37·3%
34·5%
TIME (DAYS).

TABLE 29a

A.C.T.H. AND THE ABNORMAL PROGESTERONE METABOLISM OF RHEUMATOID ARTHRITICS

Administration (Intra-musc.)	Pregnanediol mg./24 hr.	
	Subject 'Ca'	Subject 'McN'
Saline (3rd inj.)	0.26 0.24	0.25 0.30
A.C.T.H. 75 mg.	0.29 0.25	0.26 0.28
A.C.T.H. 75 mg.	0.28 0.31	0.38 0.34
A.C.T.H. 75 mg. plus Prog. 60 mg.	0.58 0.61	0.80 0.73
A.C.T.H. 75 mg.	12.75 12.40	15.25 15.35
A.C.T.H. 75 mg.	4.55 4.50	5.85 5.60
Saline	2.70 2.75	1.03 1.01
Saline	2.08 2.11	0.49 0.45
Saline	0.83 0.86	0.31 0.34
	0.65 0.61	0.30 0.31

TABLE 29b

A.C.T.H. AND THE ABNORMAL PROGESTERONE METABOLISM OF RHEUMATOID ARTHRITICS

SUBJECT 'Ca'

Administration	Before A.C.T.H.	After A.C.T.H. (a)	(b)
	Pregnanediol mg./24 hr.		
Progesterone 60 mg.	0.25 -	0.19 0.18	0.28 0.23
-	5.89 5.70	6.30 6.28	9.60 11.00
-	8.50 8.35	6.75 7.00	5.50 5.65
-	4.10 4.50	3.35 3.25	3.35 3.45
-	2.50 2.63	1.01 0.98	0.31 0.34
-	0.56 0.48	0.20 0.16	

TABLE 29c

A.C.T.H. AND THE ABNORMAL PROGESTERONE METABOLISM OF RHEUMATOID ARTHRITICS

SUBJECT 'McN'

Administration	Before A.C.T.H.	After A.C.T.H.
	Pregnanediol mg./24 hr.	
Progesterone 60 mg.	0.31 0.33	0.24 0.23
-	15.50 15.10	16.40 16.60
-	5.75 5.50	4.95 5.13
-	1.71 1.68	0.98 0.80
-	1.05 1.01	0.21 0.20
	0.29 0.28	

SECTION IV

PRELIMINARY ATTEMPT TO INFLUENCE THE ABNORMAL PROGESTERONE METABOLISM OF RHEUMATOID ARTHRITIC SUBJECTS

In the Introduction the possibility was considered that the intermediary metabolisms of progesterone and cortisone might be closely similar consisting mainly of a progressive metabolic reduction of their carbonyl substituents and it was suggested that the abnormality of progesterone metabolism is attributable to an abnormality of the appropriate enzyme reducing system. In Part I, Section II, the possible rôle of α-tocopherol as a participant in reversible oxidation-reduction systems was considered in some detail and it was now of considerable interest to investigate whether the abnormal metabolism of progesterone might be influenced by the administration of such a potential coenzyme. In the present work ascorbic acid was chosen for this purpose for the following reasons.

(1) The rôle of ascorbic acid in cell metabolism is obscure but its participation as a coenzyme in redox reactions is more plausible than is that of α-tocopherol. Its possibilities in this connection hinge upon the reversibility of the system ascorbic/dehydroascorbic acid and /

and upon the value of its potential.

It has been claimed that certain enzymes are activated by ascorbic acid notably succinic dehydrogenase and cytochrome oxidase (Harrer and King, 1941).

(2) Three aspects of the occurrence and physiological action of ascorbic acid appear to have especial relevance to the present work.

(i) There is scattered evidence to suggest that the vitamin may be concerned in steroid biogenesis. Thus the corpora lutea and adrenal cortices are rich in ascorbic acid (its concentration in the pars intermedia of the pituitary is even higher) and it has been shown that the ascorbic acid concentration of the corpus luteum is directly proportional to its functional activity (Pincus and Berkman, 1937; Pincus and Werthesen, 1938; Biskind and Glick, 1936). Following the isolation of pure A.C.T.H., Sayers et al. observed that a marked fall in the ascorbic acid and cholesterol content of the adrenal /

adrenal cortex follows administration of the hormone to rats (1943, 1944) and Sayers and Sayers (1946) observed the same changes in hypophysectomised rats and developed a sensitive method for the bioassay of A.C.T.H.

(ii) It is well established that ascorbic acid participates in the formation of the colloidal intercellular substances which comprise those of cartilage, the matrical of bone and the collagen of fibrous tissue (Jeney and Torö, 1936; Mitchell, 1945).

(iii) It is recognised that ascorbic acid is implicated in the metabolism of amino-acids. For example, Levine et al. (1941) demonstrated an abnormality of l-tyrosine metabolism in premature infants and showed that this could be resolved by the administration of the vitamin. This observation has been confirmed and extended by Morris et al. (1950). It is of considerable interest, therefore to learn that there is some evidence /

evidence for the existence of an abnormality of amino-acid metabolism associated with rheumatoid arthritis. (Stephens et al., 1949)

(3) In 1949, Lewin and Wassen reported dramatic alleviation of signs and symptoms of rheumatoid arthritis occurring within one hour of a combined intra-muscular injection of 11-desoxycorticosterone and an intravenous injection of ascorbic acid. Attempts to repeat these results have led to very variable results and controversy rages between those for and those against the view that such therapy is indeed beneficial. At the time of writing the opinion of those in favour appears to be outweighed but the fact that the situation should have arisen provided another reason for the present investigation. Furthermore, Rinehart et al. (1938) claim that the fasting plasma-concentration of ascorbic acid is invariably and markedly low in rheumatoid arthritis, and that the administration of ascorbic acid to such cases produces at best a delayed increase in the plasma ascorbic acid level, and in some cases no response.

As a preliminary investigation it was decided to investigate the proportion of administered progesterone excreted as urinary pregnanediol by subjects saturated with /

with orally administered ascorbic acid. Three rheumatoid arthritic post-menopausal women were investigated.

Experimental Procedure

(i) Control progesterone metabolism experiment in which 120 mg. of progesterone was administered by intra-muscular injection and the proportion excreted as urinary pregnanediol calculated as in Section I.

(ii) Ascorbic acid: 2 gm. orally daily for four days.

Progesterone: 60 mg. intra-muscularly on the second and third days.

Pregnanediol determinations throughout the experiment.

The results are shown in the following table and are detailed in Table 30.

Proportion of Administered Progesterone Recovered as Urinary Pregnanediol

Subject	Before Admin. Ascorbic Acid	During Admin. Ascorbic Acid
'H'	31.0	28.0
'Ca'	36.4	33.2
'Mc'	33.0	28.5

Conclusion /

Conclusion

It is clear that in this preliminary series the administration of large doses of ascorbic acid did not have a significant effect on the abnormality of progesterone metabolism in rheumatoid arthritics. It would not be anticipated, however, that the oral administration of the vitamin would favour its participation in a redox system. Accordingly, variations in the experimental procedure and especially parenteral administration of the vitamin may yield a different result.

Whatever the result of such experiments there seems little doubt that the attractive nature of the hypothesis that a coenzyme deficiency is fundamental to an abnormality in the metabolic reduction of the steroid calls for the investigation of other potential participants in reversible oxidation-reduction systems.

The results of experiments of this nature - which are in progress - suggest that the hope that the abnormality of steroid metabolism can be profoundly influenced is not ill-founded, and this experimental attack is being pursued, not without a certain optimism.

TABLE 30

PROGESTERONE (120 mg. INTRA-MUSC.) AFTER ADMINISTRATION OF ASCORBIC ACID TO POST-MENOPAUSAL RHEUMATOID ARTHRITIC WOMEN

Administration	Pregnanediol mg./24 hr.		
	Subject 'Ca'	Subject 'H'	Subject 'Mc'
Progesterone 60 mg.	0.16 0.25	0.24 0.28	0.22 0.20
Progesterone 60 mg.	7.60 7.45	6.25 6.35	6.80 6.95
-	13.25 13.00	14.85 14.60	17.40 17.25
-	11.65 11.73	9.23 9.03	7.00 7.15
-	5.18 5.33	3.78 3.83	3.38 3.35
-	3.11 3.12	0.92 0.94	0.60 0.62
-	0.33 0.38	0.35 0.25	0.18 0.21

DISCUSSION

It has been necessary in presenting exploratory work of this nature to introduce the experiments by fairly detailed discussion. The conclusions which it seems reasonable to draw from these experiments have also been considered but the underlying biochemical mechanisms and the potential clinical implications remain to be discussed. At the present stage in this work, and in the absence of previous studies of a similar nature, such speculation will be brief.

(1) The abnormality of steroid metabolism associated with rheumatoid arthritis

The present results leave no doubt as to the existence of an abnormality of progesterone metabolism in rheumatoid arthritic subjects. Furthermore, the failure to demonstrate this abnormality in subjects with other types of arthritis and with other causes of elevation of the E.S.R., and the suggestive correlation between the degree of the abnormality and the clinical status of the patient indicate that the abnormality may be intimately related to the pathology of the disease. It will be of the greatest interest, therefore, to study the intermediary metabolism of cortisone in rheumatoid arthritic subjects.

The /

The difficulties associated with such a study were discussed in the Introduction notably the unknown nature of cortisone metabolites and the possibility that, once recognised, their quantitative determination might prove difficult if not impossible. Thus the prospects of a quantitative study of cortisone metabolism are poor although the possibility should be considered that cortisone may be partly metabolised to some easily determinable 17-ketosteroid.

The observation of large amounts of 17-hydroxycorticosterone ('F') in the urine of subjects receiving the adrenocorticotrophic hormone (Mason, 1948) suggests the possibility that this steroid may be derived from 17-hydroxy-11-dehydrocorticosterone ('E') by reduction of the 11-carbonyl substituent but does not constitute proof, since 17-hydroxycorticosterone may be secreted independently of the 11-dehydro compound or may even be derived from the enigmatic biologically active amorphous fraction.

In view of these difficulties it is of immediate interest to supplement the study of the metabolism of progesterone in rheumatoid arthritic subjects by a study of other steroid precursors of urinary pregnanediol (Fig. 14).

(a) <u>11-desoxycorticosterone</u> /

(a) 11-desoxycorticosterone

This steroid is, of course, more closely related to cortisone than is progesterone and it has been shown by Cuyler et al. (1940) and by Horwitt et al. (1944) that the proportion of administered 11-desoxycorticosterone recovered in the urine as pregnanediol is similar to that which follows the administration of progesterone. 11-desoxycorticosterone would have been selected for the original study had it not been for the lack of accurate data concerning the proportion of administered steroid excreted as urinary pregnanediol by normal subjects and because it seemed doubtful if doses, large enough to permit accurate determination of the proportions so excreted, could be administered with safety in view of the salt-retaining properties of this mineralo-corticoid. Following the results obtained with progesterone, an attempt is being made to overcome these difficulties.

(b) Δ^5-pregnen-3β-ol-20-one

This steroid was administered to human subjects by Pearlman and Pincus (1942) and pregnane-3α,20α-diol was isolated from the urine. Furthermore, it has been claimed (Davison et al. 1950) /

1950) that beneficial results follow its administration to cases of rheumatoid arthritis and spondylitis ankylopoietica. These observations suggested that a study of the intermediary metabolism of this steroid in rheumatoid arthritic subjects might be of interest. The amounts of pregnane-3α,20α-diol isolated by Pearlman and Pincus were very small, however, and it will be recalled that small amounts of that steroid have been isolated from the urine of normal men and ovariectomised women (p. 7). It was perhaps not surprising, therefore, to find in the course of the present work that after the oral administration of 120 mg. of pregnen-3β-ol-20-one to a normal man an insignificant increase over the control period "blank" value of apparent pregnanediol recovery was observed. It will be necessary to repeat this experiment in a rheumatoid arthritic subject but it seems unlikely that this steroid will prove useful for the present purpose.

(c) Cholesterol

Similarly, the demonstration by Bloch (1945) of the derivation of urinary pregnanediol from administered cholesterol is not directly applicable to the present work since the quantitative relationship /

relationship is obscure. Bloch suggests that the direct conversion of cholesterol to progesterone is a normal process. Studying the isotope concentration of the urinary pregnanediol and of the blood cholesterol he estimated that about one-half to one-third of the pregnanediol excreted by a pregnant woman receiving deuterium-labelled cholesterol arose from that source.

A less direct approach to the problem would be afforded by a quantitative study of the metabolisms of administered testosterone or of oestradiol in rheumatoid arthritis. The former study would be complicated, however, by variations in the urinary level of 17-ketosteroids of endogenous origin and the latter rendered difficult by the imperfections of current methods for the determinations of the urinary metabolites of the oestrogenic hormones. In view of the differing metabolic pathways involved, failure to demonstrate an abnormality of the intermediary metabolism of these steroids would in no way invalidate the working hypothesis that there is an abnormality of cortisone metabolisms in rheumatoid arthritis.

It /

It has been suggested in the Introduction that this abnormality reflects an abnormality of the enzyme system - reductase - responsible for the metabolic reduction of progesterone and possibly of cortisone. On the other hand, it is conceivable that the abnormality is concerned with glucuronide synthesis or hydrolysis (p. 49). It seems likely that these two possibilities could to a certain extent be differentiated by a comparison of the administration of progesterone and of pregnane-3α,20α-diol to rheumatoid arthritic subjects. Further speculation on this, the most fundamental aspect of the problem, may properly be reserved, however, until these results and the results of attempts to modify the metabolic abnormality (section IV) have been obtained.

(2) Clinical Implications

Of immediate relevance is the possibility that a calculation of the proportion of administered progesterone excreted as urinary pregnanediol may constitute a new and valuable objective criterion in rheumatoid arthritis.

Further investigation should establish whether there is a significant correlation between the extent of /

of the metabolic abnormality and the "activity" or prognosis of the disease, and whether a progesterone metabolism experiment might reflect the potential responsiveness of individual cases to adreno-cortical hormone therapy. It was perhaps not surprising that the metabolic abnormality was absent in spondylitis ankylopoietica but it is less easy to rationalise its absence in Still's disease. Until the mechanism of the metabolic abnormality is more clearly understood this can only be attributed to the characteristic pathology of that entity and extension of this aspect of the work on diseases related to rheumatoid arthritis may indicate a clinical value for a progesterone metabolism experiment in differential diagnosis. It will be important to study the metabolism of progesterone in non-arthritic diseases which have been shown to respond to adreno-cortical hormone therapy.

Further discussion of the clinical and therapeutic implications of this work is highly speculative, at the present time, but the possibility cannot be overlooked that such abnormalities of steroid metabolism are of aetiological significance. During the past two decades evidence has accumulated indicating the validity of the ancient concept of psycho-somatic aetiology. Selye (1944, 1949) has crystallised his ideas on one aspect of the subject by his concept of "adaptation" /

"adaptation" and Pincus (1947) may have elucidated part of the somatic pathway by the study of changes in the pattern of urinary steroid excretion during conditions of stress. An essential feature of the psychosomatic concept is the belief that prolongation of a physiological function leads to changes in the structure of the functioning tissue and thus to irreversible disease. As the physiological importance of the adreno-cortical hormones is recognised, their participation in disease causation becomes an ever more disturbing possibility. It is just conceivable that under the prolonged action of these hormones the enzyme systems engaged in steroid hormone disposal become "primed" in a similar manner to the progesterone "priming" effect on the uterus (Part I).

Is it not possible that such a deviation of intermediary steroid metabolism is fundamental to the mechanism whereby the physiological responses to stress become fixed and pathological? If this is so, then the introduction of a method for the correction of this defect might have unparalleled therapeutic possibilities.

SUMMARY

(1) The nature of the quantitative relationship between progesterone secretion and urinary pregnanediol excretion had been investigated by the quantitative determination of the metabolite after continued daily administration of progesterone to normal post-menopausal women, normal men, hysterectomised-ovariectomised women and hysterectomised post-menopausal women. These experiments prove that when the human body is subjected to the influence of progesterone for more than a few days, a progressive change in the relationship of hormone to metabolite occurs so that an increasing proportion of the administered hormone is recovered from the urine as the metabolite. This progesterone "priming" phenomenon has been shown to be attributable to a rôle of the uterus in the metabolism of progesterone. Pregnane-3α,20α-diol has been administered to post-menopausal women and the biochemical mechanism and clinical implications of these findings have been discussed.

(2) The claim by de Watteville et al. (1948) that pre-treatment with α-tocopherol augments the conversion of administered progesterone to urinary pregnanediol /

pregnanediol has been investigated. An identical experimental procedure has been adopted but normal human subjects have been studied in place of patients with carcinoma of the uterus or breast and the method of Sommerville, Gough and Marrian (1948) has been used instead of the modified method of Huber (1947). The results clearly indicate that α-tocopherol does not have the effect observed by de Watteville et al. when normal subjects are studied by the former method. Explanations are offered for these contradictory results.

(3) A synthetic orally active progestogen - ethinyltestosterone - has been administered to normal men and the urine investigated for the presence of pregnanediol. Significant amounts of pregnanediol were not recovered from the urine but large amounts of an unidentified crystalline precipitate were observed. Excretion of this substance - which may be metabolite of ethinyltestosterone - as a glucuronide would account for the results obtained by Allen, Viergiver and Soule (1944). The clinical implications of a progestogen which is not metabolised to pregnanediol have been discussed.

(4) /

(4) The excretion of urinary pregnanediol has been studied during normal pregnancy and the results have been compared with those obtained by less specific methods for the quantitative determination of the steroid. The results emphasise the necessity to establish values of pregnanediol excretion in normal pregnancy.

(5) It has been shown that the method of Sommerville, Gough and Marrian (1948) is applicable to the determination of small amounts of pregnanediol in rabbit urine. The accuracy of the method when used for this purpose has been assessed and it is concluded that as little as 0.25 mg. of the steroid can be determined in one half of a twenty-four hour urine specimen.

(6) An analogy has been drawn between the intermediary metabolisms of progesterone and cortisone (17-hydroxy-11-dehydrocorticosterone) and it was anticipated on theoretical grounds that an abnormality of progesterone metabolism might be associated with rheumatoid arthritis. Such an abnormality was demonstrated in a large series of post-menopausal women and men with rheumatoid arthritis, and consisted in the conversion of an abnormally high proportion of administered progesterone /

progesterone to urinary pregnanediol.

The abnormality was not observed in a series of patients suffering from diseases which resemble rheumatoid arthritis in one or more of their clinical features.

The belief in the possibility that the pathology of rheumatoid arthritis is associated with an abnormality of steroid metabolism rather than with a deficiency of endogenous steroid secretion was strengthened by the observation that the abnormality is not affected by the administration of the adrenocorticotrophic hormone of the anterior pituitary gland.

The biochemical mechanism and clinical implications of the abnormality of steroid metabolism have been considered and the rational of attempts to modify this abnormality has been discussed.

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THE QUANTITATIVE DETERMINATION OF SMALL AMOUNTS OF PREGNANEDIOL IN HUMAN URINE

BY I. F. SOMMERVILLE, NANCY GOUGH AND G. F. MARRIAN,
From the Department of Biochemistry, University of Edinburgh

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Various methods have been described for the quantitative determination of pregnanediol in human urine. Of these the original procedure of Venning [1937, 1938], in which sodium pregnanediol glucuronidate is extracted from the urine and weighed after purification, is still perhaps the most widely employed. This method, although somewhat time-consuming and laborious, gives satisfactory results when applied to urines containing more than *c.* 10–15 mg. of pregnanediol per 24 hr., but suffers from certain serious disadvantages where urines containing smaller amounts of the steroid are concerned. Thus, as pointed out by Astwood & Jones [1941], 'when only small amounts of material are present in the urine, the identity of the final product is sometimes questionable'. Furthermore, it may be necessary to use a full 24 hr. or even a 48 hr. specimen of urine for a single determination in order to obtain sufficient of the glucuronidate to weigh accurately.

Other methods of determining pregnanediol as its glucuronide have been described by Allen & Viergiver [1941], Jayle, Crépy & Wolf [1943], and Bisset, Brooksbank & Haslewood [1947]. These methods would seem to be somewhat less laborious and considerably more sensitive than the Venning method, but they are open to criticism on the grounds of lack of specificity. A further disadvantage of all methods in which pregnanediol is determined as its glucuronide arises from the fact that, unless very special precautions are taken, hydrolysis of the latter by bacterial action may occur during the collection of the urine and subsequently.

A method of a different kind was developed by Astwood & Jones in 1941. In this method the urine is boiled with acid to hydrolyse the glucuronide and the free pregnanediol thus liberated extracted with toluene, purified and weighed. The originality of this method lies in the procedure used for the purification of the pregnanediol in the crude toluene extract. It was shown that from the toluene-soluble fraction, after removal of acidic substances by treatment with sodium hydroxide, nearly pure pregnanediol in almost quantitative yield can be obtained by precipitation from ethanolic solution with four volumes of water or dilute sodium hydroxide solution. The sensitivity of this method was considerably increased subsequently by Talbot, Berman, MacLachlan & Wolfe [1941], who estimated the purified pregnanediol colorimetrically by means of the yellow colour which it yields with concentrated sulphuric acid. Both groups of workers reported reasonably satisfactory recoveries of about 70 % in short series of experiments in which sodium pregnanediol glucuronidate was added to men's urine in varying amounts,* but the evidence that either procedure

* It seems possible that the recoveries obtained by these authors may actually have been somewhat higher than they themselves reported, since Marrian & Gough [1946] have shown that sodium pregnanediol glucuronidate prepared and purified in the usual way [Venning & Browne, 1936] contains only about 80 % of that compound.

would be dependable when applied to urines containing less than *c.* 10 mg. of pregnanediol per 24 hr. was not entirely satisfactory.

A somewhat simplified version of the Astwood & Jones method as modified by Talbot *et al.* has recently been developed by Guterman [1944, 1945] for the detection of pregnanediol in urine as a rapid means of diagnosing pregnancy.

What will for convenience be called the 'Astwood-Talbot' method has several advantages over other methods of determining urinary pregnanediol. The colour reaction which pregnanediol gives with concentrated sulphuric acid is extremely sensitive, thus permitting the accurate determination of small amounts of the substance; and although it is not a specific reaction for pregnanediol it was shown by Astwood & Jones and by Talbot *et al.* that many of the other urinary substances which give it are largely eliminated in the ethanol-water precipitation process of purification. Since the pregnanediol is determined in the free state, elaborate precautions to avoid bacterial hydrolysis of the glucuronide in the urine are unnecessary.

The objective of the work described in this paper was to develop an accurate, specific, convenient and rapid method for the determination of the pregnanediol (1–10 mg./24 hr.) in the urine of women during the luteal phase of the menstrual cycle. Furthermore, in order to permit duplicate determinations of pregnanediol and of other urinary constituents to be carried out it was necessary that the method developed should require not more than one-fifth or one-quarter of a 24 hr. specimen of urine.

This objective has been nearly, but not quite, attained. A procedure based on the Astwood-Talbot method has been elaborated which permits of the reasonably accurate determination of more than *c.* 0·4 mg. of pregnanediol in one-fifth of a 24 hr. urine specimen. Six determinations can be completed in two 8 hr. working days, and the method is such that it could be satisfactorily employed by a trained and competent laboratory technician.

APPARATUS AND MATERIALS

Quickfit & Quartz glassware, with interchangeable standard glass joints, was used throughout in order to avoid contamination of the urinary extracts with coloured or chromogenic material that might be dissolved out of rubber or cork stoppers.

The toluene used for the extractions was 'sulphur-free' and was distilled before use. Ethanol was purified by refluxing over sodium hydroxide and distilling twice.

Pregnane-3(α), 20α-diol was prepared from human pregnancy urine and purified *via* its diacetate. The sample used in the recovery experiments melted at 236–237° (corr.).

Sodium pregnane-3(α), 20α-diol glucuronidate was prepared from human pregnancy urine by the method of Venning & Browne [1936] and freed from ketonic glucuronides by the method of Sutherland & Marrian [1946, 1947]. The preparation used in the recovery experiments melted at 282–283° (corr.) with decomposition and evolution of gas. Samples of the glucuronidate were weighed out after exposure to moist air. As shown by Sutherland & Marrian [1947] material so treated is the *trihydrate* having the composition $C_{27}H_{43}O_8Na.3H_2O$.

For certain of the recovery experiments the toluene-soluble neutral fraction of acid-hydrolysed men's urine was required. This was prepared in the following way: 24 hr.

urine specimens from a number of normal men were pooled and heated to boiling, together with some toluene, acidified with one-tenth of its volume of concentrated hydrochloric acid, and the boiling continued for 10 min. After cooling the mixture was extracted three times with one-fifth volumes of toluene, emulsions being broken by filtration through a Buchner funnel with gentle suction. The combined toluene extracts were washed twice with one-sixth volumes of N sodium hydroxide, three times with one-sixth volumes of water, evaporated to a small volume on a hot plate, and finally taken to dryness under reduced pressure in the water-bath. The brown gummy material so obtained will be referred to as 'male urine neutral fraction'.

MODIFICATIONS MADE IN THE PROCEDURES OF ASTWOOD AND TALBOT

In consequence of a very large number of preliminary experiments, the details of all of which are not reported here, a number of modifications have been made in the original procedures as described by Astwood & Jones [1941] and by Talbot *et al.* [1941]. These modifications were designed to minimize the number of transferences from vessel to vessel during the procedure and to facilitate the rigid standardization of technique which experience has shown is essential if accurate and reproducible results are to be obtained with urines containing less than *c.* 10 mg. of pregnanediol per 24 hr. urine volume.

Removal of acidic substances

Astwood & Jones and Talbot *et al.* removed acidic and phenolic material from the toluene extract of the hydrolysed urine by boiling with methanolic sodium hydroxide and then filtering off the resulting precipitate of sodium salts. In the experience of the present authors it is equally effective and much simpler to remove acidic and phenolic material by washing the toluene extract in a separating funnel with aqueous sodium hydroxide.

Purification of pregnanediol from the neutral fraction by precipitation from ethanol

The accuracy and specificity of the Astwood-Talbot method are very largely dependent upon the efficiency of the precipitation process in separating pure pregnanediol quantitatively from the neutral toluene-soluble fraction of the hydrolysed urine. Accordingly, considerable attention has been paid in the present work to investigating the optimal conditions for carrying out the precipitation and for collecting the precipitated pregnanediol.

Collection of the precipitated pregnanediol. In the Astwood & Talbot procedures and also in the method of Guterman [1944] the precipitated pregnanediol was collected by filtration. To facilitate the quantitative collection of the small amounts of precipitate and in order to avoid as far as possible transference of the material from vessel to vessel, the precipitation process in the present work has been carried out in centrifuge tubes and the precipitate collected by centrifugation. At first some difficulty was experienced in getting the relatively light pregnanediol crystals to pack sufficiently tightly in the tubes to permit the siphoning-off of the supernatant solution. This difficulty was, however, overcome by adding to the precipitation mixture before centrifugation a small amount of the filter-aid 'Hyflo-Super Cel', which effectively entrained the precipitated pregnanediol.

Number of precipitations and volume of precipitation mixture. Astwood & Jones [1941] purified the pregnanediol from the neutral fraction by one precipitation with four volumes of N/10 sodium hydroxide followed by one with four volumes of water. Talbot *et al.* [1941] and Guterman [1944] used a single precipitation with four volumes of N/10 sodium hydroxide. In the experience of the present authors, however, one precipitation with sodium hydroxide and two with water are required in order to obtain the pregnanediol in a satisfactory state of purity. This multiple precipitation technique lengthens the process somewhat, but the loss of time is more than compensated for by the resulting increase in specificity which results.

Numerous preliminary experiments showed that the most satisfactory results were obtained when the neutral fraction from one-fifth of a 24 hr. specimen of urine was dissolved in 4 ml. of ethanol and precipitated with 16 ml. of N/10 sodium hydroxide or water.

Rate of cooling after precipitation in hot solution. The efficiency of the precipitation process has been studied in many series of recovery experiments in which varying amounts of pure pregnane-3(α), 20α-diol were added to quantities of 'male urine neutral fraction' each corresponding to one-fifth of a 24 hr. urine specimen. In the early experiments of this kind it was found that satisfactory recoveries (80% or better) were obtained when more than about 4 mg. of pregnanediol were present (corresponding to 20 mg./24 hr.), but that with smaller amounts of pregnanediol the recoveries were very much lower and very irregular.

The great irregularity of the recoveries suggested that some important variable factor in the purification process was not being properly controlled. Since the rate of cooling of the mixture after precipitation in hot solution might be expected to determine the size of the pregnanediol crystals it seemed possible that this might be the uncontrolled factor.

This possibility was investigated in two series of recovery experiments in which varying amounts of pure pregnane-3(α), 20α-diol were added to portions of 'male urine neutral fraction', each of which was equivalent to one-fifth of a single 24 hr. specimen. The pregnanediol in these mixtures was purified by the triple precipitation procedure, which was as described below (p. 252) with the exception that the cooling conditions following each precipitation were varied. In one series of experiments the precipitation mixtures were cooled rapidly by immediate immersion in an ice-bath and were then left in the refrigerator overnight before centrifugation. In a second series, which were duplicates of the first, the mixtures were cooled slowly by transferring them in beakers of water at 75° to an incubator at 37° where they were allowed to remain for 2 hr. They were then cooled in the refrigerator for 30 min. and centrifuged.

The results*, which are shown in Table 1, are quite conclusive. It will be seen that rapid cooling of the precipitation mixtures gave lower and much more irregular recoveries of pregnanediol than were obtained by the slow-cooling technique. It will also be seen that the difference in recovery due to the rate of cooling was most marked with amounts of pregnanediol corresponding to less than *c.* 20–25 mg./24 hr.

Later experiments, which are not reported here, have shown that the recoveries by the slow-cooling technique are unaffected by the omission of the short period of refrigeration before centrifugation. It has also been found that a longer period at 37°

* A preliminary paper dealing with these findings was read to the Society for Endocrinology on 29 May 1947.

than 2 hr. does not lower the recoveries. In the method finally adopted (p. 252) the actual periods during which the precipitation mixtures are incubated have been adjusted to fit a working day of convenient length.

Table 1. *The effect of varying the rate of cooling after precipitation on the recovery of pregnanediol added to 'male urine neutral fraction'*

'Male urine neutral fraction'	Pregnanediol added (mg.)	Pregnanediol recovered (mg.)		Pregnanediol recovered corrected for 'male urine blank' (%)	
		Rapid cooling	Slow cooling	Rapid cooling	Slow cooling
A	0·00	0·056	0·056		
	0·00	0·012 → 0·031	0·055 → 0·050	—	—
	0·00	0·026	0·040		
B	0·00	0·017	0·040		
	0·00	0·015 → 0·014	0·042 → 0·040	—	—
	0·00	0·010	0·037		
A	0·27	0·032	0·123		
	0·27	0·012 → 0·023	0·092 → 0·096	0	17
	0·27	0·025	0·072		
B	0·40	0·016	0·33		
	0·40	0·021 → 0·017	0·38 → 0·34	1	75
	0·40	0·015	0·31		
A	0·53	0·43	0·45		
	0·53	0·42 → 0·41	0·46 → 0·49	66	84
	0·53	0·37	0·57		
B	0·80	0·63	0·86		
	0·80	0·82 → 0·75	0·82 → 0·84	89	99
	0·80	0·80	0·83		
A	4·0	4·0	4·2		
	4·0	3·9 → 3·5	4·1 → 4·1	86	101
	4·0	2·5	4·0		

In view of these findings it is of some interest to consider the precipitation and cooling techniques of previous workers who have used the Astwood-Talbot method. Astwood & Jones [1941] do not appear to have controlled the temperature at which precipitation was carried out very exactly, but they did allow the precipitation mixtures to cool to room temperature before cooling in the refrigerator. Such a procedure in an American laboratory, where 'room temperature' may be 25° or more might be said to provide 'slow cooling'; in the average British laboratory in winter time, however, the procedure might very well give quite rapid cooling. Neither Talbot *et al.* [1941] nor Guterman [1944] controlled the temperature of precipitation, and since in both cases the precipitation mixtures were transferred directly to the refrigerator or into an ice-bath, their procedures must have undoubtedly involved rapid cooling. It can be concluded that the procedures used by all these workers would be liable to give low and erratic results with urines containing less than *c.* 20 mg. of pregnanediol per 24 hr.

Sulphuric acid colour reaction

The finally purified pregnanediol obtained by the precipitation process from the urine of pregnant and of non-pregnant women has occasionally been found to be contaminated with traces of a blue pigment. The nature of this pigment has not been

investigated, but it is suspected that it may be of dietary origin. The presence of this pigment seriously interferes with the sulphuric acid colour reaction and it is therefore necessary to remove it before carrying out the reaction. This can be effectively done without loss of pregnanediol by warming in ethanolic solution with charcoal. In order to maintain a rigid uniformity in procedure this treatment with charcoal has been adopted as a routine whether the pigment is present or not.

Talbot *et al.* [1941] carried out the colour reaction by allowing the purified pregnanediol to stand with 10 ml. of concentrated sulphuric acid at room temperature for 20 min. In the present work, in order to standardize conditions as far as possible, the colour development has been carried out in a water-bath at 25° instead of at 'room temperature'.

METHOD FINALLY ADOPTED FOR THE DETERMINATION OF PREGNANEDIOL IN URINE

A 24 hr. specimen of urine collected with 5 ml. of toluene as preservative is made up to 2500 ml. and duplicate 500 ml. samples removed. Each sample is treated as follows: It is placed in a 1000 ml. flask and after the addition of 100 ml. of toluene brought to boiling point under a reflux condenser. To the boiling mixture is added down the condenser 50 ml. of concentrated hydrochloric acid (A.R.), and the boiling continued for exactly 10 min. The flask is then rapidly cooled in cold water and the contents transferred to a separating funnel of 750 ml. capacity. After shaking and allowing the urine layer to separate, the latter is run off into the original flask and the layer of toluene and emulsion filtered with gentle suction through a Whatman No. 1 paper on a Buchner filter funnel. The urine layer is then returned to the separating funnel and extracted twice more with 100 ml. portions of toluene, each toluene and emulsion layer being filtered in succession through the same filter funnel. The combined filtrates are then transferred to a clean separating funnel, and after running off the small urine layer that separates, the toluene extract is washed twice with 100 ml. portions of N sodium hydroxide and twice with 100 ml. portions of water. The washed toluene extract is run into a 500 ml. round-bottomed flask and is evaporated nearly to dryness on an electric hot plate and then taken completely to dryness under reduced pressure on a boiling-water bath.

The dry residue is transferred quantitatively with warm ethanol to a 20 ml. conical centrifuge tube and the ethanolic solution evaporated to dryness in a water-bath under a stream of air. To the residue in the tube are added exactly 4·0 ml. of ethanol and the tube is placed in a beaker of water maintained at 75°. After stirring with a glass rod for 1 min. to obtain complete solution, 16·0 ml. of N/10 sodium hydroxide are added drop-wise from a burette during 3 min. with stirring, the last 1 ml. being used to wash down the stirring rod into the tube. After a further 1 min. at 75°, the beaker of water containing the tube is transferred to an incubator at 37° and left overnight. Approximately 8–10 mg. of 'Hyflo-Super Cel' (Johns-Manville Co. Ltd.) are added and the mixture stirred with a glass rod. The rod is washed down into the tube with 1 ml. of a 1 : 4 (v/v) ethanol-water mixture and the tube is then centrifuged for 1 hr. (1500 r.p.m.; radius of centrifuge head: 15 cm.). The supernatant solution is finally sucked from the precipitate with the aid of a fine glass tube attached to a slowly running water-pump.

The second and third precipitations are carried out as described above, except that water instead of sodium hydroxide solution is used, and the incubation periods are reduced for convenience to 2 hr. No additional filter-aid is added before the centrifugations following the second and third precipitations.

To the final precipitate are added 5 ml. of ethanol and the pregnanediol dissolved by warming with stirring at about 75°. 'Norite' charcoal (*c.* 1–2 mg.) is then added and the warming continued for 2 min. The mixture is filtered through a small filter (Whatman No. 1 paper) into a test-tube of 1 in. diameter, the centrifuge tube and filter being washed three times with 2 ml. portions of warm ethanol. The filtrate and washings in the tube are evaporated in a water-bath under a stream of air and the residue finally dried by leaving the tube in a vacuum desiccator over calcium chloride for several hours.

The colour reaction is carried out with not more than *c.* 0·5 mg. of the finally purified product. If, therefore, the amount of the latter appears on inspection to be in excess of 0·5 mg. a suitable aliquot portion is removed after solution in a known volume of ethanol. To the dry pregnanediol 10·0 ml. of concentrated sulphuric acid (A.R.) are added from a burette, and the tube is left in a water-bath at 25° for 20 min. with occasional shaking. The intensity of the yellow colour produced is measured in a 'Spekker' photoelectric absorptiometer using a 'spectrum violet' No. 601 light filter.

The absorptiometer readings are interpreted by reference to a calibration curve made with known amounts of pure pregnane-3(α), 20α-diol varying from 0·1 to 0·5 mg. It is advisable to construct a fresh calibration curve for each batch of unknowns.

RECOVERY EXPERIMENTS

The accuracy of the finally adopted method was tested in a long series of recovery experiments in which pure sodium pregnanediol glucuronidate was added in varying amounts to men's urine. The validity of such tests of accuracy depends upon two assumptions: (*a*) that all the pregnanediol in human urine is present as the glucuronide, and (*b*) that women's urine contains no substances which are not present in men's urine which would interfere with the determination of pregnanediol. Further work will be necessary to see whether these assumptions are justifiable or not.

Twelve 24 hr. specimens of urine were collected from four normal men. Each specimen was made up to 2500 ml. and four 500 ml. samples removed. To each of two of these samples was added an identical amount of sodium pregnanediol glucuronidate dissolved in 80% ethanol, the other two samples being retained for working-up as 'male urine blanks'. All four samples from each specimen were then treated as described in the preceding section (pp. 252, 253). The results in Table 2 show that a pregnanediol content of *c.* 2 mg./24 hr. is a critical one, above which recoveries are excellent, but below which they are poor.

That the loss of pregnanediol when less than *c.* 2 mg./24 hr. is present in the urine occurs mainly during the precipitation process rather than during the hydrolysis or extraction seems to be clear from the results shown in Table 1. This loss must be largely due to an effect of other substances in the neutral fraction upon the solubility of pregnanediol in 20% ethanol, since experiments in pure solution have shown that

80–95 % of pregnanediol can be removed after the triple precipitation process when it is present in amounts corresponding to as little as 0·5 mg./24 hr. It is possible, therefore, that the critical concentration of pregnanediol below which recoveries are unsatisfactory may vary somewhat with different urines. However, the results reported here with a number of different specimens of men's urine suggest that this critical concentration is probably not far from 2 mg./24 hr. in the majority of cases.

Table 2. *Recovery of pregnanediol after the addition of sodium pregnanediol glucuronidate to men's urine*

Men's urine specimen	'Male urine blank' as apparent pregnanediol in $\frac{1}{5}$ of 24 hr. specimen (mg.) (av. of duplicates)	Pregnanediol added as glucuronidate to $\frac{1}{5}$ of 24 hr. urine specimen (mg.)	Pregnanediol recovered (mg.)		Pregnanediol recovery (corrected) (%)
			Apparent	Corrected for blank	
C4	0·016	0·2	0·017	0·001	0
		0·2	0·012	—	0
A3	0·008	0·2	0·021	0·013	7
		0·2	0·047	0·039	20
B2	0·024	0·2	0·060	0·036	18
		0·2	0·045	0·021	11
D4	0·035	0·4	0·32	0·29	72
		0·4	0·33	0·29	74
B3	0·015	0·4	0·28	0·27	67
		0·4	0·29	0·28	69
A2	0·018	0·4	0·35	0·33	82
		0·4	0·35	0·33	82
A4	0·044	1·0	0·99	0·95	95
		1·0	0·98	0·93	93
C3	0·019	1·0	0·94	0·92	92
		1·0	0·96	0·94	94
D2	0·077	1·0	1·0	0·92	92
		1·0	0·98	0·90	90
B4	0·030	2·0	1·9	1·9	95
		2·0	2·0	2·0	100
D3	0·017	2·0	2·0	2·0	100
		2·0	1·9	1·9	95
C2	0·026	2·0	1·9	1·9	95
		2·0	1·9	1·9	95

SPECIFICITY OF THE METHOD

The sulphuric acid colour reaction is not specific for pregnanediol; many other steroids give similar colours with varying intensities. The specificity of the method as a whole therefore depends upon the completeness with which other chromogenic steroids originally present in the urine are eliminated in the extraction and purification process.

Some data relevant to this extremely important point have been presented by previous workers. Thus Astwood & Jones [1941] showed that cholesterol and androsterone were completely eliminated by their double precipitation process when present in amounts not exceeding 16 and 8 mg. per litre respectively, while Talbot *et al.* [1941] showed that dehydro*iso*androsterone in amounts up to *c.* 10 mg. per litre

caused no interference. In the course of present work additional relevant data have been accumulated, but since these data are still incomplete they will be referred to at the present time only briefly.

Androsterone, *iso*androsterone, and pregnan-3(α)-ol-20-one are not completely eliminated by the triple precipitation process used, but providentially they are so much less powerfully chromogenic than pregnanediol in the sulphuric acid reaction that their presence in the final product, except in abnormally large amounts, does not introduce any serious error into the pregnanediol determination. Dehydro*iso*-androsterone and pregnane-3(α), 17, 20-triol, on the other hand, are very powerful chromogens, but, providentially again, they seem to be very readily eliminated in the precipitation process. Any possible interference by the pregnanetriol is doubly safeguarded against by the fact that this compound would be largely decomposed during the initial hydrolysis of the urine with acid.

These preliminary findings provide hope that the method may be reasonably specific for pregnanediol when applied to normal urines, but before definitely concluding that it is indeed so it will be necessary to carry out further experiments with other steroids likely to be present. At the present time the method cannot be recommended for pathological urines containing abnormally high concentrations of neutral 17-ketosteroids or of other neutral steroids of adrenal origin. In such cases the pregnanediol present might be considerably overestimated. In passing, it may be remarked that any of the methods in which pregnanediol is determined as its glucuronide are also likely to give fictitiously high results when applied to urines containing abnormally high concentrations of steroids of adrenal origin, since certain of the latter are probably excreted as glucuronides also.

DISCUSSION

The methods of urinary pregnanediol determination which have hitherto been described are either insufficiently sensitive or insufficiently specific to permit of strictly quantitative studies being carried out upon pregnanediol excretion during the menstrual cycle and during the early stages of pregnancy. The method described here should make such studies more nearly possible, and in particular should be of some value in the investigation of causes of female sterility.

Although the method was not designed for use as a means of pregnancy diagnosis, it may be useful for this purpose, and because of its more quantitative nature it may prove to be less subject to both positive and negative errors than the more rapid Guterman [1944, 1945] method. In view of the widespread interest in the latter it would perhaps not be out of place to discuss in the light of the findings in the present work some of the possible sources of the negative and positive errors to which the method seems to be subject [cf. Reinhart & Barnes, 1946].

In his latest paper on the method Guterman [1945] has arbitrarily fixed on an intensity of colour in the sulphuric acid reaction corresponding to 6–8 mg. of pregnanediol per 24 hr. as the lower limit for a positive reaction. It seems probable that at such levels of pregnanediol excretion the unsatisfactory cooling conditions in the precipitation process in Guterman's procedure might lead to low and variable yields of pregnanediol and thus to false negative results. It is likely that a controlled 'slow

cooling' technique in the precipitation process might eliminate some at least of these false negatives.

The findings in the present work suggest that false positives might be caused by the presence of abnormally large amounts of the weakly chromogenic saturated neutral 17-ketosteroids which are incompletely removed even by a triple precipitation procedure, or by the incomplete elimination in the single precipitation of the Guterman method of the strongly chromogenic dehydro*iso*androsterone. In this connexion it is noteworthy that Morrow & Benua [1946] recorded false positives in a case of arrhenoblastoma which was excreting 59 mg. of 17-ketosteroids per 24 hr. It is doubtful whether false positives caused by the presence of abnormally large amounts of 17-ketosteroids could be entirely eliminated by any simple modification in the precipitation process, but it is likely that their number could be reduced if a double instead of a single precipitation procedure were to be adopted.

In conclusion, one may perhaps question whether it is justifiable to accept as diagnostic of pregnancy any arbitrarily fixed low level of pregnanediol excretion associated with amenorrhea. It must be remembered that little is known at the present time about the metabolism of progesterone, and there are in fact few reasons to believe that pregnanediol is even the main metabolic end-product of the latter. The low yields of urinary pregnanediol obtained after the administration to human subjects of progesterone raise the possibility that the latter may be largely metabolized in the body by other routes. It is therefore questionable whether the level of pregnanediol excretion provides a reliable indication of the progesterone production in the body, as has been so widely assumed.

As pointed out by Reinhart & Barnes [1946], it is possible that the greatest source of error in the Guterman test is the 'individual variations in the metabolism of progesterone, both in the pregnant and the non-pregnant woman'.

SUMMARY

A procedure based on the methods of Astwood & Jones [1941] and Talbot *et al.* [1941] has been elaborated which permits of the reasonably accurate determination of more than *c.* 0·4 mg. of pregnanediol in a fifth of a 24 hr. sample of human urine.

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

By I. F. SOMMERVILLE and G. F. MARRIAN
Department of Biochemistry, University of Edinburgh

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Since the establishment by Venning & Browne in 1937 of the status of pregnane-3α:20α-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-3α:20α-diol to normal men has been determined.

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

* The term 'pregnanediol' is used to denote the material in urine consisting largely, but not necessarily entirely, of pregnane-3α:20α-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).

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-3α:20α-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-3α:20α-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

(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 anovulatory menstruation, while Jones & TeLinde (1941) report a recovery of 11·5 % in the preovulatory 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, 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 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)	10·4 10·0 10·8 10·0
J.P.	23	Normal man	14·7
R.	22	Normal man	9·3

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 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 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.

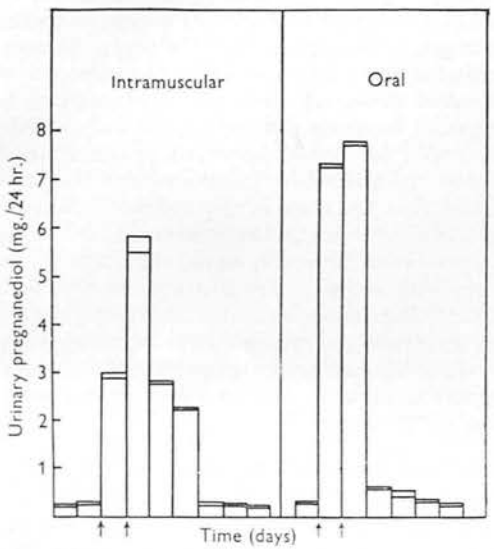

Fig. 1. Urinary pregnanediol excretion after the administration of progesterone to a normal man (subject P.) by intramuscular and oral routes. Arrows indicate administration of 60 mg. progesterone. The values obtained in the duplicate determinations are indicated by the double lines at the tops of the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

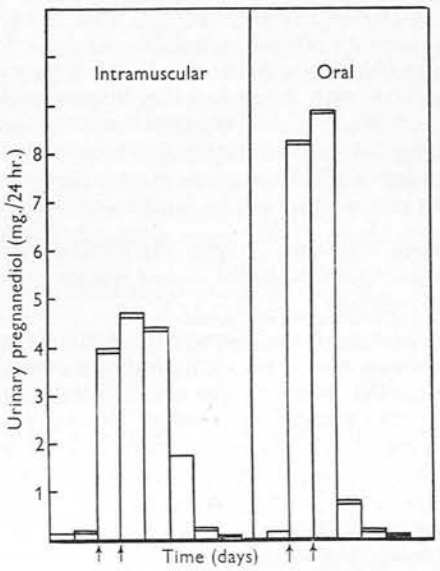

Fig. 2. Urinary pregnanediol excretion after the administration of progesterone to a post-menopausal woman (subject H.) by intramuscular and oral routes. Arrows indicate administration of 60 mg. progesterone. The values obtained in the duplicate determinations are indicated by the double lines at the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

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 toxaemic 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-3α:20α-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-3α:20α-diol. However, when sodium pregnanediol glucuronidate 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-3α:20α-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-3α:20α-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-3α:20α-diol to men on 2 successive days*

Subject	Recovery of pregnanediol following the oral administration of progesterone (%)	Pregnane-3α:20α-diol administered orally (mg.)	Recovery of pregnanediol following the administration of pregnane-3α:20α-diol (%)
R.	12·8	2 × 48	12·3
P.	18·6	2 × 56	16·3
J. P.	11·5	2 × 59	13·4

It is seen that the recoveries of pregnanediol in the urine following the oral administration of pregnane-3α:20α-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-3α:20α-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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Urinary Excretion of Pregnanediol in Human Subjects following the Administration of Progesterone and of Pregnane-3α:20α-diol. 2*

By I. F. SOMMERVILLE and G. F. MARRIAN
Department of Biochemistry, University of Edinburgh

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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 pregnanediol 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

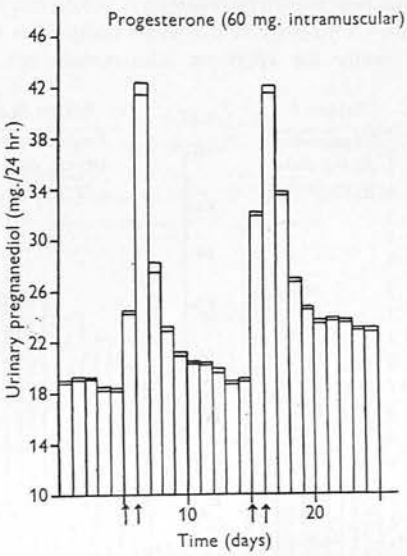

Fig. 1. Effect of progesterone on the excretion of pregnanediol during pregnancy.

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

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

$$= 41{\cdot}9 \text{ mg.}$$

$$= 34{\cdot}9\,\% \text{ of the administered progresterone,}$$

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

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

$$= 52{\cdot}8 \text{ mg.}$$

$$= 44{\cdot}0\,\% \text{ of the administered progesterone.}$$

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

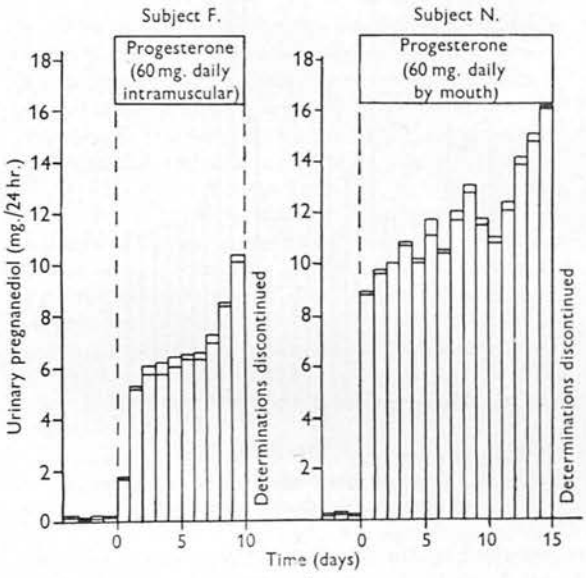

Fig. 2. Pregnanediol excretion during prolonged administration of progesterone to normal post-menopausal women.

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

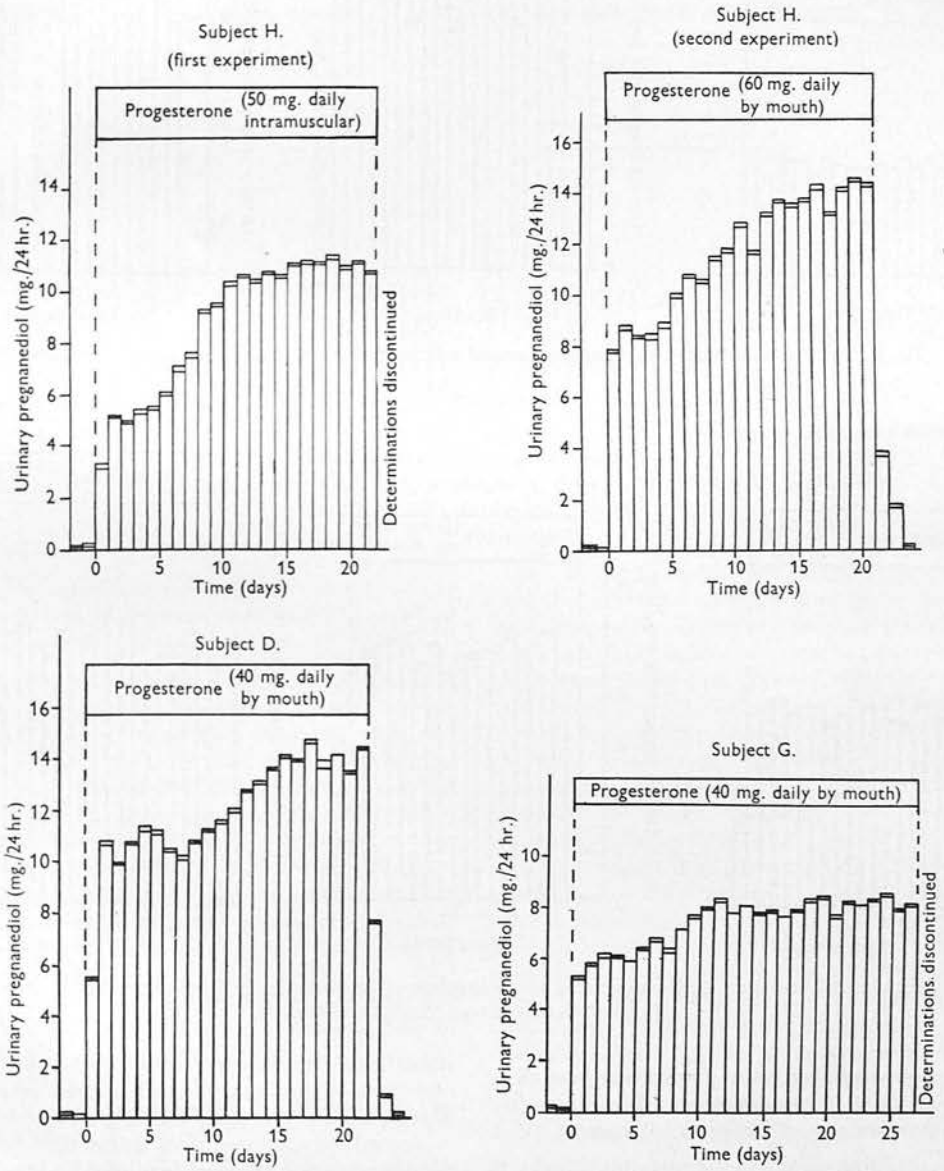

Fig. 3. Pregnanediol excretion during prolonged administration of progesterone to normal post-menopausal women.

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

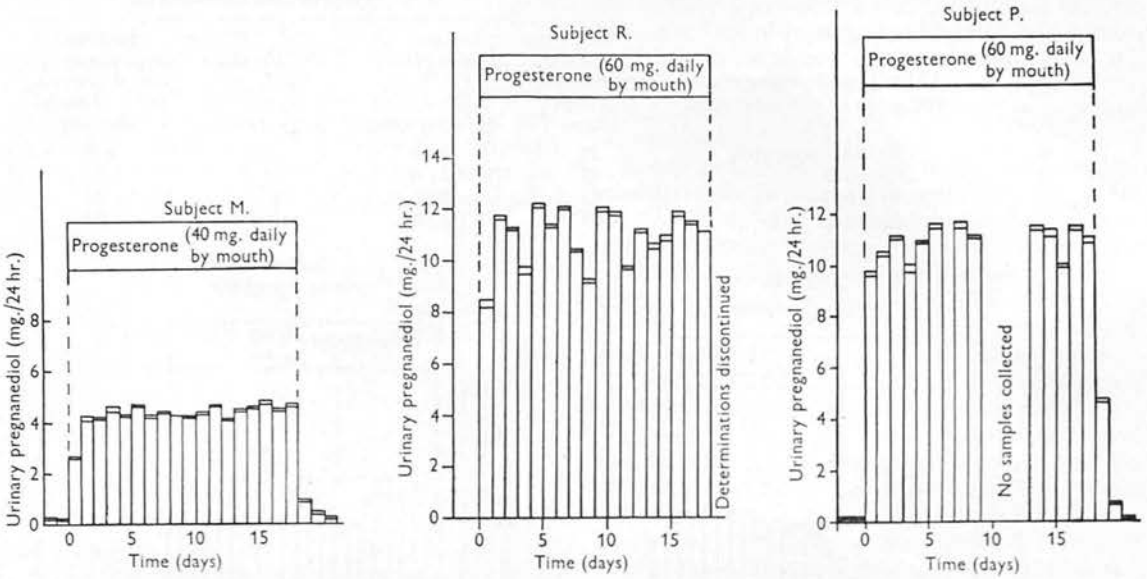

Fig. 4. Pregnanediol excretion during prolonged administration of progesterone to normal men.

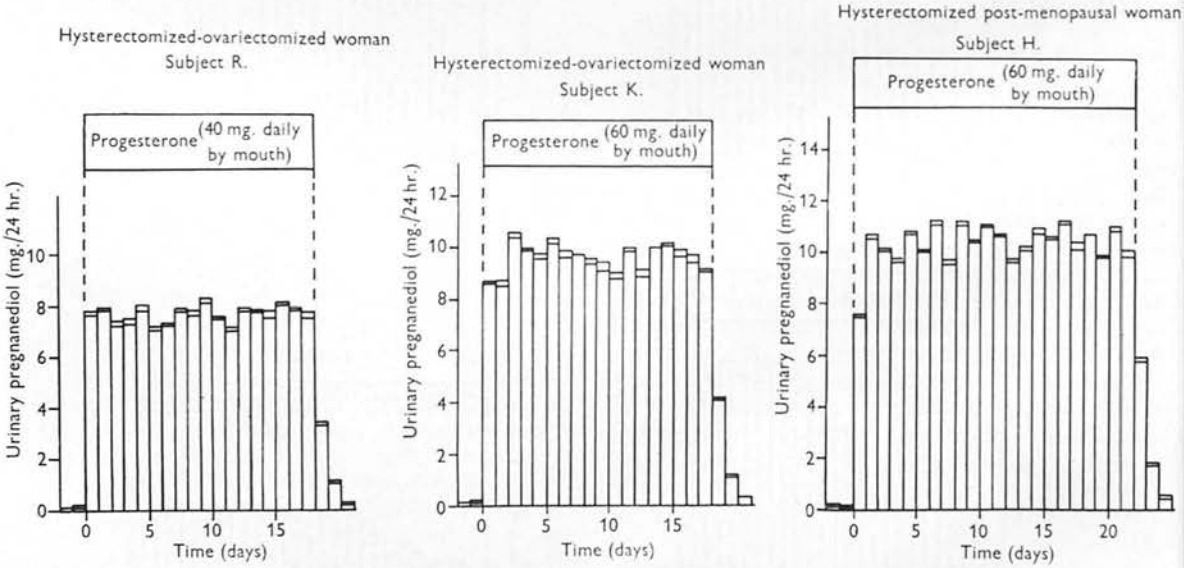

Fig. 5. Pregnanediol excretion during prolonged administration of progesterone to hysterectomized-ovariectomized and hysterectomized post-menopausal women.

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 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.

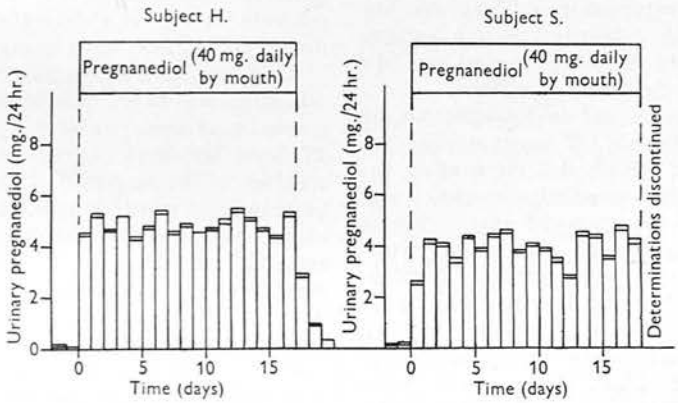

Fig. 6. Pregnanediol excretion during prolonged administration of pregnane-3α:20α-diol to normal post-menopausal women.

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.

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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ABNORMALITY IN STEROID METABOLISM ASSOCIATED WITH RHEUMATOID ARTHRITIS

I. F. SOMMERVILLE
M.B., Ph.D. Edin.

G. F. MARRIAN
D.Sc. Lond., F.R.S.

J. J. R. DUTHIE
M.B. Aberd., F.R.C.P.E.

R. J. G. SINCLAIR
M.R.C.P.E.

From the University Department of Biochemistry and the Rheumatic Unit, Northern General Hospital, Edinburgh

IT has recently been shown by Hench et al. (1949) that rheumatoid arthritics respond dramatically to treatment with 11-dehydro-17-hydroxycorticosterone (' Cortisone ') in the form of its acetate, at a dose-level of about 100 mg. per day. The high dosage required for the relief of the symptoms coupled with the fact that rheumatoid arthritics, as far as is known, do not exhibit any of the generally recognised symptoms of adrenal insufficiency, suggested to us that the condition might be associated with an abnormality in the metabolism of cortisone in the affected tissues, rather than with a deficient secretion of this hormone by the adrenal cortex.

Since the large quantities of cortisone necessary for a direct test of this hypothesis were unavailable to us, we considered that it might be profitable to study quantitatively in rheumatoid-arthritic subjects the metabolism of some more readily obtainable steroid closely related structurally to cortisone. We felt that although the discovery of an abnormality in the metabolism of such a steroid in rheumatoid arthritis would not prove the original working hypothesis, it would at least indicate the desirability of attempting a direct experimental investigation as soon as the supply of cortisone improves.

Progesterone was selected for this investigation in preference to the more closely related 11-deoxycorticosterone for two reasons : (1) at the high dosages which have to be employed in such steroid metabolism experiments the sodium-retaining action of the latter might have constituted a serious danger, whereas at these dosages progesterone produces no undesirable physio-

logical or pharmacological effects apart from mild sedation in some instances ; and (2) as a result of previous investigations by two of us (Sommerville and Marrian 1950) a considerable amount of information was already available concerning the conversion of administered progesterone into urinary pregnanediol in normal people.

EXPERIMENTAL PROCEDURE

Progesterone dissolved in arachis oil was administered to the subjects by intramuscular injection in two successive daily doses of 60 mg. Twenty-four-hour urine specimens (preserved with toluene) were collected from each subject during a preliminary control period of two days, during the two days when the hormone was being administered, and for the following four days. Each specimen was made up to a volume of 2500 ml. with water, and pregnanediol determinations in duplicate were carried out on 500 ml. samples by the method of Sommerville, Gough, and Marrian (1948). The total pregnanediol excreted as a result of the administration of the progesterone was calculated after correction of the daily apparent pregnanediol excretion for the average control-period " blank."

Experiments were carried out on 9 postmenopausal women and 4 men suffering from rheumatoid arthritis, and on 6 normal postmenopausal women and 4 normal men.* Since some salicylates are known to be excreted in the urine in conjugation with glucuronic acid, the possibility had to be considered that these might in some way influence the excretion of pregnanediol by interfering with the formation of pregnanediol glucuronide. Accordingly salicylate therapy was withheld from all the arthritic subjects for from one to eight weeks before the experiments.

RESULTS

The results indicate quite clearly that rheumatoid arthritics of both sexes excrete in the urine as pregnanediol an abnormally high proportion of intramuscularly administered progesterone. The values given are calculated from the mean of the duplicate determinations. In every case satisfactory agreement between the duplicate determinations was obtained. The percentage of administered progesterone recovered as urinary pregnanediol was as follows.

* All but one of the figures for the excretion of pregnanediol by normal postmenopausal women and men following the intramuscular injection of progesterone are taken from a previous paper by two of us (Sommerville and Marrian 1950).

	Normal	*Rheumatoid arthritic*
Men :	9·3 ..	.. 21·7
	10·0 ..	.. 23·8
	12·2 ..	.. 26·1
	14·7 ..	.. 27·4
Postmenopausal women :	12·1 ..	.. 19·0
	13·7 ..	.. 20·7
	15·2 ..	.. 22·0
	15·6 ..	.. 25·1
	15·6 ..	.. 25·7
	16·0 ..	.. 26·8
		31·0
		35·0
		36·4

Careful assessment of all clinical data revealed no clear-cut correlation between the severity of the disease and the percentage of administered progesterone excreted as urinary pregnanediol.

DISCUSSION

Until quantitative studies on the conversion of administered progesterone into urinary pregnanediol have been carried out in a wide variety of other clinical conditions, the importance of the present observations must remain in some doubt, since the abnormality in progesterone metabolism reported here may be more common than we now suppose. Furthermore, even though this abnormality should prove to be specific to rheumatoid arthritis and related conditions, it may be quite unrelated to the response of these conditions to cortisone therapy. Nevertheless, the discovery of an abnormality in the metabolism of progesterone in a clinical condition which yields to treatment with a closely related steroid hormone is not without interest ; and it now seems clear that our hypothesis that rheumatoid arthritis may be associated with an analogous abnormality in the metabolism of the adrenocortical steroid hormones deserves serious consideration and must be investigated experimentally as soon as circumstances permit.

Further speculations about the possible significance of the present results would be unwise at the moment since little is known about the metabolism of cortisone in the body, while the exact physiological significance of pregnanediol as a metabolite of progesterone is still very obscure.

Although there was no evidence of a correlation between the abnormality of progesterone metabolism and the activity of the disease process as assessed by the criteria available, further work may possibly reveal a relation between this metabolic abnormality and the fundamental pathogenesis of rheumatoid arthritis.

In the hope that others may wish to attempt the confirmation and further extension of our results, we wish to take this opportunity of pointing out that for quantitative studies of the urinary pregnanediol excretion following the administration of progesterone in doses of less than about 100 mg. per day, a method of pregnanediol determination at least as sensitive and accurate as that used in the present work must be employed. The difference in the pregnanediol excretions between normal and arthritic subjects reported here are quite definite, but it is doubtful whether they would have been observed if a less accurate and sensitive quantitative method had been used. In our opinion, older methods such as the original one of Venning (1937, 1938), or newer methods designed primarily for semi-quantitative routine diagnostic work, such as those of Guterman (1944, 1945) and of Sommerville, Marrian, and Kellar (1948), would be entirely unsuitable for quantitative studies on progesterone metabolism.

We wish to express our gratitude to the Medical Research Council for a personal grant to one of us (I. F. S.), and for a grant (to G. F. M.), from which the expenses of this work were largely defrayed. We owe much to the skilful technical assistance of Mr. D. W. Davidson.

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